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XXXV cycle

Development and validation of novel methods and technologies for sustainability and health risk assessment in circular urban water management

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List of abbreviations

BOD: Biochemical Oxygen Demand

BWD: Bathing Water Directive

CECs: Contaminants of Emerging Concern

DALYs: Disability Adjusted Life Years

DBPs: Disinfection By-Products

DWTP: Drinking Water Treatment Plant

EC: European Commission

EF: Emission Factor

EPA: Environment Protection Agency

EU: European Union

FIB: Faecal Indicator Bacteria

GPP: Green Public Procurement

GWP: Global Warming Potential

ISO: International Standard Organization

JRC: Joint Research Centre

KRM: key element of risk management

LCA: Life Cycle Assessment

RMP: Risk Management Plan

SSP: Sanitation Safety Planning

TSS: Total Suspended Solids

TOC: Total Organic Carbon

WFD: Water Framework Directive

WHO: World Health Organization

WRSP: Water Reuse Safety Plan

WSP: Water Safety Plan

UASB: Up-flow Anaerobic Sludge Blanket

UV: Ultraviolet

UWWTP: Urban Wastewater Treatment Plant

WRSP: Water Reuse Safety Plan

WWTP: Wastewater Treatment Plant

WEFC: Water-Energy-Food-Climate

Scientific production of the PhD work

TOTAL PUBLISHED ISI PAPERS AND REVIEWS: 5

CITATIONS: 52

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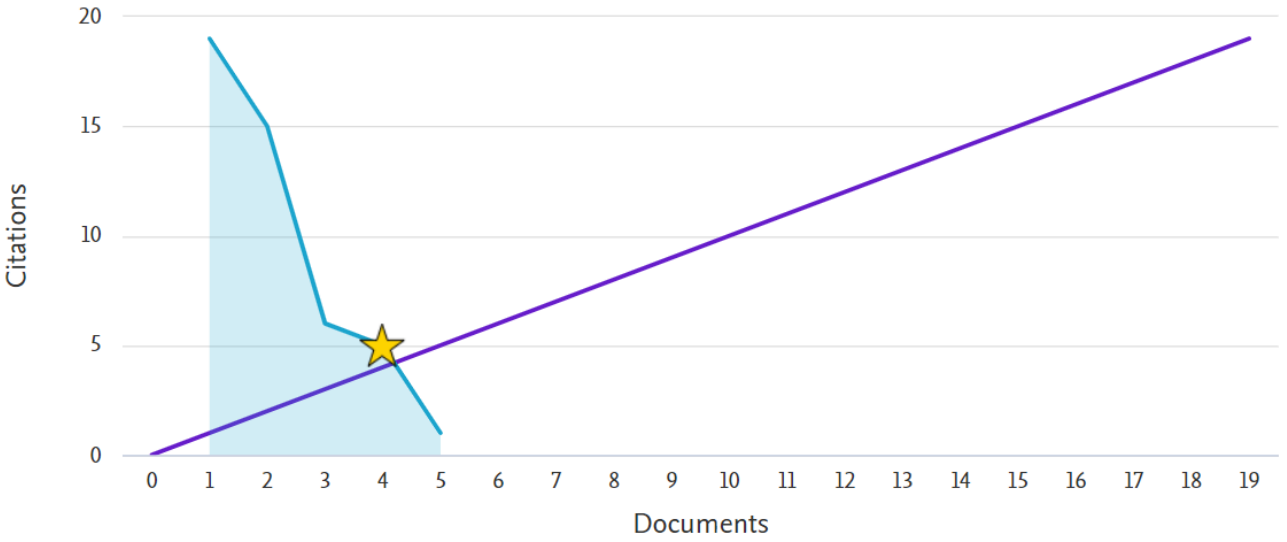


Figure 1: trend of h-index, adapted from Scopus

Akyol, Ç., Ozbayram, E. G., Accoroni, S., **Radini, S.**, Eusebi, A. L., Gorbi, S., Vignaroli, C., Bacchiocchi, S., Campacci, D., Gigli, F., Farina, G., Albay, M., & Fatone, F. (2021). Monitoring of cyanobacterial blooms and assessing polymer-enhanced microfiltration and ultrafiltration for microcystin removal in an Italian drinking water treatment plant. *Environmental Pollution*, *286*. https://doi.org/10.1016/j.envpol.2021.117535

Radini, S., Marinelli, E., Akyol, Ç., Eusebi, A. L., Vasilaki, V., Mancini, A., Frontoni, E., Bischetti, G. B., Gandolfi, C., Katsou, E., & Fatone, F. (2021). Urban water-energy-food-climate nexus in integrated wastewater and reuse systems: Cyber-physical framework and innovations. *Applied Energy*, *298*. https://doi.org/10.1016/j.apenergy.2021.117268

Foglia, A., Andreola, C., Cipolletta, G., **Radini, S.**, Akyol, Ç., Eusebi, A. L., Stanchev, P., Katsou, E., & Fatone, F. (2021). Comparative life cycle environmental and economic assessment of anaerobic membrane bioreactor and disinfection for reclaimed water reuse in agricultural irrigation: A case study in Italy. *Journal of Cleaner Production*, *293*. https://doi.org/10.1016/j.jclepro.2021.126201

Marinelli, E., **Radini, S.**, Akyol, Ç., Sgroi, M., Eusebi, A. L., Bischetti, G. B., Mancini, A., & Fatone, F. (2021). Water–energy–food–climate nexus in an integrated peri-urban wastewater treatment and reuse system: From theory to practice. *Sustainability (Switzerland)*, *13*(19). https://doi.org/10.3390/su131910952

Marinelli, E., **Radini, S.**, Foglia, A., Lancioni, N., Piasentin, A., Eusebi, A. L., & Fatone, F. (2021). Validation of an evidence-based methodology to support regional carbon footprint assessment and decarbonisation of wastewater treatment service in Italy. *Water Research*, *207*. https://doi.org/10.1016/j.watres.2021.117831

Eusebi, A. L., Foglia, A., Marinelli, E., Lancioni, N., **Radini, S.**, Fatone, F., Piasentin, A., Durigon, R., Alto, P. F., & Servizi, T. (2022). WWEECarb Un metodo scientifico validato per quantificare l'impronta di carbonio del servizio di depurazione. *Servizi a Rete Marzo-Aprile 2022*.

Radini, S., Marinelli, E., Foglia, A., Andreola, C., Sgroi, M., Eusebi, A. L., Fatone, F. (2022). Riuso delle acque reflue in Italia – Stato attuale, piani di gestione del rischio e soluzioni digitali. *Servizi a Rete Settembre-Ottobre 2022*.

Pubblications in progress

Radini, S., González-Camejo, J., Andreola, C., Eusebi, A. L., Fatone, F. (2022). Risk management plans to boost safe urban wastewater reuse for agricultural purposes in Europe. *Journal of Water Process Engineering*. (submitted).

Foglia, A., González-Camejo, J., **Radini, S.**, Sgroi, M., Li, K., Eusebi, A. L., Fatone, F. (2022). Transforming wastewater treatment plants into reclaimed water facilities in water-unbalanced regions. An overview of possibilities and recommendations focusing on the Italian case. *Journal of Cleaner Production*. (submitted)

Radini, S., Seis, W., Dupain, P., Housni, S., Caradot, N., Fatone, F. (2022). Integrated approach combining deterministic and statistic models for early warning systems development. (in preparation).

Radini, S., Sgroi, M., Andreola, C., Marinelli, E., Eusebi, A. L., Fatone, F. (2022). Assessment of the safe reclamation of a municipal wastewater effluent for agricultural reuse: QMRA analysis and standard limits compliance. (in preparation).

Scientific contributions to national and international conferences

- SUWANU EUROPE International conference on Pathways to extend sustainable water reuse practices in Europe, 18th – 19th May 2021. Supporting risk assessment and management of reclaimed water reuse in agriculture in an integrated approach.
- 5th IWA Specialized International Conference Ecotechnologies for Wastewater Treatment (ecoSTP), 22-26th June 2021. Normalized approach for carbon footprint determination in wastewater treatment service.
- SIDISA 29 June – 2 July 2021, Normalized measurement of carbon footprint in full-scale wastewater treatment plants.
- IWARR, 4th IWA Resource Recovery Conference, 5 – 8th Sep 2021. A roadmap for water reuse risk management and safety plan in integrated wastewater and reuse systems.
- ECOMONDO, 26th October 2021. Inquinamento da microplastiche nei grandi laghi e nelle relative infrastrutture idriche urbane: risultati a medio termine di EU LIFE BLUE LAKES.
- NAIADES - Event detection in the water sector, 15th February 2022. Digital solutions to early warn and support decisions for safe water reuse.
- IWA World Water Congress 12-15th September 2022.
    - Digital solutions and early warning system for decision support and risk management in water reuse for irrigation.

    - Normalized Approach for Carbon Footprint Determination: long term measurements in real wastewater treatment plants.
- ECOMONDO 8-11th November 2022.
    - Microplastiche nei laghi e nelle infrastrutture idriche urbane: Life Blue Lakes.
    - Digital Twin, Early Warning System, sustainability assessment and serious game in Water Reuse Risk Management Planning in Peschiera Borromeo.

Scientific and innovation engineering skills

- **TRAINING COURSE: Virtual Instruments (LABVIEW):** monitoring and management of industrial systems. This course was about the use of virtual instruments, such as LabVIEW, for implementing models. In particular, it was pointed out the use of loops, creation of data structure, use of decision-making structures, modularity, acquisition of measurements from hardware, access of files in LabVIEW, use of sequential and state machine programming.
- **TRAINING COURSE: Setting up whole plants models in BIOWIN:** ability to set up and operate wastewater treatment process simulator that ties together biological, chemical, and physical process models. Steady state and dynamic simulations.
- **TRAINING COURSE: implementation of water safety plans (PSA):** ability to apply a risk-assessment and risk-management approach, addressing all steps in the water supply chain– from catchment to consumers.
- **TRAINING COURSE: Modelling and simulation of wastewater treatment plants with WEST:** ability to set up and operate wastewater treatment process simulator that ties together biological, chemical, and physical process models. Steady state and dynamic simulations.

Abstract

Technical solutions addressed towards a circular approach of urban water systems are presented, including: i) risk management and digital support for water reuse in agriculture and safe bathing water; ii) evaluation of the sustainability through the definition of the nexus between water reuse, carbon and energy footprint and agriculture production, also with the support of decision-making tools such as a methodology to define the territorial carbon footprint of wastewater service or LCA; iii) determination of compounds of emerging concern in the infrastructures connected to strategic water basins, with particular focus on microplastics.

A detailed analysis was conducted for water reuse for irrigation in agriculture, including the implementation of dedicated risk management plans. Risk assessment was performed in three case studies in Italy both with semi-quantitative and with quantitative approaches, showing that treatment disinfection units and modern irrigation techniques allowed the reduction of health risks and could be implemented in a multi-barrier approach to reach the acceptability levels.

Measures to reduce the level of health risks were considered and tests on innovative sensor for microbial determination were conducted. Methodologies to assess sensors performances against standard lab analyses were investigated. Comparing sensor results with lab measurements, it emerged that sensors could reach good levels of agreement, with a correlation coefficient of 0.8 on wastewater matrix.

As concern safe use of bathing water, models for bathing water predictions were integrated to evaluate their applicability in the development of an early warning system for bathing water in Paris. A deterministic model was used to obtain simulations on the future configuration of the sewer system in Paris, while a statistic model was implemented to obtain predictions on *E.coli* concentrations in the bathing site of Pont d'Iena.

The evaluation of alternative water and wastewater uses was coupled with an analysis of the sustainability of those solutions, to evaluate the effective feasibility of their implementation. The sustainability of the circular approach to urban water management was evaluated through the definition of the connections that constitute the nexus among water reuse, carbon and energy footprint and agriculture irrigation.

A methodology for parametric carbon footprint assessment of territorial wastewater service has been developed and tested in four Italian water utilities. It emerged that direct emissions linked to processes and indirect emissions to water body were the most impacting categories. Moreover, mitigation strategies such as the use of renewable energy sources could contribute to the reduction of the emissions. Another supporting tool is represented by the LCA, that was used to evaluate different scenarios and management strategies. Sustainability of resource recovery technologies for phosphorus recovery and the valorisation of the related products in the market were evaluated.

The integrated approach on urban water management and the increased awareness on the associated health risks boosted the research towards the need to investigate on the presence and the impacts of emerging contaminants, such as microplastics. Since a standardised methodology for sampling and characterisation of microplastics in urban water is still missing, a protocol was

developed and proposed to water utilities for the determination and the quantification of MPs in drinking water and wastewater connected to strategic lake water basins. Accordingly, also the presence of cyanobacteria in drinking water treatment plant was investigated.

KEY WORDS: water reuse, risk management, carbon footprint, microplastics.

Sintesi

Nel presente lavoro vengono presentate soluzioni tecniche indirizzate verso un approccio circolare del servizio idrico urbano, tra cui: i) gestione del rischio e supporto digitale per il riutilizzo delle acque reflue in agricoltura e per le acque di balneazione; ii) valutazione della sostenibilità attraverso la definizione del nesso tra riuso dell'acqua, impronta di carbonio ed energia e produzione agricola, anche attraverso il supporto di tool decisionali quali una metodologia applicata per la determinazione del carbon footprint territoriale del servizio di depurazione e LCA; iii) determinazione dei composti emergenti nelle infrastrutture connesse a bacini idrici strategici, con particolare attenzione alla determinazione delle microplastiche.

È stata condotta un'analisi dettagliata per il riutilizzo dell'acqua per l'irrigazione in agricoltura, compresa l'attuazione di piani di gestione del rischio dedicati. La valutazione del rischio è stata eseguita in tre casi di studio in Italia sia con approcci semi-quantitativi che quantitativi, dimostrando che le unità di disinfezione del trattamento e le moderne tecniche di irrigazione possono consentire la riduzione dei rischi per la salute e potrebbero essere implementate in un approccio multi-barriera per raggiungere i livelli di accettabilità.

Sono stati condotti test su sensori innovativi per la determinazione microbica e sono state analizzate metodologie per valutarne le prestazioni. Confrontando i risultati dei sensori con le misurazioni di laboratorio, è emerso che i sensori potrebbero raggiungere buoni livelli prestazionali, con un coefficiente di correlazione pari a 0.8 sulla matrice delle acque reflue.

I modelli per le previsioni delle acque di balneazione sono stati integrati per valutarne l'applicabilità nello sviluppo di un sistema di allerta precoce per le acque di balneazione a Parigi. È stato utilizzato un modello deterministico per ottenere simulazioni sulla futura configurazione del sistema fognario di Parigi, mentre è stato implementato un modello statistico per ottenere previsioni sulle concentrazioni di *E. coli* nella località balneare di Pont d'Iena.

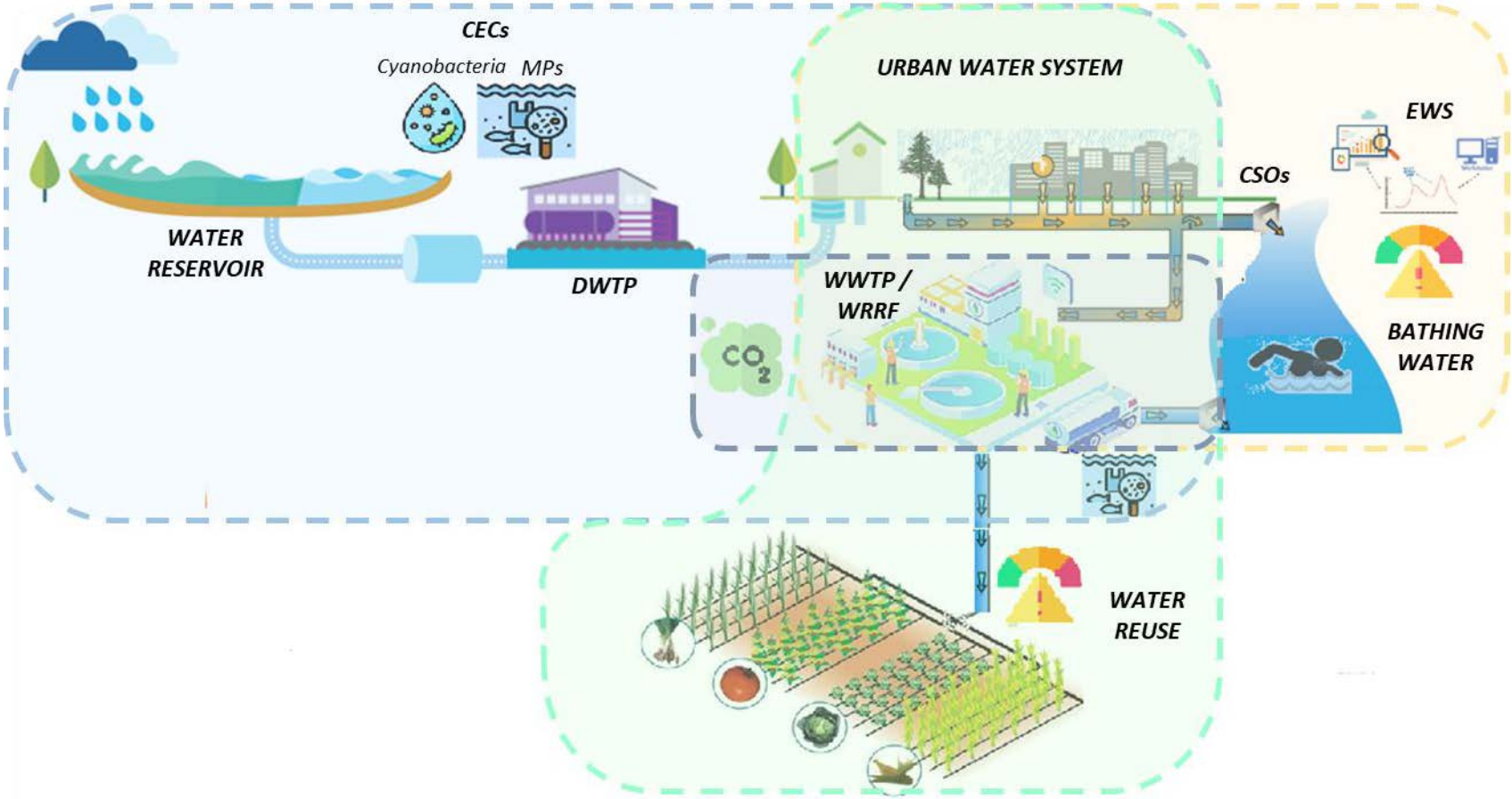
La sostenibilità dell'approccio circolare alla gestione dell'acqua urbana è stata valutata attraverso la definizione delle connessioni tra riutilizzo dell'acqua, impronta di carbonio ed energia e irrigazione agricola.

Una metodologia per la valutazione parametrica dell'impronta di carbonio del servizio delle acque reflue territoriali è stata sviluppata e testata in quattro società idriche italiane. È emerso che le emissioni dirette legate ai processi e le emissioni indirette al corpo idrico sono le categorie più impattanti. Inoltre, strategie di mitigazione come l'uso di fonti di energia rinnovabile possono contribuire alla riduzione delle emissioni. Un altro strumento di supporto è rappresentato dalla LCA, che può essere utilizzata per valutare diversi scenari e strategie di gestione. Sono state valutate la sostenibilità delle tecnologie di recupero delle risorse per il recupero del fosforo e la valorizzazione dei relativi prodotti nel mercato.

L'approccio integrato alla gestione delle acque urbane e la maggiore consapevolezza sui rischi per la salute associati hanno spinto la ricerca verso la necessità di indagare sulla presenza e gli impatti di contaminanti emergenti, come le microplastiche. Poiché una metodologia standardizzata per il campionamento e la caratterizzazione delle microplastiche nelle acque urbane è ancora assente, è

stato sviluppato e proposto alle aziende idriche un protocollo per la determinazione e la quantificazione delle microplastiche nelle acque potabili e reflue connesse a bacini lacustri strategici. Analogamente, è stata studiata anche la presenza di cianobatteri in un impianto di potabilizzazione afferente da un lago italiano.

PAROLE CHIAVE: riuso, analisi di rischio, impronta di carbonio, microplastiche.



CECs = Contaminants of Emerging Concern; MPs = Microplastics; DWTP = Drinking Water Treatment Plant; WWTP = Wastewater Treatment Plant; WRRF = Water Resource Recovery Facility; EWS = Early Warning System; CSOs = Combined Sewer Overflows.

Figure 2: Graphical Abstract.

1. Introduction

Water scarcity and water stress are issues of growing concern at international and European level.

In the worldwide, about 40% of the global land area is classified as arid, semi-arid or dry sub-humid, containing over 2 billion people that experience high water stress (Delli Compagni et al., 2020; Farhadkhani et al., 2018; WWAP, 2019). In Europe, more than 11% of the population and 17% of the territory have been affected by water scarcity (https://ec.europa.eu/environment/water/reuse.htm). Freshwater availability is getting scarcer due to effects related to climate change issues, like heatwaves or drought periods (EIT Water Scarcity, 2021). Apart from limiting water resources for human consumption, water scarcity implies some environmental impacts such as aquifer exhaustion from over abstraction, salinisation of groundwater, increasing contaminants concentration and water pollution, increased erosion of cultivated soils on slopes and decrease of biodiversity in natural water bodies.

One of the major pressures on water stress is water over-abstraction, which is enhanced by irrigation and domestic demand. Agriculture, in fact, is one of the greatest water users, accounting for around 70% of the total extracted water (Jaramillo and Restrepo, 2017). New sources of water thus need to be found to alleviate water scarcity trends. In this respect, the reuse of wastewater in irrigation is a practice that could help to cope with future demands in a sustainable way, also increasing circular economy in the water sector (EIT Water Scarcity, 2021; Foglia et al., 2021). Alternative sources of water supply, such as wastewater reuse, may potentially reduce the pressure on freshwater sources, with significant environmental, social and economic benefits, as stated in the Communication "Blueprint to safeguard Europe's water resources" (European Commission, 2012). In this respect, among the objective of UN Sustainable Development Goal on Water (SDG 6) it is targeted an increase in safe water reuse by 2030.

Reclaimed wastewater can contribute to compensate water needs, providing regular supply (Deviller et al., 2020; Garcia and Pargament, 2015), reducing the exploitation of natural water resources and alleviating the pressures of wastewater treatment plants (WWTP) discharges into sensitive areas. The ecological status of water bodies could be improved with relatively lower investment costs, energy consumption and greenhouse gas emissions, when comparing it with desalination or water transfer practices. Moreover, treated wastewater is much less affected by droughts and seasonal variabilities, and its nutrient content may reduce the use of synthetic fertilizer. In addition, it helps to alleviate poverty in water-scarce developing countries, guaranteeing socio-economic and political stability (Adegoke et al., 2018; Ofori et al., 2021; Sorinolu et al., 2021).

Currently, every year about 1 billion cubic metres of treated urban wastewater is reused in Europe, even if its potential is estimated at six times higher. Despite the potential benefits, water reuse is still scarcely implemented, not only due to factors related to the technological processes, but also for socio-political issues and the lack of standardised regulations. It is thus essential to ensure environmental and health safety in the use of reclaimed water to shift this paradigm, also by developing Water Reuse Risk Management Plans (WRRMPs).

The implementation of WRRMPs is also stated by the EU Regulation 741/2020, with the aim to ensure the healthy use of reclaimed water in agriculture and, consequently, to change the view of wastewater, now considered as a waste, to a source of water.

Conventional municipal WWTPs have been basically aimed to reduce pollutants below legal limits with a linear economy approach. But WWTPs can be implemented into reclamation facilities maximising the production of reclaimed water, together with energy and nutrient recovery, moving towards circular economy principles (Al-Saidi et al., 2021; Pikaar et al., 2022).

It must be highlighted that, other than reclaimed water, other resources such as nutrients, cellulose, biopolymers and fertilizers can be recovered from wastewater, with the subsequent increase in the profitability and sustainability of the process (Palmieri et al., 2019; Robles et al., 2021).

On the same trend, urban water quality is also continuously improving, thanks to an improved management along the water and wastewater cycle, the application of an integrated framework of regulations (WFD) and the construction of dedicated infrastructures for water treatment and management. This aspect is also reflected in bathing waters, with 83% of sites characterized by excellent water quality status, according to EU regulation, and a decreasing number of poor-quality sites, varying from 2% in 2013 to 1.3% in 2020 (EEA 2021). The general increase of water quality responds not only to a deeper consciousness on health and environmental impacts, but also on its potential for tourism and local economy development (EEA, 2021; Kaas et al., 2011). An integrated management and the implementation of monitoring and decision support tools appear thus fundamental to guarantee safe water uses.

In addition, there are increasing concerns regarding greenhouse gases (GHG) emissions during water treatment processes and discharge of contaminants of emerging concern (CEC) such as pharmaceuticals, antibiotics, endocrine disruptors, microplastics (MPs), and antibiotic resistance bacteria (ARB) (Marinelli et al., 2021b; Tran et al., 2018; Wu et al., 2022). Wastewater treatment sector needs to be transformed and modernised to satisfy current and future social and environmental demands and to increase circularity in the water sector (EEA, 2022).

For all those transformations to occur, wastewater sector needs to be approached from different perspectives, considering technological, techno-economic, socio-political and legal aspects in a comprehensive way.

1.1 Aims and structure of the thesis

The present thesis presents the work carried out during the PhD period, contextualising it under the framework of the existing pressures and the challenges that need to be addressed, the state of the art and the research objectives. Specifically, the aim of the thesis is to promote solutions to support the shift from a linear perspective of capture-use-discharge of the water source towards a circular and sustainable management of urban water, including water reuse, bathing water, nexus and carbon footprint assessments and resource recovery.

In detail, the first part of the thesis is dedicated to risk assessment and management for water reuse in agriculture and for urban bathing water. A contextualisation of the current practices, together with the existing guidelines and the EU regulation on water reuse were analysed. A proposed

methodology was developed in the context of the PRIMA FIT4REUSE project, while specific contributions were provided to the development of the JRC “Technical Guidance Water Reuse Risk Management for Agricultural Irrigation Schemes in Europe”. Risk management was supported in three different case studies, focusing on risk assessment methods (Peschiera Borromeo), risk management plan definition (Fregene) and monitoring planning (San Benedetto del Tronto), depending on the respective needs and requirements. Support of innovative sensors to monitor reclaimed water quality was also analysed, under the context of the European H2020 project Digital Water City. Digital tools were also addressed to integrate deterministic and statistic methods for the development of an Early Warning System (EWS) for safe bathing water management in Paris.

The sustainability of the territorial water management was investigated, with a particular focus on the relationships that define the nexus between water reuse, emissions, energy consumption and agriculture production. A detailed methodology for carbon footprint assessment on territorial contexts was developed and presented. Moreover, an LCA was conducted on Peschiera Borromeo WWTP to analyse and quantify the benefits of water reuse. In order to investigate also in other practices for a sustainable urban water management, other than water reuse, a specific analysis was conducted on phosphorus recovery technologies from wastewater and products valorisation.

The circular approach to water management in recent years has also raised some issues, such as the presence of emerging contaminants. In the context of the LIFE Blue Lakes project, microplastics (MPs) in drinking water and wastewater treatment plants impacting on Italian lakes (Castreccioni and Garda) were investigated. Protocols and instrumentation for monitoring and determination of MPs were developed and tested, and the acquired expertise is going to be shared and disseminated to Italian and German water utilities. The presence of Cyanobacteria was also investigated in the case study of Castreccioni drinking water treatment plant (DWTP), representing a strategic infrastructure for the supplying of drinking water in Marche Region.

The thesis structure is schematised below.

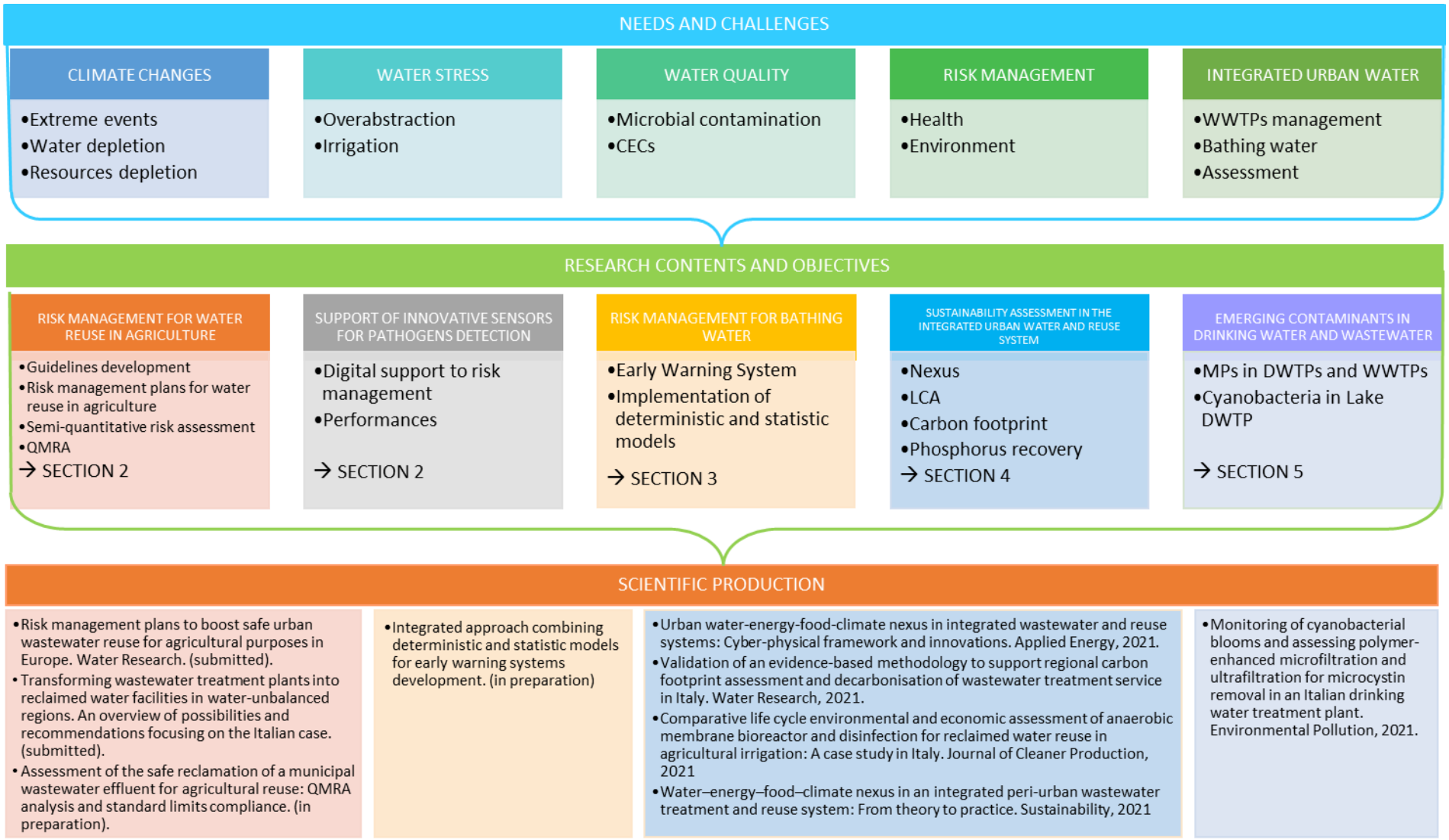


Figure 3: Structure of the thesis

1.2 Context of the thesis

The PhD scholarship research topic is entitled '*Real-time data monitoring and co-processing for water reuse, water-energy-carbon-food-ecosystem nexus assessment in integrated urban water reuse systems'*. The research activities were contextualized within several European, National and water utility funded projects and mainly focused on data processing, risk management for water reuse, carbon and energy footprint assessment and on water-energy-carbon-food-ecosystem nexus assessment in integrated urban water reuse systems.

The PhD activities carried out were particularly contextualized under the following European projects:

- Horizon 2020 project Digital-Water.city (DWC https://www.digital-water.city/). The research activities aimed at the development of digital solutions for safe wastewater reuse in agriculture and focused on the analysis of real-time data from sensors installed in wastewater treatment plants, combined with offline data and information related to plant operations. System analysis was integrated into a risk management plan, as a control measure to minimize health-related risks. The activities also included the development of an Early Warning System (EWS) for bathing water to provide information and prediction on water quality, supporting decision making and risk management.
- LIFE BLUELAKES project (https://lifebluelakes.eu/en/). The activities were focused on the evaluation and quantification of microplastics (MPs) occurrence in drinking water and wastewater treatment plants, also through the definition of sampling procedures, also based on innovative sampling and filtering systems, and the development of Technical Protocols for Urban Water Service Infrastructure. Activities also included the organisation of training activities for professionals and stakeholders operating in the water sector.
- PRIMA FIT4REUSE (https://fit4reuse.org/). The project aimed to develop innovative, sustainable and safe treatment technologies, and on the use of treated wastewater and desalinated water in agriculture and for aquifer recharge. Activities were related to the development of guidelines for water reuse risk management plans.
- EIT CLIMATE KIC PROSUMER focused on techno-economic and environmental feasibility study of Phosphorus recovery and reuse in fertilizers applied to Italian Prosumers. Activities mainly relied on comparative studies and information collection about the available technologies to recovery P in form of struvite, CaP or organic fertilizers and their efficiencies in relation to the waste stream, its features and the characterization of the recovered resources, for the development of a database about technologies for struvite or other forms of phosphorus recovery.

Moreover, this thesis was developed in the context of several water-utility funded projects. The most important are here reported: i) Calculation of the indicator called "carbon footprint of the wastewater treatment service", in accordance with UNI EN ISO 14064-1; ii) Technical-scientific support to water utilities for the assessment of a Water Reuse Risk Management Plan, in a real-scale application of reclaimed wastewater for agriculture irrigation; iii) Technical-scientific support to water utilities for the assessment of a Water Safety Plan; and iv) Technical and scientific support for process engineering for the design of the sludge centre of the wastewater treatment plant of Castelfranco Salvatronda.

European projects:















Collaboration with water utilities:















2. Risk management for water reuse in agriculture irrigation

Minimising the health and environmental risks associated to water reuse is a complex task that needs comprehensive knowledge of the process. Risk assessment is the core step of management and implementation plans in water reuse. The risks associated with reclaimed water differ on the type of reuse and entail both human health and environment protection.

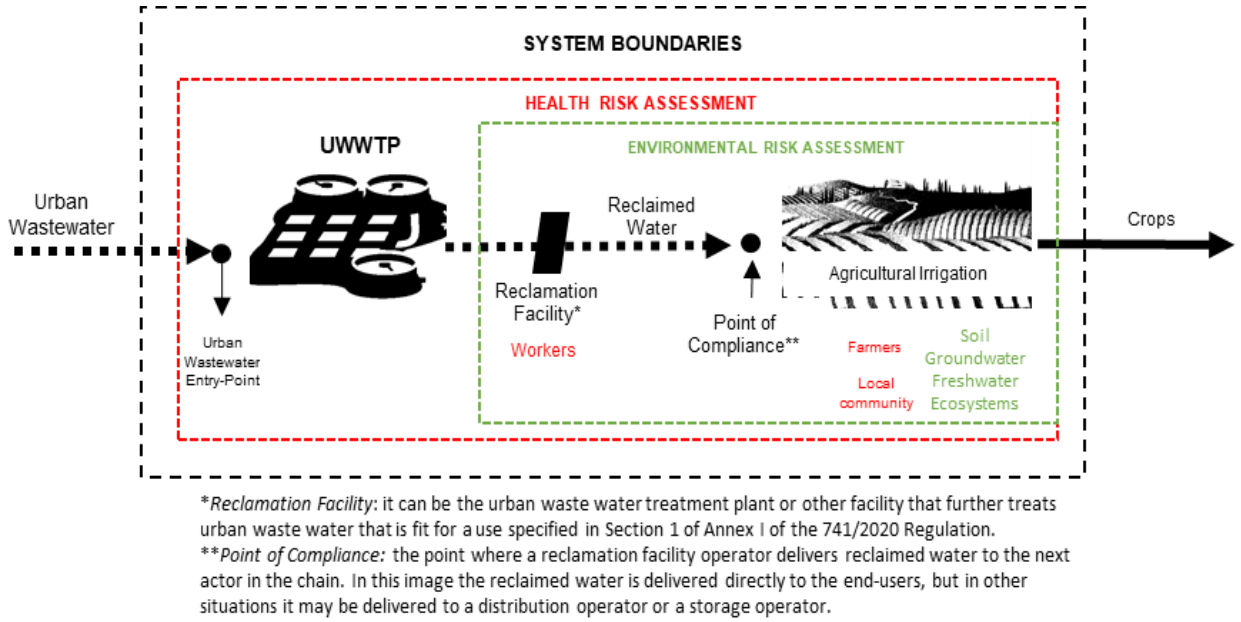


Figure 4: Schematic representation of a water reuse system.

2.1 State of the art for water reuse

In Europe, current practices for water reuse are less than 3% of the treated urban wastewater vs a potentiality of 15%, while in Italy reclaimed water is the 4% of treated municipal wastewater vs a potentiality of 24%. Water unbalanced and decentralised regions in Southern Europe seem to be the most active and the ideal actors to lead the transition to a circular wastewater sector in Europe since it could improve local economies, making them less dependent to other economic activities that are highly water consuming such as agriculture and tourism (Lavrnić et al., 2017).

Table 1: Worldwide data related to the production, collection, treatment and reuse of wastewater for agricultural purposes, adapted from Aquastat, 2022.

Area	Country	Produced municipal wastewater ($\cdot 10^{-9}$ $m^3 \cdot y^{-1}$)	Collected wastewater (%)	Treated wastewater (%)	Reuse of Treated Wastewater ($\cdot 10^{-9}$ $m^3 \cdot y^{-1}$)	Reused wastewater (%)	Reused wastewater in agriculture (%)	Water withdrawal for irrigation ($\cdot 10^{-9}$ $m^3 \cdot y^{-1}$)
Asia	China	48.510	64.2	100	7.720	15.7	16.3	385.200[a]
	Japan	16.930	71.00	68.3	0.390	3.4	3.0	53.300
Africa	Egypt	7.078	91.8	60.5	2.400	56.0	12.1	45.110
	Tunisia	0.312	88.8	87.8	0.042	15.5	33.0	1.552
	South Africa	2.420	100	90.9	3.220	-	-	11.397
	Ukraine	1.513	-	59.1	0.006	0.7	-	4.052

Central - Eastern Europe	Romania	1.011	96.34	88.2	-	-	-	0.441
	Russian Federation	12.320	71.43	74.7	-	-	-	7.000
	Croatia	0.336	100	83.7	-	-	-	0.030
	Poland	2.168	96.36	61.3	0.006	0.5	-	0.084
Southern Europe	Cyprus	0.035	100	96.5	0.034	99.7	50.0	0.174
	Italy	3.926	-	99.4	0.090	2.3	96.7	16.000
	Malta	0.026	100	92.3	0.001	5.8	50.0	0.024
	Portugal	0.577	100	46.8	0.006	2.2	-	3.395
	Spain	5.206	100	90.0	1.184	25.3	-	18.645
Northern Europe	Denmark	0.368	100	100	-	-	-	0.051
Western Europe	France	4.000	94.25	94.25	0.822	21.8	-	2.588
	Germany	5.213[b]	100.0	99.42	-	-	-	0.363
	UK	4.089	99.0	99.0	0.328	8.1	-	0.084
Middle East	Iran	3.548	32.8	24.9	0.328	37.1	-	86.000[a]
	Israel	0.500	100	90.0	1.028	-	-	1.173[a]
	Saudi Arabia	1.546	74.0	100	0.508	31.8	100	19.000[a]
Northern America	Mexico	7.960	84.5	54.9	1.796	41.1	76.8	65.87
	Canada	6.074	97.3	95.5	-	-	-	2.950
	USA	60.410	78.2	75.1	5.548	12.2	-	163.0
Southern America	Argentina	2.458	64.9	11.8	0.182	62.8	-	27.930[a]
	Brazil	10.0810	65.7	51.9	0.100	1.8	8.0	32.160
	Colombia	2.397	100	6.4	-	-	-	16.000
Oceania	Australia	2.094	87.3	95.5	0.280	14.0	50.0	11.099

[a] *Referred to annual quantity of self-supplied water withdrawn not only for irrigation, but also for livestock and aquaculture purposes.*

[b] *Amount of wastewater collected.*

2.1.1 Water reuse in Italy

Italy is the first EU country in water withdrawal for civil use (with more than 9 billion m^3/y), and the second in relative terms: 152.4 m^3/inhabitant withdrawn for drinking water (The European House - Ambrosetti, 2022). With respect to the use of water, irrigation is the main user like most of the Mediterranean countries.

The indication of the pressure of the population on natural water resources can be quantified by the Water Exploitation Index (WEI), which is around 17% (mid stressed) for Italy, placing it at the sixth place of the most stressed Countries in Europe (Aquastat, 2020). Nonetheless, this value is influenced by the significant differences between the north and the south parts of the country, being some Southern regions in severe water stressed conditions. This situation is predicted to worsen significantly in the incoming years, being over half of EU river basins expected to be affected by water scarcity by 2030 (WRE, 2022).

Italy has a powerful water sector that generates 17% of Italian GDP, being one of the most water-intensive countries in Europe (The European House - Ambrosetti, 2022). In addition, the interest in wastewater reuse is projected to continue expanding in the following years in this water-unbalanced country (EIT Water Scarcity, 2021; WRE, 2022).

Water reuse solutions have grown significantly in Italy recently, achieving up to 82 water reuse schemes for agricultural purposes in 2017 (WRE, 2022). However, it still remains as a minor water source for irrigation. In fact, in some of the most important water utilities, the percentage of wastewater reused does not even reach 10% of the total wastewater treated. Although this number is higher than other European countries that are normally under 5%, it can be stated that water reuse in Italy is far from its maximum potential. Currently, of the $3.9 \cdot 10^{-9}$ m^3/y of treated municipal wastewater, only $0.09 \cdot 10^{-9}$ m^3/y are reused, mainly for agricultural irrigation, as schematised in Table 1.

However, the development of technological solutions is still limited in Italy in comparison to other EU members, occupying the position 20 out of the EU-27 members in the Digital Economy and Society Index (DESI). Some barriers to the deployment of digital tools in the water sector are related to: i) the relatively high investment in new digital equipment and resources, and in the training of their employees; ii) digital tools for water management have not been traditionally considered as a priority for many companies and policy makers; iii) the payback of digital investments is not immediate, so it is sometimes hard to be perceived by users and/or investors; iv) limited stakeholder engagement; v) data reliability and security issues; vi) sometimes limited access to reliable internet network, e.g.,. in decentralised systems.

These advanced digital tools are essential for the wide development of water reuse practices. For all these reasons, bigger utilities are expected to increase their capacity to reuse wastewater faster than other smaller utilities that cannot (or are not so willing to) promote water reuse technologies. This could be highly relevant in a future scenario where water reuse practices are expected to be essential in the economy and resource management of a country like Italy. For instance, if sustainable policies such as Green Public Procurement (GPP) are implemented, utilities that produce reclaimed water could have some competitive advantages (e.g., in public contract acquisition) in comparison to other “less sustainable” companies.

2.2 Technologies for water reuse

Conventional WWTPs have traditionally aimed at the removal of the organic matter, suspended solids and nutrients, but only a few of them can achieve the high water-quality standards of “EU Regulation 741/2020 on minimum requirements for water reuse”. Particularly, the new European regulation for water reuse regulation requires higher standards for disinfection, lower content of solids (i.e., total suspended solids) and organic matter (i.e., BOD_5 concentration) in the final effluent than those required for the simple discharge of wastewater in surface water bodies. In addition, the regulation on water reuse requires risk assessment for the identification of all potential hazards for the environment and human health, which may comprise the removal of micropollutants, CECs and priority substances.

Planning and design of treatment solutions to reach the appropriate objective is thus crucial to ensure the feasibility and the implementation of water reuse practices.

2.2.1 Disinfection technologies

To ensure microbiological quality, disinfection treatment is essential during wastewater reuse applications. Conventional chemical-based treatments that have been applied at full scale for wastewater disinfection include, between others, ultraviolet (UV) radiation, ozone (O3), peracetic acid (PAA) and Performic Acid (PFA) (Collivignarelli et al., 2018). Apart from them, chlorination (e.g., by addition of NaClO) is one of the most well-established disinfection techniques due to its reliability and simplicity. However, it is commonly dismissed for production of high-quality reclaimed water since, high chlorine doses, in presence of natural organic substances, produces different disinfection byproducts (DBPs), such as trihalomethanes (THMs) and haloacetic acids, known to be carcinogenic to humans and toxic to aquatic life. Furthermore, if ammonia is present in water, the formation of chloramines can lead to the formation of the highly toxic nitrosamines, and particularly N-Nitrosodimethylamine (NDMA) (Sgroi et al., 2018). Chlorine dioxide is another bactericidal agent which disinfectant power is even higher than chlorine. ClO_2 is a yellow-green gas with a pungent smell, water-soluble, but very unstable. It is produced in site by the reaction of sodium hypochlorite with hydrochloric acid. Chlorite (ClO_2^-) and chlorate (ClO_3^-) are the major reaction by-products, which are regulated during drinking water production.

As most convenient alternative, PAA has been adopted in many WWTPs in Italy, as well as in Europe (Rizzo et al., 2020), due to: i) the easy retrofit of the already existing equipment for chlorination (without the need for expensive capital investment); ii) its bactericidal, virucidal, fungicidal, and sporicidal effectiveness (as demonstrated in various industries); iii) its broad spectrum of activity even in the presence of heterogeneous organic matter; iv) absence of persistent toxic or mutagenic residuals or by-products; v) no quenching requirements; vi) small dependence on pH; vii) short contact time and; viii) effectiveness for treating primary and secondary effluents. Nevertheless, high residual PAA concentration (> 2 mg·L-1) in the effluent is commonly reason of significant toxic effects in aquatic ecosystems. PAA is typically commercially available at 5-40% w/w in an equilibrium mixture with acetic acid, H2O2, and water. Although H2O2 is also a disinfectant contributing to the disinfection power of the PAA mixture, acetic acid (AA) is a more potent antimicrobial agent than H2O2. PAA disinfection action is very effective, and its use results to be very competitive with other benchmark technologies including chlorination and UV irradiation. Major disadvantage associated with peracetic acid disinfection is the increase of organic content in the effluent due to acetic acid and thus in the potential microbial regrowth. The formation of adsorbable organic halogens (AOX) has been also reported (Antonelli et al., 2013)

Tertiary treatment with performic acid (PFA) is a relatively recent option for chemical disinfection that has been already tested in different applications including advanced primary effluent, storm water, and secondary effluent treatment. Despite not being as well established as the previous ones, it generally implies the formation of by-products such as formic acid, CH_4 and CO_2, which at the concentration levels commonly encountered are considered non-toxic (Tchobanoglous et al., 2014). Generally, PFA showed greater microbial inactivation properties than other organic peracids (such as peracetic acid (PAA) and perpropionic acid) against faecal indicator bacteria (i.e., *E. coli*, faecal coliforms and enterococci) in water and wastewater. Importantly, no genotoxic or mutagenic

substances were detected in PFA-disinfected wastewater in a full-scale municipal study (Maffettone et al., 2020).

Last but not least, UV disinfection is cited in most guidelines as the “best available technology not entailing excessive costs” for reclaimed water production. In fact, its O&M costs are even lower than PPA and chlorination when high quality must be guaranteed as in the case of wastewater reuse (Reclaimed water quality class A in EU Regulation 2020/741: 10 CFU/100ml). For this reason, it presents nowadays a great diffusion in Europe (Soller et al., 2017). UV disinfection is effective for the inactivation of most viruses, spores and cysts, and in general, needs shorter contact time (about 20 – 30 s for low-pressure lamps) with respect to other solutions. Furthermore, it is compact, with low efforts required from operators, even if maintenance is required to control fouling of tubes, which also makes it suitable for decentralised systems. Since it is a physical process, it avoids the use of hazardous or corrosive chemicals, does not require additional safety measures for handling, transport, and storage, and does not generate residuals potentially toxic for human or aquatic life (Collivignarelli et al., 2018). However, suspended solids and turbidity can obstacle UV radiation, significantly losing its effectiveness when wastewater contains SS concentrations over 30 mg·L-1. A refinement treatment is thus advisable to be installed before UV disinfection.

2.2.2 Refining technologies

For the refinement of WWTP secondary effluents, simple technologies based on the physical removal of pollutants are commonly used, such as sand filters. Apart from removing macro-pollutants such as suspended solids, these technologies have been reported to be able to subtract certain amounts of some CECs such as microplastics and other chemical substances. Membrane treatment allows the physical retention of pollutants. High-quality effluents can be thus reached, with almost no solids and high removals of pathogenic bacteria and viruses (González-Camejo et al., 2019). Due to their high performances in microbial and solids removal, coupled with their flexibility on achieving fit-for-purpose water quality by selecting the most appropriate pore size, this technology is growing higher interest in reuse applications. Membranes can be used both as tertiary treatment (after conventional wastewater treatment systems), or being part of alternative wastewater treatment processes, as occurs in aerobic membrane bioreactors (MBRs) (Grandclément et al., 2017), anaerobic membrane bioreactors (AnMBRs) (Foglia et al., 2020), or in other innovative processes that have been recently implemented to boost circularity in the water sector such as microalgae-based membrane photobioreactors (González-Camejo et al., 2021). The main factor affecting filtration is normally the membrane pore size. Lower sizes will improve removal efficiency, but they will also increase membrane fouling, which is the main issue of membrane-based systems. Different membrane-based technologies can be distinguished according to their pore size. Microfiltration (MF) and ultrafiltration (UF) are commonly applied barriers for solids and pathogens, being able to remove them to a very high extent. However, the capital and operational costs of membrane-based systems tend to be elevated, due to the high pressures required to ensure the correct flow and to avoid membrane fouling, as well as for membrane cleanings. Moreover, membrane operations are more complex than other wastewater tertiary treatment systems.

Another alternative to conventional WWTPs are nature-based solutions (NBS) such as waste stabilisation ponds (WSPs), green filters and constructed wetlands (CWs), including vertical subsurface flow (VF) CWs, horizontal subsurface flow (HF) CWs, and free water surface (FWS) CW.

This group of technologies aim to reduce the impacts of wastewater treatment by imitating natural environments, thus showing minimal energy requirements during their operations since mechanical aeration is not required, simplicity, and low environmental impacts in the construction phase (Kobayashi et al., 2020). NBS have been confirmed to be robust and low-cost eco-technologies for wastewater treatment and reuse, being ideal for the implementation of water reuse practices in small communities and other decentralised systems (Carvalho et al., 2022). They often show high removal of macro-pollutants and pathogens, especially in the summer season Russo et al., 2019). However, they are not usually very efficient in CEC removal and commonly require huge land surfaces. Combinations of NBS with other manmade treatments can thus be an option to produce reclaimed water with the appropriate quality level of irrigation in and efficient and environmentally friendly way (Thompson et al., 2022).

Summarising, the implementation of refining technologies in WRF schemes could be advisable to improve efficiencies in further downstream water reuse processes. However, depending on wastewater characteristics and the costs and impacts of the refining technologies, they might not be needed as their operation could be unfeasible.

2.2.3 Advanced treatment technologies

Advanced oxidation processes (AOPs) are treatments that mainly aim to chemically destroy contaminants of emerging concern, which are present at ppb levels in water. Disinfection of pathogen simultaneously occurs. AOPs can be developed by simultaneously applying different oxidants, including ozone, hydrogen peroxide, UV radiation, peracetic acid, chlorine, etc., normally in higher doses than those used only for disinfection. This, together with the large amounts of wastewater treated in WWTPs, imply that AOPs normally present high operating costs, which has somewhat limited their large-scale applications (Collivignarelli et al., 2018). In addition, the formation of reaction reactants with high reactivity, such as hydroxyl radicals (OH•) can occur. To overcome these issues, some authors have tested processes such solar Photo-Fenton with the goal to use solar radiation as main oxidising factor. However, this process is limited by the daylight hours, thus storage tanks would be needed to store wastewaters produced during night hours. Moreover, acid conditions are mandatory for solar phot-Fenton processes to reduce the presence of inorganic carbon as bicarbonate, which tends to slow down the process (Arzate et al., 2019).

When looking only at micropollutants oxidation, ozonation (and its combinations O3/H2O2, O3/UV) appear as the most energy efficient processes with consumptions under 1 kWh·m-3, while processes such as photocatalysis, ultrasound, and microwave-based AOPs have reported energy consumptions over under 100 kWh·m-3, being thus considered not energy efficient for water reuse (Miklos et al., 2018). On the other hand, AOPs based on combinations of technologies with UV irradiation (e.g., UV/H2O2, H2O2/O3/UV) have been reported as the most effective processes for wastewater treatment that produce negligible or null amount of DBPs (Sgroi et al., 2021). Technologies based on granular (GAC) and powder activated carbon (PAC) can be also used to remove CECs. It must be noted that membrane-based technologies such as nanofiltration (NF), which presents smaller pore sizes than MF and UF, could be theoretically as “perfect filters” that could remove CECs (Arvaniti et al., 2022). However, the costs of NF are commonly too high, thus the applications for reclaimed water production are limited nowadays.

A comparison of the proposed technologies is presented in Table 2.

Table 2: Key performance indicators (KPI) and comparison of water reuse technologies.

	KPI	CLHORINATION	PAA	PFA	OZONE	UV	AOPs	GAC/ PAC	MEMBRANE	NBS
Infrastructure and management	scale of application	large	medium	medium	medium	large	large	medium	medium	Low
	operability	Easy	easy	difficult	medium	easy	Medium	medium	High	Easy
	plant complexity	Medium	medium	High	high	low	Medium/ High	medium	High	Low
Performances in pollutant removal	bactericide action	High	high	High	high	high	High	Low	high	Medium
	virucidal action	Medium	medium	medium	medium	medium	High	Low	high	Low
	protozoa removal	Low	low	High	high	high	High	Low	high	Low
	cec removal	Low	Low	n.d.	high	Low	High	Medium	high	Low
Dosage	dose – class a (mg·min·l^{-1})[a]	100-300[j]	210-600	20-60	50-70	80-100[b]	N.A.	N.A.	Pore size UF/NF	N.A.
	dose – class b (mg·min·l^{-1})[a]	70-150	100-300	5-20	20-30	60-80[b]	N.A.	N.A.	Pore size UF/NF	N.A.
	dose – class c (mg·min·l^{-1})[a]	30-80	50-100	<5	<10	30-60[b]	N.A.	N.A.	Pore size MF	N.A.
	dose – class d (mg·min·l^{-1})[a]	10-40	20-50	1-5	<10	< 30[b]	N.A.	N.A.	Pore size MF	N.A.
Affecting parameters	suspended solids	High	low	low	high	High	High	High	High	Low
	organic compounds	High	low	medium	high	medium	Medium	High	High	Medium
	ammonia-nitrogen	Low	low	medium	low	Low	Low	Low	Low	Medium
Down stream effects	by-products	THMs, NDMA, HAAs	AOX	none	NDMA, bromate	none	None	None	none	None
	residual toxicity	large	low	medium	medium	none	none	none	none	none
	bacterial re-growth	low	high	low	low	medium	none	N.A.	medium	Medium

	KPI	CLHORINATION	PAA	PFA	OZONE	UV	AOPs	GAC/ PAC	MEMBRANE	NBS
Costs	OPEX (€·m^{-3})	Low 0.023[c]	Low 0.023[c]	Low	Medium 0.02-0.037[d]	Low 0.006	High	Medium 0.12[e]	MF: 0.03[e] UF: 0.03[e]	CW:0.40[f] 0.12[g] 0.64[h] 0.31[h] 0.85[h] HRAP: 0.42[f] 0.007[i] 0.02[i]
	CAPEX (€·m^{-3})	Low 0.002[c]	Medium 0.002[c]	Low 0.002[c]	High 0.003-0.006[d]	Medium 0.017 - 0.035[d]	High	Low 0.20[e]	MF: 0.37[e] UF: 0.42[e]	CW: 2.31[f] 3.07[g] 2.41[h] 3.23[g] 5.51[g] HRAP: 1.80[e] 2.11[h] 1.53[h]

[a] *Dose of UV expressed as mWs·cm^{-2}*
[b] (Crocetti et al., 2021)
[c] (AQUAREC, 2006)
[d] (Akhoundi and Nazif, 2018; Melgarejo et al., 2016)
[e] (Mohamad Mazuki et al., 2020)
[f] (Garfí et al., 2017)
[g] (Freeman et al., 2019)
[h] (Puigagut et al., 2007)
[i] (Arashiro et al., 2018)
[j] *Not advisable for toxicity problems*
HRAP: High-rate algal pond

2.3 Policy/governance aspects linked to water reuse

Water policies can play a highly significant role in reducing the techno-economic barriers with the goal to shift to a more sustainable wastewater sector. For instance, economic instruments such as full-cost recovery (which is part of the 'polluter-pays principle') can help to partially finance existing and future water infrastructures by obtaining higher funding directly from the water bill, thus reducing the investment pressure of water utilities. There is global consensus on the advantages of cost recovery policies to deal with water scarcity issues since, apart from increasing financial resources for investments in water infrastructures, it also achieves water saving through water pricing. In fact, it has been proved that EU countries with higher water prices normally consume less amount of water per capita than those with lower prices (The European House - Ambrosetti, 2022).

Other water policy-based tools such as Green Public Procurement (GPP) and EU Taxonomy can increase the benefits of water utilities that turn their treatment processes into more circular and greener ones by giving them some economic and/or competitive advantages in comparison to those non-sustainable companies, hence encouraging utilities to give more priority (and higher investment) to the transition to Water Reclamation Facilities (WRFs). GPP approach goes beyond current public procurement that simply focuses on satisfying low-cost approach, obviating sustainability, and gets the public buyer become an early adopter by buying (or hiring) a product, service or process that contains substantially novel and environmentally friendly characteristics (EIT

Water Scarcity, 2021). On the other hand, the EU taxonomy is a classification tool that provides information to investors about how "environmentally sustainable" an economic activity is (e.g., reclaimed water production), preventing greenwashing and helping investors to make more circular and greener choices (Traini, 2021). The requirements to define an economic activity as environmentally sustainable according to the EU taxonomy have been described by TEG (2020).

In Italy, investments are mainly covered by the water tariff (78%), which is one of the lowest in Europe. For instance, it is half of that of France and 40% of Germany's (The European House - Ambrosetti, 2022). This often disincentives water companies to transform their technologies, as this means that (indirectly) they have to assume most of the investment risks. As a consequence, the level of investment in the Integrated Water Service in Italy accounts for only 56% of the European average (Utilitalia, 2021). To shift this current situation, Italian utilities are considering in their business plans to obtain funds from the National Recovery and Resilience Plan (in Italian Piano Nazionale di Ripresa e Resilienza (PNRR)). Actions such as update and improvement of structures, reduction of losses in water distribution networks, digitalisation of the sector, and resilience of irrigation agro-systems could be funded by PNRR. It must be noticed that those actions are mostly in line with the investments previewed by the Italian water utilities in their business plans for the recent future.

Other obstacles in the use of reclaimed water are related to the engagement of policy makers and stakeholders, the high number and variety of public organisms involved in decisions related to water, and an inadequate understanding of some roles and responsibilities that can lead to conflicts and addressing efforts to the wrong way (Goodwin et al., 2019). Although collaboration between different parts is commonly intended to be addressed, this is not always achieved in practice. Due to these political issues, a lack of homogeneity in regional and national regulations about water reclamation has been detected and is identified as one of the main obstacles to the wide development of water reuse in Europe (Seco et al., 2018). Differences in regulations concern the type and the number of monitored pollutants as well as their limiting values, which can be as strict as those for drinking water in certain regulations (Lavrnić et al., 2017). This lack of homogeneity hinders the free movement of agricultural products irrigated with reclaimed water (Goodwin et al., 2019). Significant efforts have been and are doing currently by the European Union to align policies and governmental decisions in the water sector by supporting the development of guidelines and policies that help to boost water reuse. Indeed, the European Parliament and the Council has recently launched the Regulation 2020/741 on minimum requirements for water reuse (to be applied in all Member States from 26 June 2023) with the goal to standardise water reuse regulation in the Union and to overcome barriers that hinder it.

Social factors such as negative opinion regarding the use of reclaimed water are also worth being considered since they can ruin all the technical, economic, political and legal advances that can be done in the sector. A portion of the public does not trust in water reuse regulation and monitoring practices, the technical process, the organisations that manage it, and the quality and safety of the reclaimed water itself (Ofori et al., 2021). Hence, raising awareness regarding the benefits of water reuse, together with increasing transparency and assuring the safety and low impacts of water reuse are essential to increase this practice worldwide. Risk assessment and management plans (which are now mandatory in Europe) are clearly aligned to these aims. By assuring safe water reuse, the reputation and image of water reclaim facilities can be maintained at a high level. Water reuse risk

management plans (WRRMPs) thus appear as a useful tool to boost water reuse in agriculture (Semerjian et al., 2018).

Table 3 summarises the considered aspects related to wastewater treatment and reuse sector.

Table 3: Evaluation of techno-economic, socio-political and legal aspects to encourage the transformation of the wastewater sector.

Aspect	Current barriers	Paradigm shifts	How these changes would encourage the transformation of the water sector	Difficulties in the implementation
Technical	- Low development of digitalisation	- Deployment of digitalisation to improve water treatment performance and reduce malfunctions.	- Cost reduction. - Assurance of high quality of reclaimed water. - Higher reliability of water reclaimed facilities. - Extension of infrastructures' life. - Reduction of the possibility of making mistakes. - Greater speed in decision-making.	- High investment. - Personnel training. - Continuous updating. - Low level of maturity of some digital solutions.
Economic	- High investment in infrastructures and digital tools. - Low market value of by-products from wastewater (including reclaimed water).	- Revenues from by-products obtained from wastewater. - Modern technologies to improve water quality and improve treatment performance.	- Reclaimed water can be sold as wastewater by-product. - Most efficient technologies can assure the safe use of reclaimed water. - Reduction of operating costs by increasing process performance. - Reduction of pollutant, GHG emissions, energy losses and resource consumption of the water treatment process.	- Risk assessment. - Reduced market of circular water by-products. - Requirement of high-skilled personnel.

Aspect	Current barriers	Paradigm shifts	How these changes would encourage the transformation of the water sector	Difficulties in the implementation
Socio-Political	- Low prizes of water tariffs. - Limited support to circular water policies. - Limited awareness of issues related to water scarcity. - Misled concept of reclaimed water as competitor with potable water instead of as alternative water source. - "Silo" thinking: not considering the correlation of water with other sectors.	- Raise awareness. - Full-cost recovery policies. - Implementation of water policy-based tools such as green public procurement and EU Taxonomy - Implementation of NEXUS thinking.	- Partial funding of water infrastructures from increased water tariff. - Water pricing encourages water saving. - Encouraging of water companies to implement sustainable processes (not only economic ones). - Development of systemic policies that considers all the sectors influencing water management.	- Lack of consideration of the "real value of water". - Perception of risk in the use of reclaimed water. - Lack of knowledge related to the long-term effects of water reuse. - Lack of knowledge on the relationships between the sectors which influence water management.
Legal	- Legal barriers in the implementation of water reuse. - Lack of standardisation in the water reuse sector. - Lack of recognition of some water reuse practices such as fertigation. - No differentiation between centralised and decentralised systems.	- Approval of EU Regulation 741/2020 by EC. - Fit-for-purpose approach. - Elaboration of new legislation. - Specific requirements for decentralised systems.	- Application of EU Regulation 741/2020 in National legislations. - Water reuse risk management plans to assure the safe use of reclaimed water. - Circular use of water and nutrients in fertigation. - More robust water reuse systems in decentralised and isolated regions. - Innovation Deals to overcome legal barriers in the implementation of water reuse.	- Difficulties in the control of the accomplishment of legal requirements. - Too conservative legal requirements in certain occasions. - Long periods to apply EU Directives into national and regional legislation.

2.4 Existing guidelines for water reuse risk management plans

Due to the lack of specific regulations and harmonised procedures for risk management, the main international health and environmental protection agencies have provided, according to the purpose considered and the needs at each time, guidelines and manuals to address risk management for water reuse projects. Some of them (EPA, 2012; WHO, 2006) aimed to provide technical indications for the design of reclaimed facility projects. On the other hand, Australian Government Initiative (2006), Sanitation Safety Planning (SSP) (WHO, 2016) and DEMOWARE (2017) guidelines detail contents to develop risk management plans with the goal to consistently ensure the safety and acceptability of water reclamation.

Existing guidelines were analysed in order to have a complete framework of the different methodologies that were proposed by the most important health authorities to try to support water reuse practices and compensate the lack of a harmonised legislation, before the entry into force of the current EU Regulation.

2.4.1 UNEP 2005- Guidelines for municipal water reuse in the Mediterranean Region

UNEP Guidelines are specifically declined for the Mediterranean area and thus are mainly focused on water reuse for urban and agricultural reuse, since potable reuse was not seen as a common practice. That explained also the focus on microbial risks rather than chemical, since the main health hazard for water reuse in irrigation was identified in pathogens. The proposed standards are thus focused mainly on the microbiological criteria, overpassing risks associated to other micropollutants. However, to estimate the environmental impacts of chemicals on soil and crops, it is suggested to monitor organic and nutrient content, Boron and metals. Barriers such as detention time in storage systems, selected irrigation systems, setback distances from the application site, entry restrictions, avoiding or preventing cross-connections with potable water distribution systems/backflow prevention, and coded distribution systems, were suggested to minimise the direct contact of potentially contaminated water. Four use categories were distinguished, from urban reuse to unrestricted, restricted irrigation and irrigation with efficient irrigation systems. Such repartition underlined the importance of water reuse cost-effectiveness. For each category, minimum water quality requirements and monitoring frequency were established. Three water quality criteria were proposed, based on nematode eggs, faecal coliforms or Escherichia coli, and suspended solids (SS). Periodical monitoring of the soil should be established for irrigation and agricultural purposes. Those guidelines aimed to encourage water reuse practices on Mediterranean area, providing scientifically sounded recommendations and trying to cover the lack of the existing regulations of that Region in that time. The do not cover all the possible reuse practices and do not analyse all the possible risks; instead, they provide referenced thresholds for a limited number of parameters indicators of microbial contamination, as well as monitoring frequencies, sampling points and additional measures for the addressed water reuse practices.

2.4.2 Guidelines for the safe use of wastewater, excreta and greywater, volume II – Wastewater use in agriculture, WHO 2006

The Guidelines developed by WHO were mainly addressed for developing countries with scarce resources. The Guidelines aims primarily at human health protection, with particular attention for workers, local communities and product consumers. Hazards associated with pathogens such as bacteria, helminths, protozoa and viruses, skin irritants, vector-borne pathogens or chemicals such

as halogenated hydrocarbons, pesticides and heavy metals are taken into account. Health-based targets are used to define the minimum level of health protection from each hazard, depending on the level of tolerable health risk. Disability-adjusted life years (DALYs) is the population metric used to express life years lost to disease due to both morbidity and mortality. 10^{-6} DALYs is the defined health limit and health protection measures are evaluated considering the $\log_{10}$ pathogen reduction required to achieve 10^{-6} DALYs for different exposures. The Guidelines provide pathogen reductions achievable by various protection measures, considering a multi-barrier approach of treatments, technical and behavioural practices. Risk management strategies considered include wastewater treatment, produce restriction, application techniques, exposure control methods and produce washing/disinfection/cooking. Even if the main purpose is human health protection, environmental impacts are also considered. Maximum soil concentrations for different chemicals based on health risk assessment and levels that impact agricultural productivity are also presented. Planning and implementation of water reuse programmes follow progressive steps. Policy aspects are considered since the Guidelines aims to represent a reference for regulation and standards at national and international levels. Community and stakeholder participation is encouraged through education and information activities, public meetings and workshops.

Those Guidelines are particularly helpful to find referenced and quantitative information about the presence of pathogens, removal efficiencies and proposed thresholds, since they provide summary tables and supporting material. However, that big amount of data may seem to be dispersive and not very conclusive for the development of a risk management system. On the other hand, those data are mainly focused on practices and treatments available in developing countries and they provide only general information about the development of water reuse management plans.

2.4.3 Australian Guidelines for Water Recycling: Managing Health and Environmental Risks, 2006

Australian Guidelines provide a framework for risk management applied in water reuse systems. The process consists in assessing, managing, monitoring and reviewing risks. Assessment is performed through the identification of hazards and hazardous events, the estimation of their likelihood, the evaluation of impacts and the characterisation of the corresponding risks. This scheme can be replied both for health as well as for environmental risks. As concern human health protection, a particular focus is paid for microbial risks caused by pathogens. On the other side, environmental risks mainly involve chemicals that may cause plant toxicity (boron, chloride, sodium, cadmium and chlorine), soil degradation (salinity, sodium) or nutrient imbalance. Even water excess is considered. DALYs are used to quantify impacts on human health and define acceptability levels and targets. The characterisation distinguishes between the maximum risk and the residual risk, taking into account existing preventive measures, to define priorities. Management considers a multiple barrier approach and focuses on critical control points. Treatment processes are combined with on-site controls and use restrictions. Multiple barriers can be used to meet performance targets for microorganisms and keep concentrations of any chemical contaminants below target values. Preventive measures are set up to reduce risks to acceptable levels and must be monitored in order to be rapidly corrected in case of failure. Critical control points are therefore introduced to focus sampling campaigns and monitoring on limited but strategical points for the evaluation of the efficiency and the efficacy of barriers and preventive measures. Four types of monitoring are used with both human and environmental health risks: to obtain baseline data, to validate the system, to obtain operational data and to verify process effectiveness. Communication activities and public

consulting are encouraged to increase acceptance and trust. Particular focus is paid on public information and on the development of communication strategies.

2.4.4 Guidelines for Water Reuse – US. EPA 2012

The Guidelines offer information about a wide range of reuse applications and includes best management practices (BMPs) communities' involvement and provides an overview of water reuse case studies within the United States and around the world. EPA provides suggestions for a water reuse system from the planning and management phase to the public participation and consultation phase, addressing the existing regulatory framework, the main hazard in wastewater constituent, and available treatment to minimise the related risk, up to discuss the funding possibilities available for water reuse projects. Different quality classes are defined depending on the intended use and characterized by different quality requirements, monitoring programs, setback distances and additional recommendations. The main parameters to be addressed are faecal coliforms, BOD, Turbidity, pH, and residual Cl_2. In this regard, a portfolio of treatment options is provided to mitigate microbial and chemical contaminants in reclaimed water and meet specific water quality goals depending on the intended use and the related belonging class. Other objectives, such as preventing environmental degradation, avoiding public nuisance, and meeting user requirements, must also be satisfied. Protection of public health is achieved by 1) reducing concentrations or eliminating pathogenic bacteria, parasites and enteric viruses in reclaimed water; 2) controlling chemical constituents in reclaimed water; and 3) limiting public exposure (contact, inhalation, or ingestion) to reclaimed water. The USEPA guidelines address different end-use categories, from urban reuse to groundwater recharge, agricultural, industrial up to potable reuse. Those categories are deeply investigated in terms of quantity and quality impacts, historical development, and water quality standard established by different regulations; full -plant experiences are proposed, too. Where reclaimed water is intended for non-potable reuse, the major priority in the design, construction, and operation of a reclaimed water distribution system is the prevention of cross-connections. Another major objective is to prevent improper or inadvertent use of reclaimed water as potable water. Suggested treatment processes, reclaimed water quality, monitoring frequency, and setback distances for water reuse in various categories are provided for each water reuse category. Specific thresholds are proposed for faecal coliforms, that are selected as indicator organism, turbidity, since pathogens can be particulate-associated, pH, organic matter content, and Cl_2 residual concentration. These guidelines, unlike their predecessors, underline the need for further investigation on CECs before any final decisions can be made on their threshold limits.

2.4.5 Sanitation Safety Planning, WHO 2016.

Sanitation Safety Planning (SSP) Manual implements the WHO 2006 Guidelines by providing a structure for risk assessment and management in the stepwise process. SSPs provide a systematic approach in six Modules towards assessing, managing and monitoring risks from wastewater origin to its final use. The first module, "Prepare for SSP", consists of establishing priorities and objectives, defining boundaries and components to include in the plan and setting up the working group; the Module 2 includes a detailed description of the sanitation system; Module 3 is the core phase of risk assessment, that includes the identification of hazards and hazardous events, refine exposure groups and exposure routes, assess existing control measures and prioritise the exposure risks. Module 4 provides information to develop and implement an incremental improvement plan; while Module 5 lays out operational monitoring. Finally, Module 6 supports the development of

programmes and review plans. The concepts of coordination and incremental improvement over time are central to the SSP approach. This manual supports the development and the implementation of water reuse management plans, providing suggestions on what to consider in each module. Some examples of case studies are also presented, even if they refer mainly to developing countries. The manual recalls to previous (WHO 2006) or parallel guidelines (QMRA, 2016) for the semi-quantitative or quantitative assessment of health risk.

2.4.6 Water Reuse Safety Plans - a manual for practitioners, DEMOWARE 2017

The report promotes the adoption of a risk management framework in water reuse and is intended to enable operators and authorities to develop viable management and safety concepts for existing water reuse systems. The added value of this manual is to provide a simple methodological approach that can be followed for any kind of water reuse practice, not only for agriculture, to support operators and authorities in the design and development of water reuse schemes. Following the SSP approach, the Water Reuse Safety Plan (WRSP) relies on a structured analysis of the system (hazards and related risks), suggests multi-barrier approaches to control risks, and highlights the importance of communication, cooperation, and review. The manual does not provide limits nor specifies thresholds, delegating these aspects to current regulations or existing guidelines. All the available risk assessment methods (Team based descriptive risk assessment, risk matrix, QMRA and QCRA) are valid to identify and evaluate the risk. Nevertheless, a more sophisticated QMRA/QCRA can be a better choice when resources are available. In general, three types of control measures can be applied that either target water quality or work on the exposure routes, including treatment, non-treatment but technical measures, non-technical (behavioural) measures. The operational monitoring should include measuring parameters at control points, online monitoring systems and real-time data reporting are advisable since fast response are required to detect a failure in the system early. Finally, the communication is addressed, to engage the public and other stakeholders. This manual provides a practical methodological guideline for the development of WRSPs and, even if it does not specify quantitative information about minimum qualitative limits, monitoring frequencies or exposure assessment, they give useful instructions for the implementation of safety plans.

In Table 4 a comparison of the existing guidelines for water reuse risk management is proposed.

Table 4: Comparison of guidelines for water reuse risk management plans.

	UNEP (2005)	WHO Guidelines (2006)	Australian Guidelines (2006)	US EPA (2012)	ISO 16075	ISO 20426	SSP (WHO 2016)	QMRA (WHO 2016)	DEMOWARE (2017)
Water reuse practices	- Urban and agricultural reuse. - 4 quality classes.	Agricultural reuse of wastewater, greywater and excreta	- Urban reuse. - Irrigation in agriculture. - Industrial reuse	- Urban. - Industrial. - Agricultural. - Impoundment. - Environmental. - Groundwater recharge. - Potable reuse.	Agricultural and urban irrigation projects;	Non-potable reuse: agricultural, urban, industrial, recreational and environmental	Agricultural reuse of wastewater, greywater and excreta	Water Safety Management. all uses.	All possible reuses
Risk addressed	Microbial	- Health risks (priority) - Environmental aspects	Health and environmental	Health and environmental	Health and environmental	Health	Health	Microbial	- Health risks (priority) - Environmental aspects
Risk assessment	No. Reuse based on limit values.	Microbial analysis, Epidemiological study, QMRA	Qualitative and Quantitative risk assessment	No. Reuse based on limit values. Methods for assessment specified in other documents (https://www.epa.gov/risk/conducting-human-health-risk-assessment#tab-5)	- No. Reuse based on limit values	Qualitative and Quantitative health risk assessment	Qualitative and Quantitative risk assessment.	Quantitative risk assessment	Qualitative and Quantitative risk assessment
Risk management	Multi-barrier approach	Multi-barrier approach	Multi-barrier approach	Multi-barrier approach	Multi-barrier approach	Multi-barrier approach	Multi-barrier approach	Multi-barrier approach	Multi-barrier approach

	UNEP (2005)	WHO Guidelines (2006)	Australian Guidelines (2006)	US EPA (2012)	ISO 16075	ISO 20426	SSP (WHO 2016)	QMRA (WHO 2016)	DEMOWARE (2017)
Water quality criteria	- Intestinal nematodes. - E.coli. - Faecal coliforms. - Suspended solids.	- Reference pathogens: Campylobacter, Cryptosporidium, Rotavirus. - Risks expressed in DALYs.	Reference pathogens, soil and plant toxic compounds. Risk expressed in DALYs.	Faecal coliforms, BOD, Turbidity, pH, residual Cl_2, metals and soil characteristics	BOD, TSS, Turbidity, thermotolerant coliforms, intestinal nematodes, organic matter, nutrients salts and heavy metals	E.coli, BOD, TSS or Turbidity Chlorine residual	Reference pathogens (e.g., Campylobacter, Cryptosporidium, Rotavirus). Risk expressed in DALYs.	Reference pathogens (e.g., Campylobacter, Cryptosporidium, Rotavirus). Risk expressed in DALYs.	Microbial, toxic, Compounds of agronomic relevance, and Compounds of emerging concern (no limits)
Monitoring	Minimum frequencies and sampling points	Validation, Operational, Verification	Validation, Operational, Verification at critical control points	Frequencies depending on intended use.	Operational monitoring on wastewater, crops, soil and environment in ISO 16075	Parameters to be monitored and log removals	Validation, Operational, Verification	-	Operational
Social aspects	No contemplated	- Public perception. - Economic feasibility. - Policy aspects	- Acceptance. - Communication strategies.	- Public Outreach. - Participation, and Consultation.	No contemplated	No contemplated	Supporting programs	No contemplated	- Acceptance. - Communication.

	UNEP (2005)	WHO Guidelines (2006)	Australian Guidelines (2006)	US EPA (2012)	ISO 16075	ISO 20426	SSP (WHO 2016)	QMRA (WHO 2016)	DEMOWARE (2017)
Pros	Specific for the most common practices in the Mediterranean region.	Referenced data, e.g., epidemiologic studies, concentrations in wastewater, crops and soil, log removals.	Complete of referenced characterisations, limits, examples and recommendations for risk management	- Referenced data - Provide suggestions from the planning and management phase to the public participation and consultation phase. - Address the existing regulatory framework to minimise the related risk - Discuss the funding possibilities available for water reuse projects.	Provide design and technical specifications for treatment, storage, distribution and irrigation infrastructure.	Provide guidelines to evaluate qualitative and quantitative risk assessment	Provide examples	Address the core phase of Risk assessment in a quantitative approach	Simplify the structure of a water reuse safety plan
Cons	- Not addressed all possible reuses. - Not addressed environmental risks.	Focused mainly on developing countries.	- Complex. - Lot of data required	- Focused on US applications.	No social aspects included. No guidelines on risk assessment.	No social aspects included. No limit values specified	- Focused on developing countries.	Do not consider non-technical aspects, monitoring and implementation	- Non quantitative indications.

DALYs: Disability-adjusted life years; SSP: Sanitation Safety Planning; UNEP: United Nations Environment Programme; USEPA: United States Environmental Protection Agency; WHO: World Health Organisation.

2.5 Regulation (EU) 2020/741 on minimum requirements for water reuse

With the aim to increase the amount of reclaimed water used in the continent and to solve the conflicts related to the lack of standardisation, the European Parliament and the Council approved recently the Regulation 2020/741 *on minimum requirements for water reuse*. This Regulation was established following the principles of previous guidelines, also trying to overcome their limitations and to adapt water reuse practices to the current socio-political scenario. The EU Regulation establishes quality limits, as well as the frequency of monitoring and treatment performance targets for reclaimed water reuse in agriculture applications, depending on four different quality classes in a fit-for-purpose approach (Table 5).

Table 5: Requirements for water reuse in EU Regulation 741/2020

Minimum reclaimed water quality class	Crop category (1)	Irrigation method	Indicative technology target	E. coli		BOD5		TSS		Turbidity		Legionella		Intestinal nematodes		Performance targets for the treatment chain (log10 reduction) (6)
				water quality requirements (4)	Minimum monitoring frequencies	water quality requirements (5)	Minimum monitoring frequencies	water quality requirements (5)	Minimum monitoring frequencies	water quality requirements (5)	Minimum monitoring frequencies	water quality requirements (4)	Minimum monitoring frequencies	water quality requirements (4)	Minimum monitoring frequencies	
A	All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw	All irrigation methods	Secondary treatment, filtration, and disinfection	≤ 10 (number/100 ml)	Once a week	≤ 10 mg/l	Once a week	≤ 10 mg/l	Once a week	≤ 5 NTU	Continuous	Legionella spp.: < 1 000 cfu/l where there is a risk of aerosolisation	Twice a month	Intestinal nematodes (helminth eggs): ≤ 1 egg/l for irrigation of pastures or forage	Twice a month or as determined by the reclamation facility operator according to the number of eggs in wastewater entering the reclamation facility	E. coli ≥ 5,0 Total coliphages/ F-specific coliphages/ somatic coliphages/ coliphages (7) ≥ 6,0 Clostridium perfringens spores ≥ 4,0 / spore-forming sulfate-reducing bacteria (8) ≥ 5,0
B	Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals	All irrigation methods	Secondary treatment, and disinfection	≤ 100 (number/100 ml)	Once a week	In accordance with Directive 91/271/EEC (Annex I, Table 1)	In accordance with Directive 91/271/EEC (Annex I, Section D)	In accordance with Directive 91/271/EEC (Annex I, Table 1)	In accordance with Directive 91/271/EEC (Annex I, Section D)	-	-					-

Minimum reclaimed water quality class	Crop category (1)	Irrigation method	Indicative technology target	E. coli		BOD5		TSS		Turbidity		Legionella		Intestinal nematodes		Performance targets for the treatment chain (log10 reduction) (6)
				water quality requirements (4)	Minimum monitoring frequencies	water quality requirements (5)	Minimum monitoring frequencies	water quality requirements (5)	Minimum monitoring frequencies	water quality requirements (5)	Minimum monitoring frequencies	water quality requirements (4)	Minimum monitoring frequencies	water quality requirements (4)	Minimum monitoring frequencies	
C	Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals	Drip irrigation (2) or other irrigation method that avoids direct contact with the edible part of the crop	Secondary treatment, and disinfection	≤ 1000 (number/100 ml)	Twice a month					-	-					-
D	Industrial, energy and seeded crops	All irrigation methods (3)	Secondary treatment, and disinfection	≤ 10000 (number/100 ml)	Twice a month					-	-					-

(1) If the same type of irrigated crop falls under multiple categories of Table 1, the requirements of the most stringent category shall apply.

(2) Drip irrigation (also called trickle irrigation) is a micro-irrigation system capable of delivering water drops or tiny streams to the plants and involves dripping water onto the soil or directly under its surface at very low rates (2–20 litres/hour) from a system of small-diameter plastic pipes fitted with outlets called emitters or drippers.

(3) special attention should be paid to the protection of the health of workers or appropriate preventive measures shall be applied.

(4) the indicated values for E. coli, Legionella spp. and intestinal nematodes are met in 90 % or more of the samples; none of the values of the samples exceed the maximum deviation limit of 1 log unit from the indicated value for E. coli and Legionella spp. and 100 % of the indicated value for intestinal nematodes.

(5) the indicated values for BOD5, TSS, and turbidity in Class A are met in 90 % or more of the samples; none of the values of the samples exceed the maximum deviation limit of 100 % of the indicated value.

(6) The reference pathogens Campylobacter, Rotavirus and Cryptosporidium may also be used for validation monitoring purposes instead of the proposed indicator microorganisms. The following log10 reduction performance targets shall then apply: Campylobacter (≥ 5,0), Rotavirus (≥ 6,0) and Cryptosporidium (≥ 5,0).

(7) Total coliphages is selected as the most appropriate viral indicator. However, if analysis of total coliphages is not feasible, at least one of them (F-specific or somatic coliphages) shall be analysed.

(8) Clostridium perfringens spores is selected as the most appropriate protozoa indicator. However, spore-forming sulphate-reducing bacteria are an alternative if the concentration of Clostridium perfringens spores does not make it possible to validate the requested $\log_{10}$ removal.

The EU Regulation 2020/741 fills a legislative gap defining requirements and conditions for water reuse applications. For the first time, there is harmonised legislation that standardise technological and quality standards across Europe. Before that, only few Member States addressed water reuse with specific regulations, differing significantly. The regulation follows a fit-for-purpose approach, instead of the fit-for-all method that was addressed by some EU Members such as Italy. Thus, not only quality limits are established, but also crop quality and irrigation techniques must comply accordingly. With respect to National legislations, EU Regulation set a lower number of parameters to be monitored. In fact, Regulation 2020/741 indicates the minimum parameters that all EU members have to consider in their national regulations, although it gives flexibility to EU members to increase the number of pollutants to be analysed. This approach aims to facilitate the economic feasibility of water reuse applications, which is supported by the limited number of parameters selected to accomplish quality requirements. However, Regulation 2020/741 gives EU members the opportunity to limit concentrations of other pollutants such as ARB, antibiotics, microplastics and others. These compounds have been scarcely considered in previous legislations, but are specifically contemplated in this EU Regulation.

Water quality classes (class A–D) are defined in terms of pathogen, BOD_5, and TSS concentrations, as well as turbidity. In this respect, pathogen content is assessed by the *E.coli* concentration in water (bacteria indicator), while total coliphages (alternatively, F-specific coliphages or somatic coliphages) and Clostridium perfringens spores (or spore-forming sulphate reducing bacteria) act as virus and protozoa indicators, respectively. The limiting values of these parameters vary according to the quality class. Limits of *Legionella* spp. and intestinal nematodes are also indicated for certain reuse practices. Water quality classification also depends on the crop category to be irrigated and on the irrigation technique. Class D can be only applied to non-food crops; class C includes irrigation of food crops with edible parts above the ground and irrigated with drip irrigation, while class B can also use other irrigation methods. The most stringent is class A which includes irrigation of food crops consumed raw in direct contact with reclaimed water.

Monitoring requirements are also depending on the reclaimed water quality class, apart from the specific parameter analysed. *E.coli* monitoring varies from twice a month (classes C – D) to once a week for classes A – B, while BOD and TSS should be analysed once a week, while turbidity must be continuously monitored. Validation and routine monitoring shall be performed before a new reclamation facility is put in operation.

One of the most innovative aspects of this Regulation is the inclusion of a water reuse risk management plan among the mandatory requirements (Article 5 and Annex II). This aspect underlines the priority of health and environmental protection, which must be always guaranteed above all.

WRRMP should identify and manage risks in a proactive way to ensure that reclaimed water is safely used and managed and that there are no significant risks to the environment or human or animal health. The Water Reuse Risk Management plan is to be based on the key risk management principles as defined in Annex II of the Regulation 2020/741 following a

preparatory step (description of the entire reuse system and the parts involved), a system assessment and risk management step (identification of the potential hazards and hazardous events for human health and the environment, the population involved and the exposure routes), operational monitoring step (monitoring specifications for different matrices involved) and implementation step (governance, management and communication plans). The risk assessment may be carried out using qualitative or semi-quantitative risk assessment. The quantitative risk assessment shall be used when there is sufficient supporting data or in projects having a potential high risk for the environment or public health. Depending on the risk assessment outcomes, additional requirements concerning heavy metals, CEC and ARG could be required. Technical guidance to supplement the key risk management tasks will be developed in the form of a delegated act by June 2022 from the Commission.

Other innovative aspects of this regulation in comparison to previous ones are: i) monitoring requirements are explicitly expressed, which in some national regulations, such as in the Italian one, they were demanded to local authorities; ii) the definition of reclamation facility operator, with the clear intention to shift the conventional paradigm of wastewater treatment to wastewater reuse; iii) to highlight the relevance of public awareness regarding the benefits in water reuse, following the recommendations of previous guidelines; iv) the promotion of fertigation, a practice that combine irrigation and nutrient recovery and is against traditional wastewater treatment scheme where nutrients are aimed to be removed from water instead of being recovered (Jiménez-Benítez et al., 2020); v) the proposal of financial incentives for water reuse and research in the topic.

Even if the Regulation 2020/741 harmonised water reuse practices providing standards and requirements, some aspects are not deeply addressed yet: i) concerning risk management, key elements are listed but not detailed, so technical guidelines are needed; ii) the methodology for risk assessment should be at least semi-quantitative, but no further information is provided. Some examples of control measures are given, but they are not well defined on how to evaluate their contribution to risk minimisation; iii) the legislation specifies that the reuse system description must consider all the integrated system from the wastewater source to its final use, including possible external contributions such as dilution of treated wastewater with other sources, but the Regulation does not specify how to address those aspects. This creates a significative gap in some areas such as Italy, where distribution systems are managed by irrigation consortia that normally use surface water to irrigate crops which quality is not monitored nor regulated, thus they do not have the infrastructure to do so.

With respect to water quality, there is a detailed description on how to assess microbiological risks. Nonetheless, indicators such as *E.coli*, legionella and intestinal nematodes are not always monitored in WWTPs. Lack of data may discourage new water reuse projects since no information can be provided about their occurrence, their trends and treatment efficiencies. For this, even if minimum log removal requirements are provided by the Regulation, it is hard for reuse facility managers to assess total removal efficiencies of the complete plant as microbial parameters are measured (when they are measured), only at the effluent. Moreover, pathogen determination is quite expensive and not always precise as it is affected by many variable conditions and the accuracy of the instrumentation. In fact, they sometimes

provide results with an uncertainty higher than the threshold. In addition, standard methods for their determination usually require excessively long times of around 24-48 hours. This would imply a very long time of response in case pathogen proliferations would occur. Furthermore, some techniques for pathogen analysis are based on their simple presence in the sample, not distinguishing between active and non-active microorganisms (especially for eggs and spores). This hinders the accomplishment of legal limits and increase disinfection costs without improving the safety reuse of water. Monitoring programmes should be thus supported by digital tools, including innovative sensors to monitor microbiological contamination or Early Warning Systems to provide real-time signals and allow rapid interventions in case possible hazardous outcomes are detected. However, the Regulation only considers standard lab measurements to verify the accomplishment of quality requirements, limiting the use of digital tools only for internal monitoring procedures.

Apart from the aforementioned, there is a lack of info on how to manage risks from other pollutants such as CEC, ARG, and metals (Deviller et al., 2020). In any case, the monitoring all these compounds would imply technical issues and high costs in terms of analysis. Some authors state that these costs should be shared by stakeholders (Salgot and Folch, 2018) but no information is provided about cost-sharing in the Regulation. There are no references about pollutant reduction in origin either. This should be promoted in this (or complementary) regulations since water quality of reclaimed facility influents are one of the most relevant factors influencing final water quality (Deviller et al., 2020).

Another important aspect (that is not detailed in the Regulation) is the monitoring requirements in soils and/or in crops. No information is provided about which parameters should be monitored, nor frequencies nor sampling points. This is crucial to both assessing the environmental impacts of water reuse practices and to evaluate potential health risks, especially for toxic compounds that may accumulate in crops and humans.

Following the proposed Regulation, determining whether additional requirements are needed requires the operator to compare the outcomes of the risk assessment to acceptable levels of risk or water quality. Regulation 2020/741 provides no further guidance on this, except for referring to other EU regulations under task 4. Translating these regulations or other environmental and health criteria to minimum requirements for reclaimed water is a complex task, even for experts in water risk assessment, exceeding the current expertise of the Water Boards and the competent authorities. Some authors have stated their concerns that these difficulties, together with the costs of reuse associated, might unintendedly stimulate indirect reuse (Dingemans et al., 2018). Hence, supporting (supra)national guidelines are needed for operators and regulators to efficiently prepare WRRMPs that protect human health and the environment without hindering the water reuse process excessively (Dingemans et al., 2018). it must be also highlighted that despite Regulation 2020/741 follows a multibarrier approach (Ofori et al., 2021), the way to address it is not explicitly described.

2.5.1 EU supporting guidelines on health and environmental risk management

Annex II of the Regulation 741/2020 on minimum requirements for water reuse for agricultural irrigation reports key elements to develop and improve risk management plans,

to be prepared by the reclamation facility operator, together with other responsible parties and/or end-users. On this basis, the EU Commission provided in August 2022 their Guidelines to support the application of Regulation 2020/741 on minimum requirements for water reuse. The Guidelines lays down the implementation of Water Reuse Risk Management Plans, providing guidance and technical support. The structure is divided into 11 Key Risk Management elements, grouped into 4 Modules.

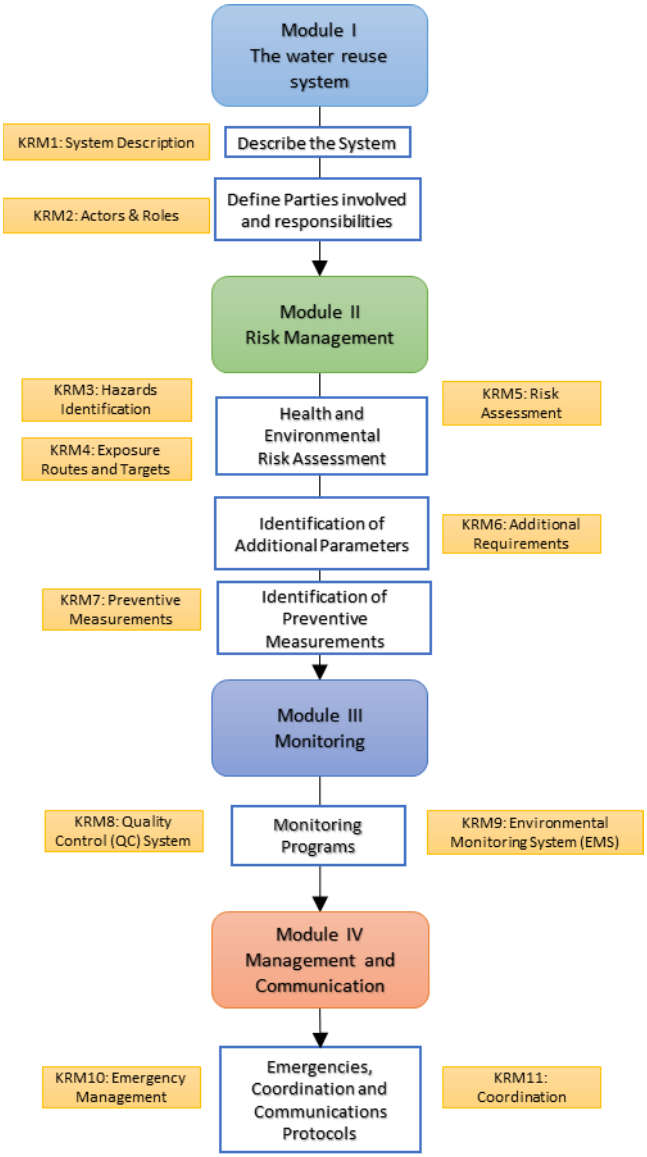


Figure 5: Schematic of key elements for water reuse risk management plans

Moreover, the Joint Research Centre (JRC) of the European Commission developed "Technical Guidance Water Reuse Risk Management for Agricultural Irrigation Schemes in Europe". Key risk management elements previously declined in the EU Regulation were furtherly detailed in the JRC technical guidelines for the application of the key risk management principles for the assessment and management of health and environmental risks linked to a water reuse system.

The first step consists in the preparatory phase, where objectives, influencing factors, boundaries and actors involved must be defined. The Guidance suggests the quantitative information to collect, as well as the factors to be considered. This is a crucial step, since it allows the contextualisation of site-specific particularities into the larger regional, national and European framework. The second Module is about system assessment and risk management, where system functionality, exposure routes and exposed groups must be defined, and hazards and hazardous events are identified. Here, information can be found on health and environmental risk assessment, as well as exposure assessment, targets and pathways. Indications are provided both for qualitative and for quantitative risk assessment approaches. Socio-economic impact assessments are also presented. The third Module is dedicated to operational monitoring and specifies requirements, recommendations and practices for the monitoring of the different matrices of water, crop and soil. Finally, in Module IV, information can be found on governance, management and communication plans to support water reuse planning and implementation.

Contributions were also provided by experts of the water reuse sector, representatives and authorities of EU Member States, and stakeholders. Dedicated workshops (Risk Management for Agriculture Irrigation in Europe – Ruggedness testing of Guidelines), and consultations with members of Ad-Hoc working groups on water reuse were organized by JRC. Best-practises and case studies from European Member States, which were presented during those events, are also reported in these guidelines. The Technical Guidance also included some examples of water reuse case-study schemes, which should allow a better understanding on how to address the indications provided and how to perform in practice a Water Reuse Risk Management Plan with the final goal to implement safe water reuse worldwide.

2.6 Material and methods

First of all, risk management requires a description of the system defining its main components and boundaries. Usually, the system is schematised in a flow diagram, in order to get a clear but complete overview of the global picture and all the parts involved. The system is then contextualised considering the regulatory framework of standards and requirements, together with the geographic, climatic and socio-economic conditions of the local area. Once the system is defined, the next phase consists of the identification of all hazards and hazardous events, the characterisation of their likelihood, and the severity of the related consequences. Risk assessment is usually performed following a semi-quantitative approach, attributing weights and scores in order to quantify the considerations and the evidence emerged. Possible exposure routes and exposed groups are defined, with particular attention to vulnerable subjects. Preventive and control measures are evaluated, considering their effectiveness on risk minimisation. Treatments, technologies and behavioural measures are taken into account, considering a multi-barrier approach. Monitoring level, procedures, maintenance programs and emergency plans are implemented in the evaluation to get the residual risks. Finally, results are prioritised in order to define the most appropriate actions and implementations needed.



Figure 6: Schematic methodology for risk management

Health risks are partially addressed already by existing legislation concerning agricultural product safety, such as the Regulation on the Hygiene of Foodstuffs n.852/2004. However, this legislation does not specify the requirements for treated wastewater used to irrigate agricultural products, which can also present other impacts on human health due to the direct exposure of farmers and people from the surroundings to the pollutants contained in water. Environmental risks due to the presence in water of chemical contaminants, inorganic salts, nutrients, and heavy metals and detergents must be also considered as well. In general, such risks should be analysed carefully prior to using wastewater as a water source through a risk-based approach. In the case of irrigation, risks associated to water reuse could include: i) health risks associated to consumers of agricultural products irrigated with reclaimed water, including those related to the health of animals consuming crops irrigated with reclaimed water; ii) health risks associated to humans exposed to reclaimed water (workers, bystanders and residents in nearby communities); iii) risks for the local environment, i.e., surface waters, groundwaters, soil and ecosystems.

2.7 Proposed methodology in FIT4REUSE project

FIT4REUSE PRIMA project (Safe and sustainable solutions for the integrated use of non-conventional water resources in the Mediterranean agricultural sector) aims to develop innovative solutions tailored for maintaining nutrients only when needed (crop irrigation seasons), while removing pathogens and emerging compounds for a safe wastewater reuse. In this context, structured guidelines were developed to address water reuse risk management plans, specifically focused on Mediterranean areas and including the main results that the FIT4REUSE technologies achieved for wastewater treatment and reclamation.

Considering the analysed background, specific guidelines were developed and schematised into sections, in order to support the users in the implementation of water reuse risk management plans. Suggestions and examples were provided to facilitate their comprehension and their implementation in real scenarios. Specific details were moreover provided in the risk assessment section, considering the different approaches that can be followed. Guidelines for semi-quantitative analysis were proposed, as well as indications on quantitative microbial risk assessment. The proposed guidelines focus on health risk assessment and thus particular attention is paid to microbial risks. However, suggestions on how to address environmental risk characterisation are also proposed. Health-based targets, following EU, national and local regulations and guidelines from international authorities, are selected to assess risk acceptability level. Water resource availability, together with BATs, feasibility and sustainability of solutions, will be also considered to modulate the risk acceptability levels, inside the limits already individuated by regulations and guidelines.

A risk management framework involves identifying and managing risks in a proactive and dynamic way, planning preventing measures to minimize the consequences related to the occurrence of hazardous events and iterating the process to continuously adapt it to system changes.

The general framework for the implementation of the WRRMP is summarized. This includes in total 11 stages, grouped in three main parts, as listed below:

PART A:

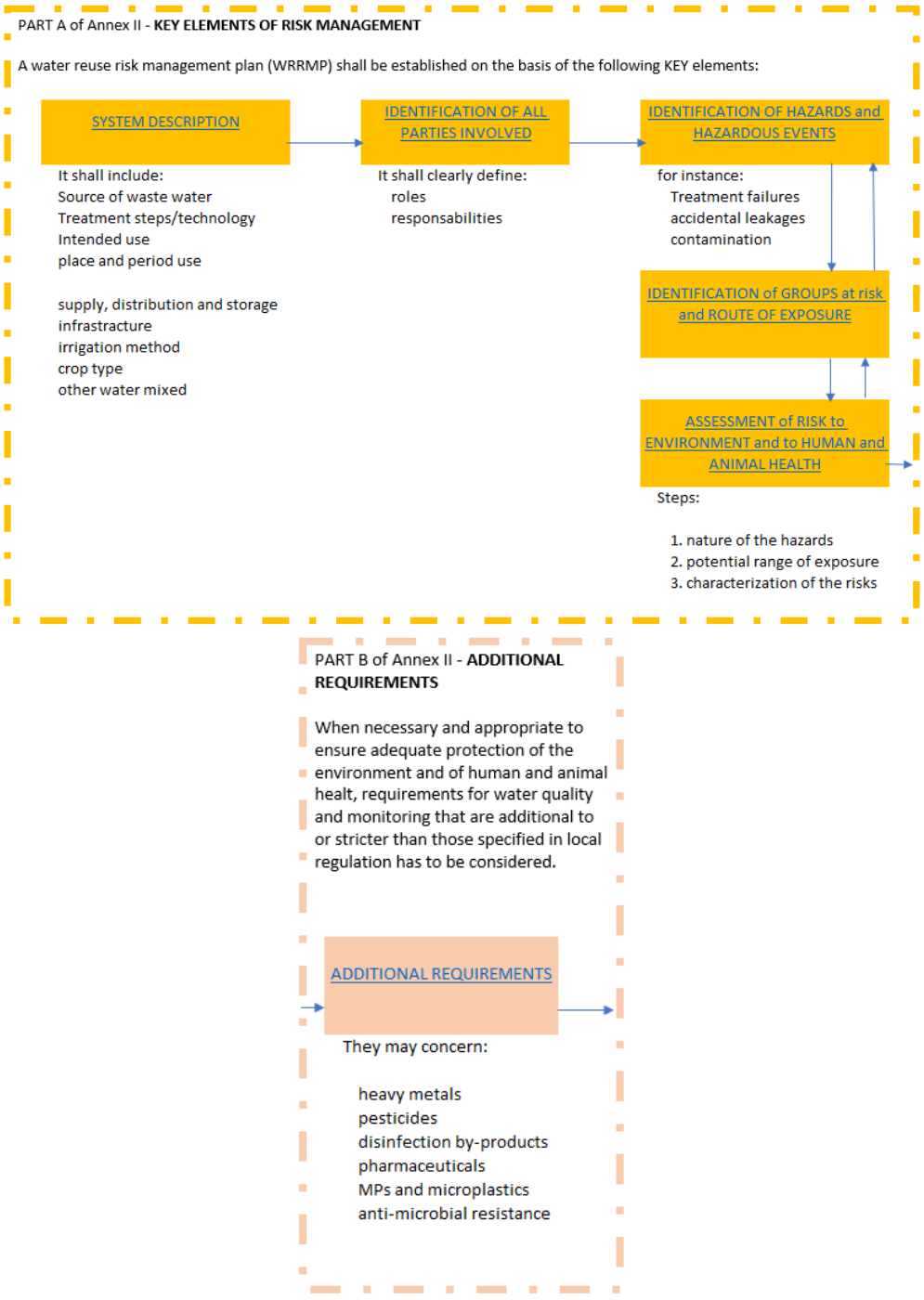
- System description
- Identification of all parties involved
- Identification of hazards and hazardous events
- Identification of groups at risk and route of exposure
- Risk assessment

PART B:

- Additional requirements

PART C:

- Preventive measures
- Quality control and procedures
- Environmental monitoring system
- Emergency plan
- Coordination plan


PART A of Annex II - KEY ELEMENTS OF RISK MANAGEMENT
A water reuse risk management plan (WRRMP) shall be established on the basis of the following KEY elements:
SYSTEM DESCRIPTION
It shall include:
Source of waste water
Treatment steps/technology
Intended use
place and period use
supply, distribution and storage
infrastracture
irrigation method
crop type
other water mixed
IDENTIFICATION OF ALL PARTIES INVOLVED
It shall clearly define:
roles
responsabilities
IDENTIFICATION OF HAZARDS and HAZARDOUS EVENTS
for instance:
Treatment failures
accidental leakages
contamination
IDENTIFICATION of GROUPS at risk and ROUTE OF EXPOSURE
ASSESSMENT of RISK to ENVIRONMENT and to HUMAN and ANIMAL HEALTH
Steps:
1. nature of the hazards
2. potential range of exposure
3. characterization of the risks
PART B of Annex II - ADDITIONAL REQUIREMENTS
When necessary and appropriate to ensure adequate protection of the environment and of human and animal healt, requirements for water quality and monitoring that are additional to or stricter than those specified in local regulation has to be considered.
ADDITIONAL REQUIREMENTS
They may concern:
heavy metals
pesticides
disinfection by-products
pharmaceuticals
MPs and microplastics
anti-microbial resistance

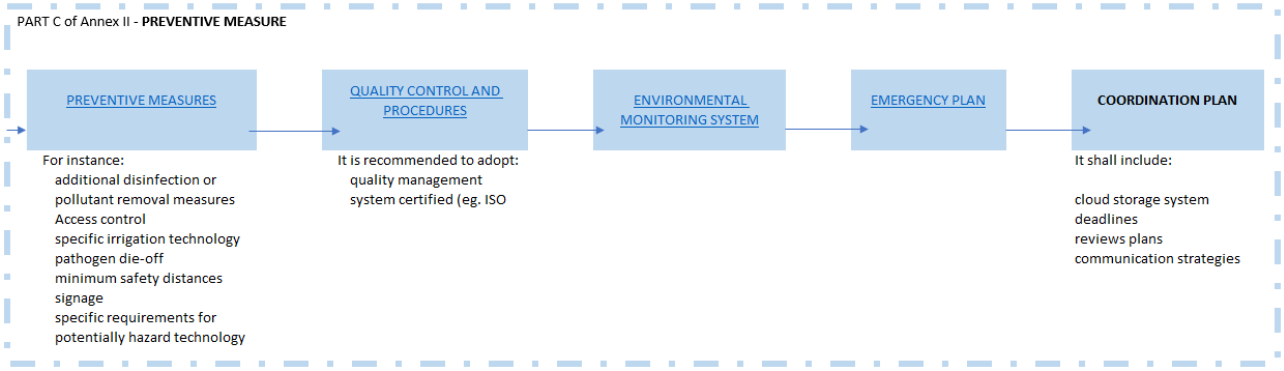


Figure 7: Proposed scheme for FIT4REUSE guidelines on water reuse risk management planning

2.7.1 Decentralized and rural context

According to the World Health Organization (WHO, 2016), 264 million people lived in rural regions in the pan-European region in 2015, with 40% of the population without access to wastewater collection and treatment infrastructure.

Small-scale decentralized collection and treatment systems can now provide small and rural communities with a long-term solution that is also dependable, versatile, and cost-effective. Decentralized methods may also help to improve water management conditions of sustainability and resilience (Leigh & Lee, 2019). Innovative and nature-based technologies and concepts for water and wastewater systems currently exist, however they have largely been deployed in pilot/demonstrative projects due to institutional constraints (Trapp et al., 2017).

Although decentralized water/wastewater treatment systems have a lot of promise in terms of technological, ecological, and economic sustainability, their adoption generally falls short. In fact, critical processes linked to how the water industry might change toward decentralized infrastructure are not well understood, and technology techniques alone will not be enough to support more sustainable systems. There is no question that water infrastructure innovation occurs at both the technical and institutional or organizational levels. As a result, these solutions should be reviewed under legislative/policy frameworks in order to replicate these technologies and close water-related cycles. The European Union's (EU) legislations are, in most circumstances, the starting point for the EU Member States and accession nations.

Providing regular and sustainable water supply for irrigation to areas prone to droughts, such as Mediterranean region, besides having an evident positive effect on the agro-food sector, can also increase the amount of freshwater available for potable uses and can therefore prevent negative impacts on population of rural and peri-urban areas. Indeed, FIT4REUSE Project aims to provide safe, locally sustainable and accepted ways of water supply for the Mediterranean agricultural sector by exploiting non-conventional water resources, namely treated wastewater and desalted water.

According to the water quality achieved and required, reclaimed water could be used not only for irrigation, but also for aquifer recharge providing a barrier against saltwater intrusion in coastal areas and storing additional water resources.

In this sense, integrated innovative and nature-based technologies of FIT4REUSE could be applied to rural scenarios such as Mediterranean areas. Therefore, risk management plans have to implemented considering that in rural areas technology level, in general, does not include innovative high-cost applications or digital solutions and not all data could be always available. Reference indicators can be selected to represent a class of contaminants that are harder or more costly to be measured, to obtain affordable, even if indirectly acquired, data.

In FIT4REUSE context the main wastewater treatment processes tested by all the partners were:

- ***Single stage CW***

Laboratory scale, single-stage, horizontal flow (HF) and vertical flow (VF) CWs and filters (void volume of around 5-10 L). All the tested technologies were assessed by monitoring BOD, COD, ammonia, nitrate, phosphorous and suspended solids. The monitoring was extended to emerging pollutants such as endocrine-disrupting compounds (EDCs) (Bisphenol A as a representative) and pharmaceutical compounds (diclofenac as a representative). In order to face the rapid emergence of antibiotic-resistant bacteria, the antibacterial activity of the proposed technologies was quantified and compared against tetracycline-resistant bacteria

- ***Hybrid CW***

Various types of CWs (hybrid systems) were combined, at small pilot scale, in order to achieve higher treatment performances, especially for pathogens and nitrogen removal.

- ***UASB/AnMBR/MIPs***

Upflow anaerobic sludge blanket (UASB) process was tested: (a) with or without association to membrane technology (such as Anaerobic Membrane Bioreactor); (b) with different pre-treatment methods for TSS and COD removal: static biofilter and dynamic filter (rotating belt); (c) with or without additional disinfection. Molecularly imprinted polymers (MIPs) were synthesized via bulk polymerization using diclofenac as a template and assembled in adsorption columns.

- ***Adsorption substrate for nutrients recovery/removal***

Ion exchange processes, to be implemented after the UASB reactor, were developed for the removal and recovery of NH_3 and P. For this purpose, different types of ion exchange resins, including natural and engineered zeolites, were screened in terms of selectivity for NH_3 and P, adsorption capacity, easiness of regeneration. In addition, the technical and economical effectiveness of mesolites engineered with Fe nanoparticles for P removal and recovery were evaluated.

- ***Improved aeration***

In addition, different types of aeration (uniform, phased or only at the beginning/end of the systems) were applied to create optimal aerobic and anaerobic conditions that can increase the removal efficiency of organic matter and total nitrogen

- ***Nano zero-valent iron***

Nano Zero Valent Iron (nZVI) were synthesized by reducing Fe^{2+} using polyphenolic plant extracts and modified to improve aqueous mobility, then tested for heavy metals and pharmaceuticals removal from wastewater effluents through fixed-bed column installations. Nano Zero Valent Iron (nZVI) were tested as a polishing step to remove selected emerging contaminants from anaerobic effluent.

2.7.2 Characteristics and removal efficiencies: multibarrier approach

For each one of the FIT4REUSE technologies, treatment performances were evaluated through the collection of monitored parameters, depending on the specific purpose of the technology in exam.

Table 6 and Table 7 schematise the main parameters collected and shared by the different partners.

Monitored parameters varied depending on the main purpose of the technology in exam, with sampling frequencies in the range of about 1-2/week.

Technologies were tested at a pilot scale, with capacity varying from 30 – 500 l/d, even if they could be implemented in scale-up with treatment capacity up to 500 – 1000 PE. The expected optimal plant capacity meets the small-medium sizes typical of the decentralised systems and the rural areas.

The proposed different technologies can be implemented to face different quality requirements or site-specific conditions and so to reduce chemical and/or microbiological hazards and prevent human-health and environment risk. As an example, MIPs and nZVI solutions can be implemented to decrease chemical hazards by removing CECs. Experimental tests showed that MIPs treatment could achieve a 55% of Diclofenac removal, while nZVI could reach up to 80%–98% of different pharmaceuticals removal. As concern microbial contamination, AnMBR, single-stage or hybrid CWs can achieve, respectively, 4–5 log removal, 1–2.7 log removal and 3.5 log removal for E. coli. Moreover, most of the technologies are also effective in macro pollutants removal preventing environmental risk of nutrient accumulation. Single and hybrid CWs are characterized by a removal efficiency of about 80%–93% on COD, BOD5 and TSS, while more variable removal rates were observed for Nitrogen and Phosphorus, varying from 20% up to 85%. Also, UASB and AnMBR technologies can remove macropollutants, with TSS removals close to 100%. As concern nutrients, some of the technologies, such as UASB and AnMBR, can release them into the liquid phase, allowing possible recoveries in the following steps or direct application in agriculture through ferti-irrigation techniques. In the same way, adsorption substrate technologies aim for nutrient recovery, reaching up to 95–96% of phosphorus and nitrogen removals.

It must be noticed that there is not a technology that can be singularly applied and that can face alone all the hazards of different nature. For this reason, possible combinations were proposed, in order to achieve reclaimed water quality standards using a multi-barrier approach.

The considered combinations are below schematised:

- 3+e+1+f+h: UASB + MIPs + CW + nZVI + UV
- 4+a+e+f+h: AnMBR +ion exchange + MIPs + nZVI + UV
- 3+e+2+d+f+h: UASB + MIPs + CW + Improved aeration + nZVI + UV
- 4+a+e+1+f: AnMBR + ion exchange + MIPs + CW + nZVI

Table 6: Characteristic parameters of FIT4REUSE technologies for wastewater reuse

Name	Description	Nature based / Intensive solution	Monitored parameters	Monitoring frequency	COD effluent	BOD_5 effluent	TSS effluent	*E. Coli* effluent	CEC effluent	TN effluent	TP effluent	Effluent salinity
				samples/week	mg/l	mg/l	mg/l	FCU/100ml	ng/l	mg/l	mg/l	µs/cm2
1	SINGLE STAGE CW	Nature based	COD, TSS, E.coli, TN, TP	1	25.8		10	4650		26.7	1.18	
	SINGLE STAGE CW		pH, CE, COD, NH_4^+, NO_3^-, NO_2^-, TSS,pathogenic bacteria	Weekly /Monthly	490 ± 45	341 ± 61	286 ± 77	Total coliforms: 9.3 10^6; Fecal coliforms: 7.5 10^5; *E.coli :* 6.4 10^2; Total streptococcus: 1.3 10^5; Fecal streptococcus: 1.4 10^4	not detected	NH_4^+:83± 3,5	PO_4^{-3} :12±2.15	2.9 ± 1.02
	SINGLE STAGE CW (coupled with nZVI)		CODt, CODs, BOD5, TSS,VSS, TN,NH4-N, PO4-P, TP, pH, Conductivity, Microbial parameters, Ecs, Heavy metals, Temperature	1/week (macro), 2/month (nutrients), 2/week (pH, Cond.), 1/month (microbial and metals), 1/minute	65.2	30.7	12.9	4.41E+05	Naproxen : 1257 ng/L, Bisphenol - A : 3191 ng/L, Diclofenac: 3747 ng/L	47	6.9	1272
2	HYBRID CW / Improved aeration	Nature based	COD, TSS, E.coli, TN, TP	1	14.1		9.8	850		30.4	0.74	
	HYBRID CW		pH, CE, DCO, NH_4^+, NO_3^-, NO_2^-, TSS,pathogenic bacteria	Weekly /Monthly	507 ± 52	375 ± 47	256 ± 81	Total coliforms: 9.3 10^6 ; Fecal coliforms: 7.5 10^5 ; *E.coli :* 6.4 10^2; Total streptococcus: 1.3 10^5; Fecal streptococcus: 1.4 10^4	not detected	NH_4^+:79± 6,5	PO_4^{-3} :13±3.05	3.1 ± 1.2
3	UASB	Intensive	pH, Conductivity, COD, BOD5, TSS, NH4, PO4, TN, TP; eCOli	2/week	175	102	175	10^4-10^5	none	34	5	1480±150
			pH, CE, DCO, NH_4^+, NO_3^-, NO_2^-, TSS, VSS, VFA, gas production	Weekly /10 days	617 ± 205	465 ± 89	223± 11.5		not detected	NH_4^+:15± 6.5	PO_4^{-3}: 9,6± 2.55	3.6 ± 1.75
4	AnMBR	Intensive	pH, Conductivity, COD, BOD5, TSS, NH4, PO4, TN, TP; eCOli	2/week	61	39	0	<10	none	28	3	1480±150
a	Adsorption substrate for nutrient recovery/removal	Intensive	N and P adsorption outlet concentration, N and P desorption outlet concentration, operating P, Adsorption and desorption Yield,	Adsorption and desorption Outlet Concentration taken every 0.30 h, other parameters								

Name	Description	Nature based / Intensive solution	Monitored parameters	Monitoring frequency	COD effluent	BOD_5 effluent	TSS effluent	*E. Coli* effluent	CEC effluent	TN effluent	TP effluent	Effluent salinity
				samples/week	**mg/l**	**mg/l**	**mg/l**	**FCU/100ml**	**ng/l**	**mg/l**	**mg/l**	**µs/cm2**
			Adsorption and desorption operating capacity, Resin efficiency	calculated after each BT								
			Bisphelnol A, Diclofenac						BPA: 50 -104 ng/l/ DFC:10ng/L			
e	Molecularly imprinted polymers (MIP)	Intensive	Diclofenac	1/week								
f	Nano zero valance iron (nZVI)	Intensive	organic micropollutants (diclofenac, naproxen, bisphenol-A) & heavy metals (Cr(VI), As)	6 samples per week initially (1st week of operation) and then 3 samples per week	31.8	N.A.	0.63	90	Naproxen: 428 ng/L Bisphenol A:1017 ng/L Diclofenac: 626 n/L Cr(VI): 30 µg/L or 30000 ng/L As: 39 µg/L or 39000 ng/L	7.33	1.71	1178
h	UV disinfection		Pathogenic bacteria	Every two weeks				Total coliforms: $9.3*10^6$; Fecal coliforms: $7.5*10^5$; *E.coli :* $6.4*10^2$; Total streptococcus: $1.3*10^5$; Fecal streptococcus: $1.4*10^4$				

Table 7: Removal efficiencies of FIT4REUSE technologies for wastewater reuse

Name	Description	Main objective	ACTUAL PLANT CAPACITY - SIZE	Expected optimal plant capacity (in a possible scale-up scenario)	COD removal	BOD_5 removal	TSS removal	*E.coli* Log reduction	CECs removal	N removal	P removal
			PE	PE	%	%	%	-	%	%	%
1	SINGLE STAGE CW	Macropollutants removal; Pathogens removal			87.8		82.1	2.73		50.3	76.8
	SINGLE STAGE CW		800 cm^2 - 2300 cm^2		81%	78%	89%	Total coliforms: 0.5 Log; Fecal coliforms: 0.4 Log; *E. coli:* 4 Log; Total streptococcus: 0.6 Log; Fecal streptococcus: 0.5 Log		48%	22%
	SINGLE STAGE CW (coupled with nZVI)		flowrate= 500 L/d if we assume 150 L/d/person it results in approximately 3.3 PE		80.4	80.4	90	1	Naproxen: 65%, Bisphenol - A: 59%, Diclofenac: 4%	23.7	22.4
2	HYBRID CW / Improved aeration	Macropollutant removal; Pathogens removal			93.3		82.8	3.47		43	84.9
	HYBRID CW		3100 cm^2		92%	88%	98%	Total coliforms: 1 Log; Fecal coliforms: 1 Log ; *E.coli :* 4 Log; Total streptococcus: 2 Log		61%	27%
3	UASB	Macropollutant removal; Nutrient release (UASB+AnMBR)	flowrate= 70 L/d	5000-10000	53%	39%	67%	0.5	none	5%	5%
			75000 cm^2		37.20%	42%	49%			86%	

Name	Description	Main objective	ACTUAL PLANT CAPACITY - SIZE	Expected optimal plant capacity (in a possible scale-up scenario)	COD removal	BOD_5 removal	TSS removal	*E.coli* Log reduction	CECs removal	N removal	P removal
			PE	PE	%	%	%	-	%	%	%
4	AnMBR	TSS removal; Pathogens removal; Nutrient release (UASB+AnMBR)	flowrate= 70 L/d	5000-10000	65%	61%	100%	4-5	none	10%	15%
a	Adsorption substrate for nutrient recovery/removal	Nutrient recovery	N-removal: 0,0305 m3/d P-removal: 0,00694 m3/d	P-removal: 0.238 m3/d						96	95
									Olive seeds: DFC (77%), BPA (87%)/ Clay: DFC (38%), BPA (60%)		
e	Molecularly imprinted polymers (MIP)	CECs removal							55% Diclofenac removal		
f	Nano zero valance iron (nZVI)	CECs removal	flowrate=26 L/d if we assume 150 L/d/person it results in approximately 0.17 PE	500	2	N.A.	2	0.37	Naproxen removal: 91% Bisphenol A removal: 80% Diclofenac removal: 87% Cr(VI) removal: 94% As removal: 98%	10%	10%
h	UV disinfection							3-4			

One of the critical steps in risk management is the validation of the processes, especially if the plant is still not existing and running. The aim of process validation is to ensure effective operation and control of the reclaimed water system by evaluating available scientific and technical information (including historical data and operational experience) and, where necessary, undertaking investigations to validate system-specific operational procedures, critical limits, and target criteria. Therefore, FIT4REUSE outcomes from the long-term pilot validation, mainly consisting in technologies efficiencies data (Table 7), are actionable insights that could be also compared to most frequently used reference sources.

As previously stated, there is not a single technology that could be applied in any case and that could remove all the microbial and chemical hazards. Thus, combinations were proposed accordingly, in order to achieve reclaimed water quality standards in a multi-barrier approach.

Taking as an example the combination of UASB, MIPs, CW, nZVI and UV (Figure 8), both WHO data and FIT4REUSE results highlighted the effectiveness in preventing microbial hazards and maintaining the human-health risk under acceptable level for a safe reuse of reclaimed water in agriculture (Table 8). It is worth mentioning that only bacteria (E. coli, faecal coliform, etc) are the only monitored microbiological parameters in such rural areas. At the same time, a multi-step treatment chain improves system resilience, e.g., if operational issues occur within constructed wetland, UV disinfection will anyway guarantee a safe water reuse.

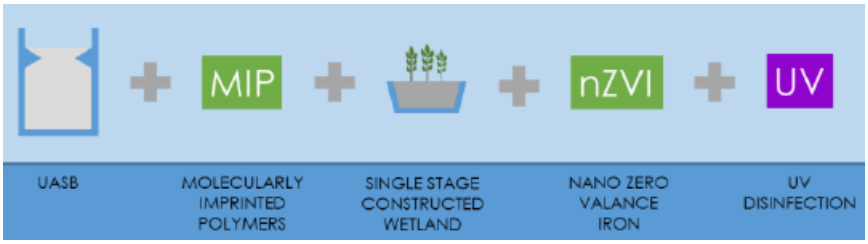


Figure 8: Example of FIT4REUSE combination of technologies (3+e+1+f+h)

Table 8: Log_{10} removals achievable using multibarrier approach

	UASB		MIP	SINGLE STAGE CW		nZVI	UV		SYSTEM		Agriculture reuse target
Reference	**a)**	**b)**		**a)**	**b)**	**b)**	**a)**	**b)**	**a)**	**b)**	**c)**
Virus	0 – 1			1 – 2			1 - >3		**2 - >6**		**≥ 6**
Protozoa	0 – 1			0.5 – 2			2 - >4		**2.5 - >7**		**≥ 4**
Bacteria	0.5 – 1.5	0.5		0.5 – 3	1 – 4	0.4	>3	4	**4 - >7**	**6 – 9**	**≥ 5**

a) WHO, 2006; b) FIT4REUSE results; c) EU Regulation 2020/741

Additionally, suggested treatment trains combine minimization of microbial hazards (by removing pathogens) and chemical hazards (by decreasing CECs content and recovering nutrients if exceeding soil and crop requirements). Moreover, such treatment barriers are coupled with agronomic practices acting as onsite preventive measure and end-use restriction.

Therefore, the combination of FIT4REUSE solutions, according to the multi-barrier approach, can achieve effective results in terms of risk management and minimization.

2.8 Water reuse risk management in ACEA Fregene

The water utility ACEA manages the territorial district of ATO2 in Lazio, Italy, and asked for a technical support for a study for the development of a risk management plan for wastewater reuse for irrigation use in the Consorzio di Bonifica Litorale Nord (CBLN) basin.

Risk assessment for the water reuse system of Fregene is still under development and the suggested approaches for health and environmental risk assessments is in line with the existing guidelines. The proposed approach includes: i) the characterization of the integrated system, from wastewater catchment to treatment, delivery and reuse in agriculture, also with the support of checklists; ii) the identification and the analysis of possible hazards and hazardous events and iii) the quantification of risks by the attribution of scores to probability and severity of the detected hazardous events.

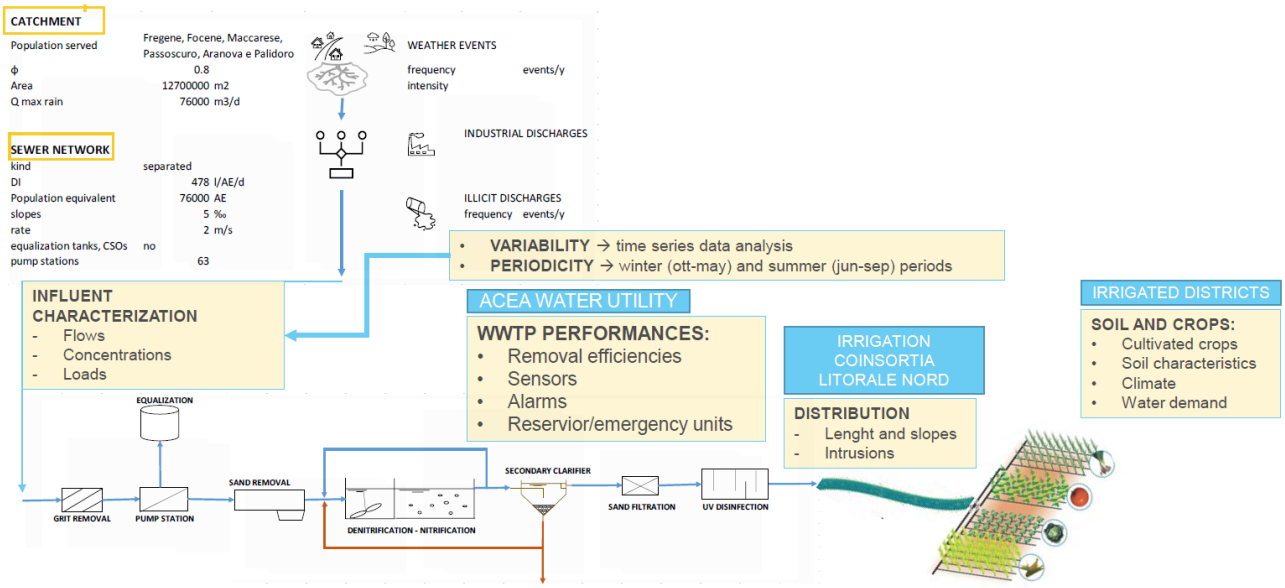


Figure 9: Schematic of the ACEA Fregene water reuse system.

The territory is mainly dedicated to agriculture and the presence of industries is limited to the agro-food sector. Agricultural activities mainly generate widespread discharges, together with run-off water that can infiltrate the sewer. Carrots are among the most common crops and are generally grown for 2-3 cycles a year. Among the main farms in the area, Maccarese extends for 3500 ha, of which 200 ha are irrigated. The irrigation periods are generally from February to November. The irrigation techniques depend on the type of crop, from micro-irrigation or localized sprinkling for vegetables, to surface and sprinkler for corn. The area near to the plant is included in the WWF Oasis of Macchiagrande, Foce dell'Arrone and Vasche di Maccarese. The Fregene WWTP is located in the northern outskirts of the town of Fregene, Municipality of Fiumicino. Currently, the plant is authorized to discharge a flow rate of 29064 m^3/d at the receiving water body called Arrone River.

It should be noted that currently the waters of the Arrone, together with those taken from the Tevere, are used for the irrigation of the land served by the Reclamation Consortium. This situation is common to many cases in Italy, since many plants drain into canals, canals or other surface water covers that are part of an irrigation network or from which water is drawn for agriculture. In this case we talk about of "indirect" reuse. In the Ministerial Decree 185/03 no reference is made to

criteria or limits to regulate the practice of indirect reuse. On the one hand, it is desirable that this method of reusing water is maintained and enhanced. However, it should be considered that it also creates problems of qualitative compatibility for agriculture, as well as for natural waterways; therefore, its confirmation, or even its incentive, can only presuppose an improvement in the current quality standards for the discharge of purified wastewater into surface water. A further issue concerns the level of monitoring of the quality of the water currently used for irrigation, compared to that established by current regulations. The annex to the Ministerial Decree 185/03 reports, among the microbiological parameters, *Escherichia coli*: its concentration in the wastewater leaving the recovery plant must never exceed 100 CFU/100 ml (maximum punctual value) with 80% of the samples it must be below 10 CFU/100 ml. It should be noted, however, that a survey on the quality of water in the Emilia-Romagna Region indicates that for 27 significant watercourses (including the Po River), almost all used for irrigation purposes, the 75th percentile of the Escherichia concentration coli in the two-year period 2001-02 stands between a minimum value of 163 CFU/100 ml and maximum values up to 92500 CFU/100 ml.

Fregene WWTP collects municipal wastewater from a 13 km^2 catchment area with limited industrial presence that collects urban wastewater in separated sewer network. The area is characterised by high touristic fluxes over the summer season, which causes a significant variability in the influent. The WWTP has a capacity of 76000 P.E. and it is composed by pre-treatments, biological and secondary treatments, sand filtration and UV disinfection. The reclaimed water is conveyed to an open channel from where it is withdrawn and reused for irrigation in agriculture, mainly for horticultural crops.

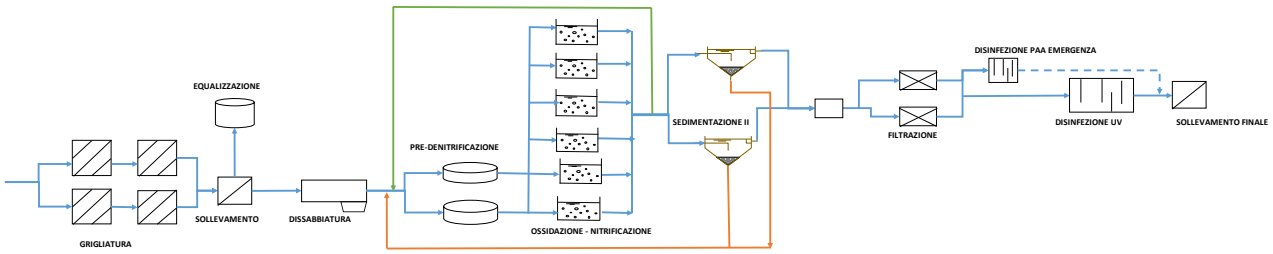


Figure 10: Layout of Fregene WWTP

Sewer network is in part separated, but it is influenced by the presence of parasite infiltration. For this reason, analysis on flowrates and rain gauges was conducted.

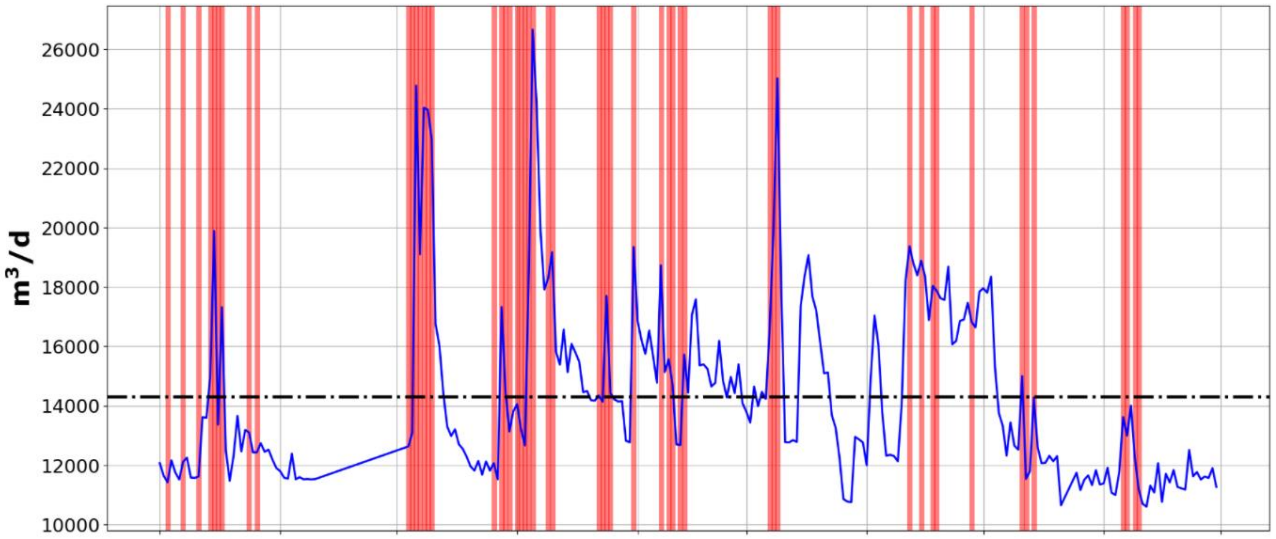


Figure 11: Influent flowrates (blue line) with indication of rainy days (red)

From the comparison between rainfall data and influent flowrates, it was therefore possible to extract the dry period, from which it was identified the typical daily trend of the influent flows related only to the contribution of municipal wastewater (black water).

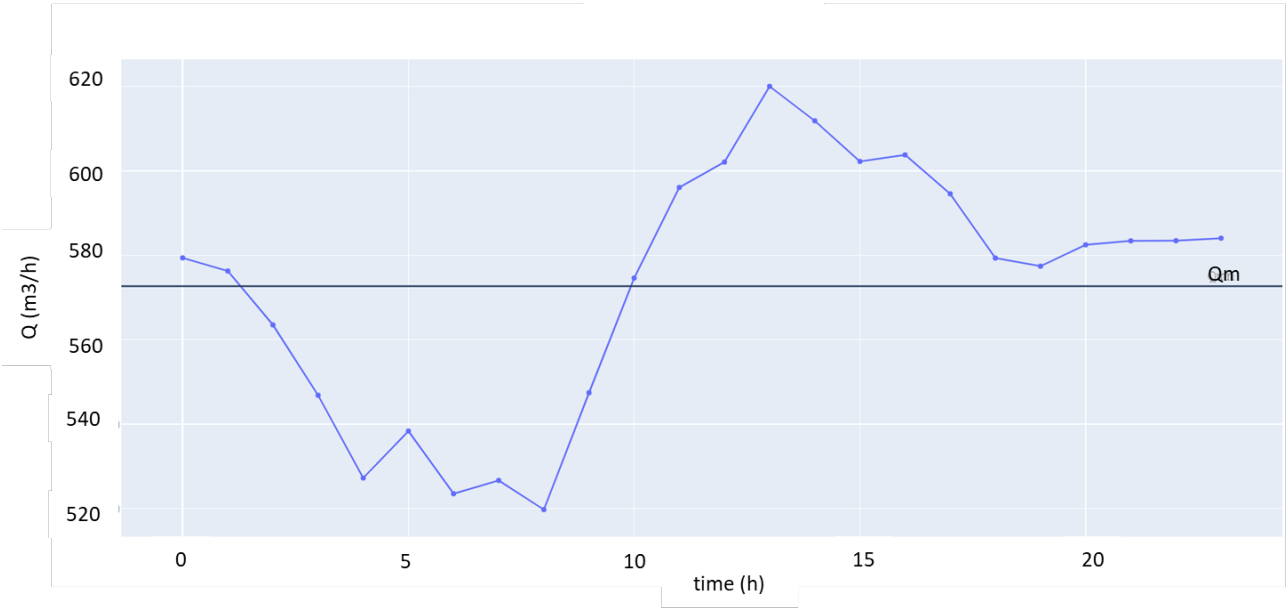


Figure 12: Seasonality of flowrates in dry period

Monitored parameters included organic macro-pollutants, nutrients, metals and trace elements, as well as the determination of *Escherichia coli*, as indicator of pathogenic contamination. The measured concentrations were compared with the limits set by current Italian legislation on the reuse of wastewater.

In particular, it was noted that for some parameters, such as Benzene, Cyanides, Chromium VI and *Escherichia coli*, the detection limits of the analysis laboratory were greater than the limit imposed by the reuse legislation. In addition, parameters required by Italian legislation, such as the SAR index, Beryllium, Cobalt, Thallium, Vanadium, Active Chlorine, Mineral Oils, Pentachlorophenol, Benzo (a) pyrene and Salmonella, were not monitored by routine analyses. Also as concern parameters for which the regulatory limits are expressed as a sum, such as tetrachlorethylene and

trichlorethylene and trihalomethanes, the detection limits of the individual parameters were equal to or exceed the threshold stated in the regulation.

The values of total suspended solids are generally below the threshold of 10 mg/l, with the exception of 5 values, of which the last reaches 40 mg/l.

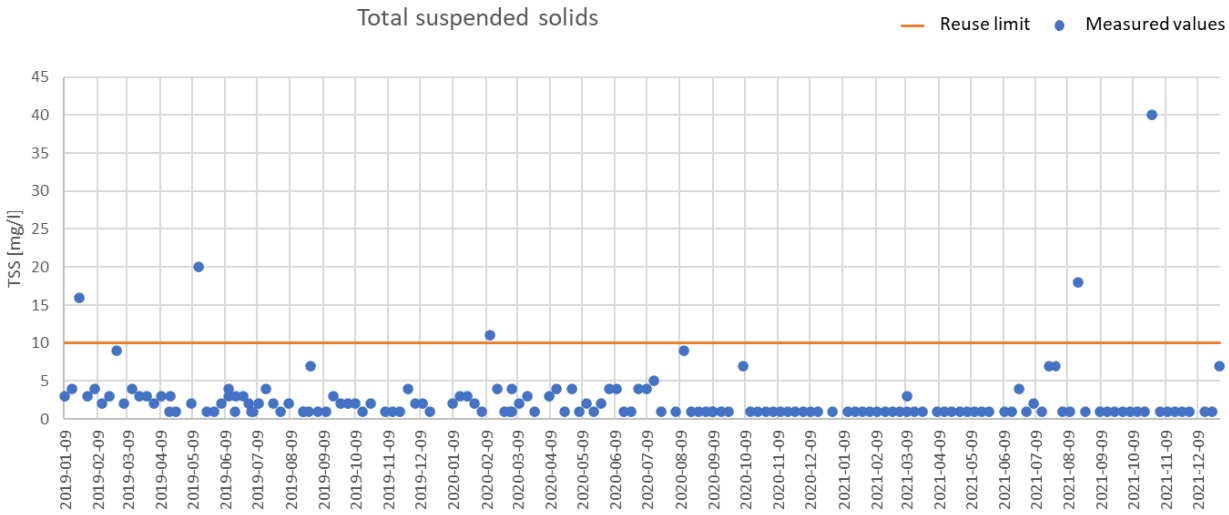


Figure 13: Effluent TSS concentrations vs reuse limits

As concern nutrients, exceedances are observed for ammonia nitrogen, total nitrogen and total phosphorus, if we consider the limits shown in the table of Ministerial Decree 185/2003. However, the legislation also provides for the possibility of extending the limits of nitrogen and phosphorus in the case of reuse in agriculture, respectively to 35 and 10 mg/l. In this case, the total nitrogen and phosphorus values measured comply with the requirements.

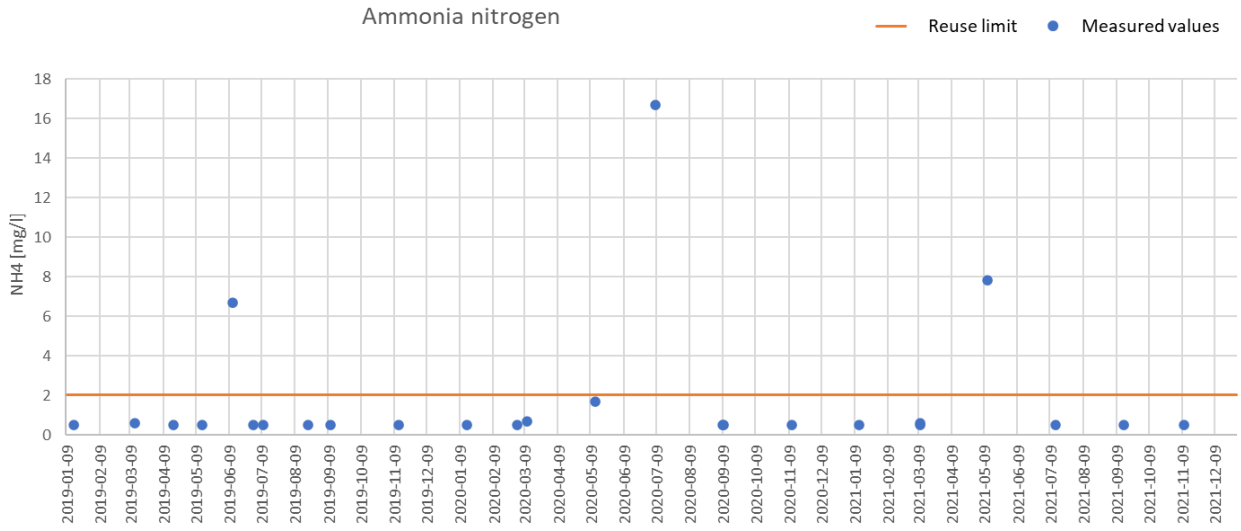


Figure 14: Effluent NH_4 concentrations vs reuse limits

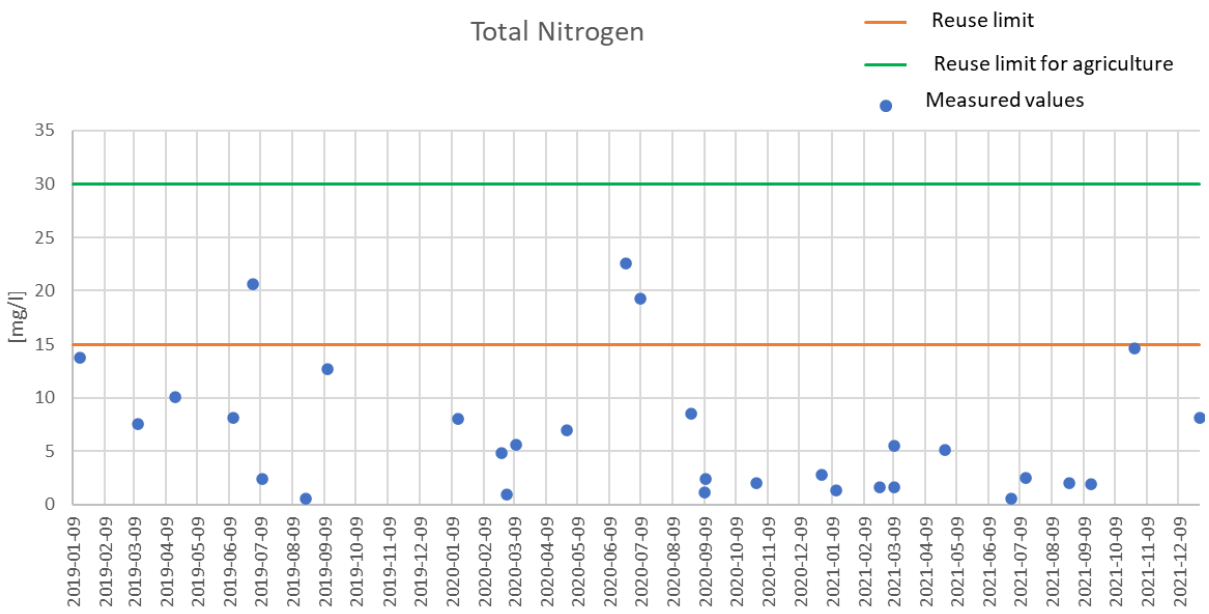


Figure 15: Effluent Total Nitrogen concentrations vs reuse limits

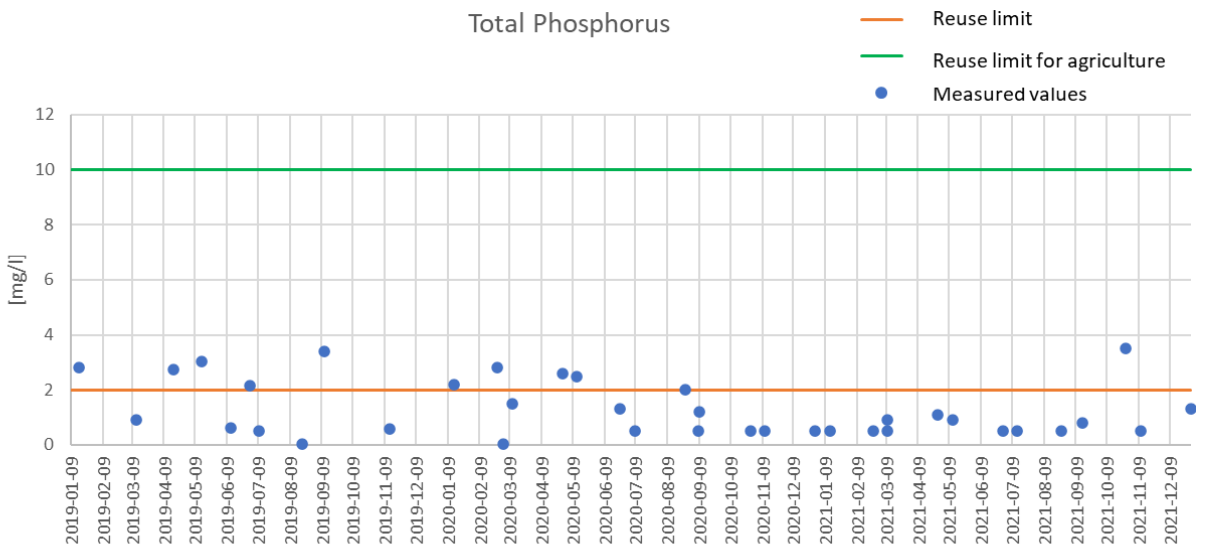


Figure 16: Effluent Total Phosphorus concentrations vs reuse limits

In the historical analyzes provided, the Escherichia Coli parameter was analyzed with a view to the discharge authorization, which provides for a limit of 5000 UFC / 100 ml. Therefore, sometimes the laboratory detection limit is 100 CFU / 100ml. For this reason, it is not possible at the moment to compare the available data with the current limits of the Italian legislation, which set a threshold value of 10 CFU / 100 ml. On the other hand, considering the maximum admissible value, equal to 100 CFU / 100 ml, there are 16 exceedances, of which five values, which respectively exceed between 104 and 105 CFU / 100 ml, are particularly out of scale with respect to the average. However, it should be noted that the frequency of exceeding is higher in the previous years than in 2021.

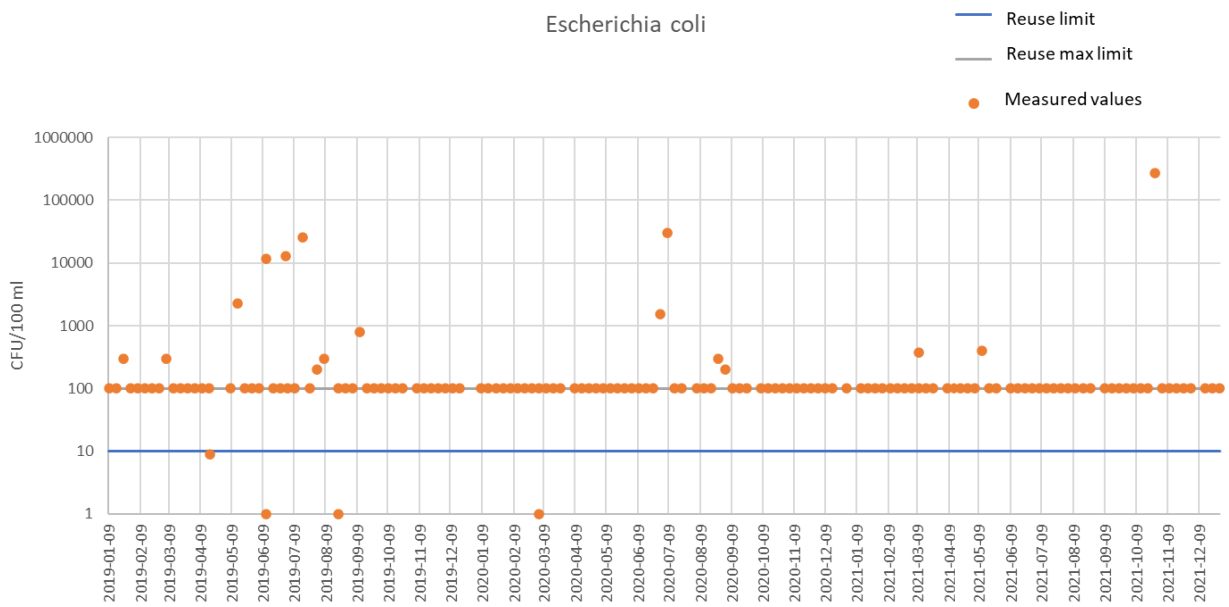


Figure 17: Effluent *E.coli* concentrations vs reuse limits

Table 9: Summary of non-compliances with regulatory thresholds for water reuse (and specific for water reuse in agriculture)

	Non compliance TSS	Non compliance Ammonia Nitrogen	Non compliance Total Nitrogen	Non compliance Total Phosphorus	Non compliance max *E.coli*
Number	5	3	3 (0 for agriculture)	10 (0 for agriculture)	16 (max)
Probability of exceeding [%]	3	12% (0%)	10% (0%)	27% (0%)	11 (max)
Maximum value	40	16.7	22.6	3.5	$2.7*10^5$
Limit regulation	10	2	15 (30)	2 (10)	10-100

Considering the evidence emerged from the system analysis, a risk matrix is going to be implemented, including all the possible hazardous events that may occur at each critical point of the system. Both health and environmental hazards were identified considering the effluent characteristics and the applicable EU and national legislations. Populations and environments at risk, along with the routes of exposure, were identified.

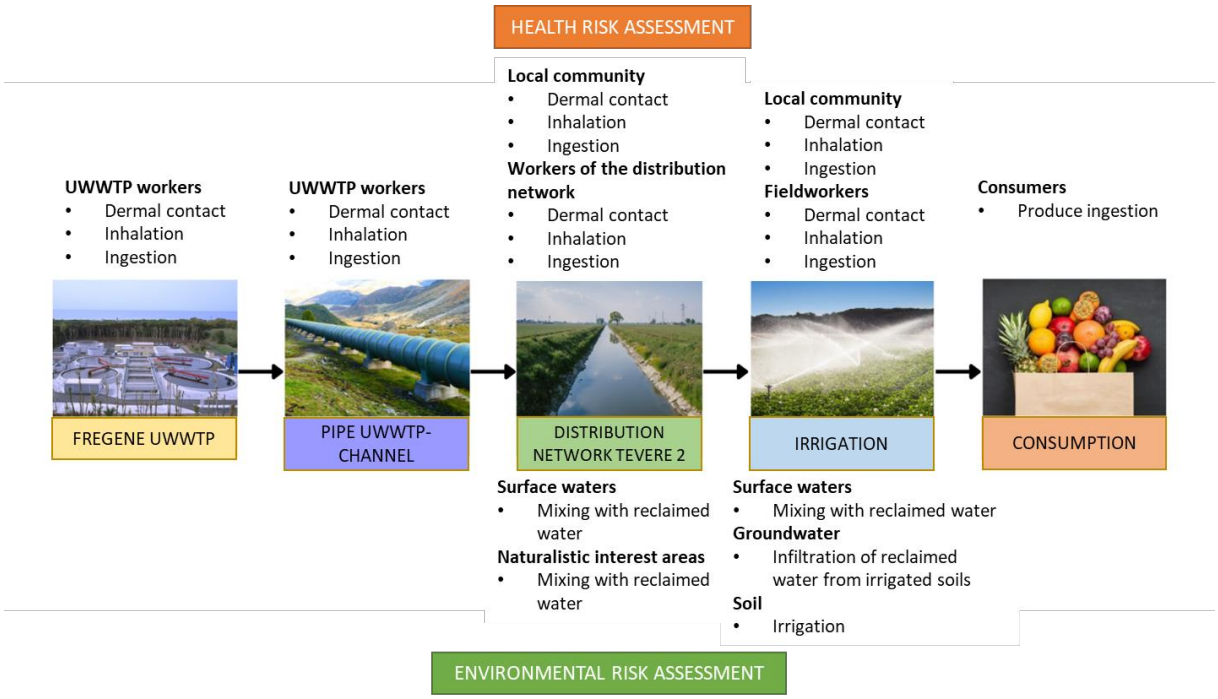


Figure 18: Identification of exposed population and routes of exposure at the *ACEA Fregene* water reuse system

For the environmental risk assessment, possible hazards were identified, such as Boron, Salinity, Chlorides, Sodium, heavy metals, Carbonates, Nutrients and suspended solids. Effluent concentrations were compared with regulatory requirements to verify the acceptability of the environmental risks. However, in case further analyses were required, a quantitative chemical risk assessment (QCRA) is proposed. This method is based on the verification of the following equation:

$$RQ = \frac{PEC}{PNEC} < 1$$

Where:

— RQ: Risk quotient

— PEC: Concentration at the endpoint exposed, defined through measured or/vs estimated data

— $PNEC$: Predicted no effect concentration

Preventive measures are made up by the sequence of treatment units and by the quality control system (see Module III) applied in the plant, with the support of real-time sensors. Moreover, at the final effluent, a by-pass system can diverge treated wastewater to discharge instead of reuse, in case of non-compliances. Monitoring programs are performed through quality control system, including operational and routine monitoring. The WWTP is provided with a network of sensors connected to remote-control system for continuous monitoring and management of the processes.

Operational monitoring includes laboratory analyses, which are periodically performed for influent and effluent characterization to verify compliances with D. Lgs 152/2006 (according to the WFD).

Table 10: Monitoring system set out at the ACEA Fregene water reuse system

Control point	Parameters	Frequency
Influent and Effluent	Internal controls of macro-contaminants: pH, Conductivity, TSS, BOD5, TN, TP, Ammonia, Nitrites and Nitrates	Instantaneous and/or averaged samples, 2/week
	External (ARPA) controls of parameters foreseen in D. Lgs 152/2006 (according to WFD).	Instantaneous and/or averaged samples, 1/week
Biologic treatment	DO, T, Nitrates, REDOX	Online sensors (continuous monitoring)
Electromechanical equipment	Status (on/off) and alarms	Online sensors (continuous monitoring)
Effluent	Flowrates, Ammonia, Phosphates, Chlorides, Conductivity, Nitrates, Turbidity and pH	Online sensors (continuous monitoring)
WWTP	TSS predictions by EWS	Continuous monitoring

The emergency management is automated. Alarms are connected to remote control, as well as maintenance programs. The work orders are managed through the SAP system and are sent via tablet to local staff, selected on the basis of proximity, professionalism or equipment required. The system manages operators' agendas and organise the most appropriate team to carry out work orders.

2.9 Water reuse risk management in San Benedetto del Tronto

A territorial analysis of wastewater reuse was carried out in the Municipality of San Benedetto del Tronto, located in Marche Region (central Italy). The urban catchment (25.4 km^2) is characterised by a relevant touristic area, with a population of 47000 inhabitants which can reach up to 800000 non-residents tourist presences over the summer period (year 2019). The seaside is certified with the Blue Flag, by Foundation for Environmental Education. The condition is typical of several municipalities of costal area of Mediterranean Sea with specific vocation of bathing tourism.

Moreover, due to the earthquakes occurred in recent years, the Region started to suffer water scarcity, making the issue of water availability. The aquifer presents conditions of over-exploitation and, as a consequence, seawater intrusion phenomenon occurs during drought periods (mainly between May and September).

In the territory, the Natural Reserve Sentina is a Natura 2000 site that covers about 177 ha and 1.7 km of coastline, including some agricultural activities and high hydro-demanding habitats.

For the natural organization of the places, Sentina is considered a homogeneous system of land, river, and lake areas, a landscape of waters and sand, covering an area of 177.55 ha. Sentina Regional Nature Reserve represents for San Benedetto del Tronto an invaluable treasure for its natural environments and life quality. It is characterized by about 1700 meters of coastline with small dune system and natural vegetation. The main part of the area is composed by natural wetlands characterized by small pools of fresh or brackish water, permanent or seasonal. This heterogeneity provides food and shelter to many species of birds, among them there is the Black winged stilt, the symbol of reserve and valuable visitor to the Sentina, but also many other species including egrets, the Kingfisher and several species of ducks and passerines. The ponds and streams are inhabited by amphibians, turtles and reptiles. Moreover, different plant species of considerable

interest are still present. This environment, also called “salt steppes”, is particularly rare and it is characterized by soil with high salt concentration due to the nearby of the sea. The natural area is also site of educational and dissemination activities such as birdwatching, walking or biking, guide visits for families, schools and tourists and educational environmental events and activities.

One part of the reserve is used for agricultural purposes for local products. The intensive agriculture area of Sentina reserve is extended for about 89 hectares, characterized by cultivation of arable land, vineyards, olives groves and permanent crops (Figure 19).

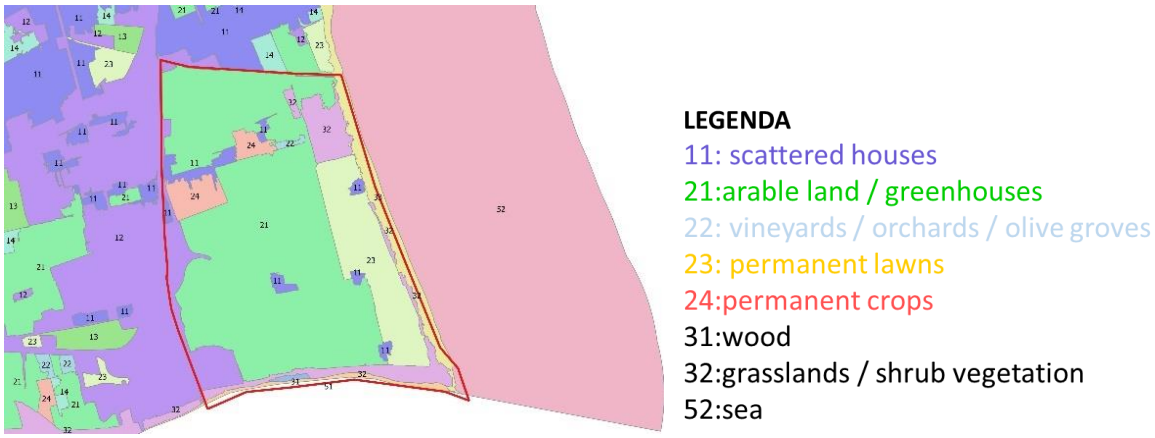


Figure 19: Intensive agriculture area of Sentina reserve and related cultivation

This is the part of landscape that has suffered most from human activities and shifted far away from the ecosystem prior to the mechanization of agriculture. These habitats are largely hydro-demanding with necessities of constant water, to date provided by conventional sources.

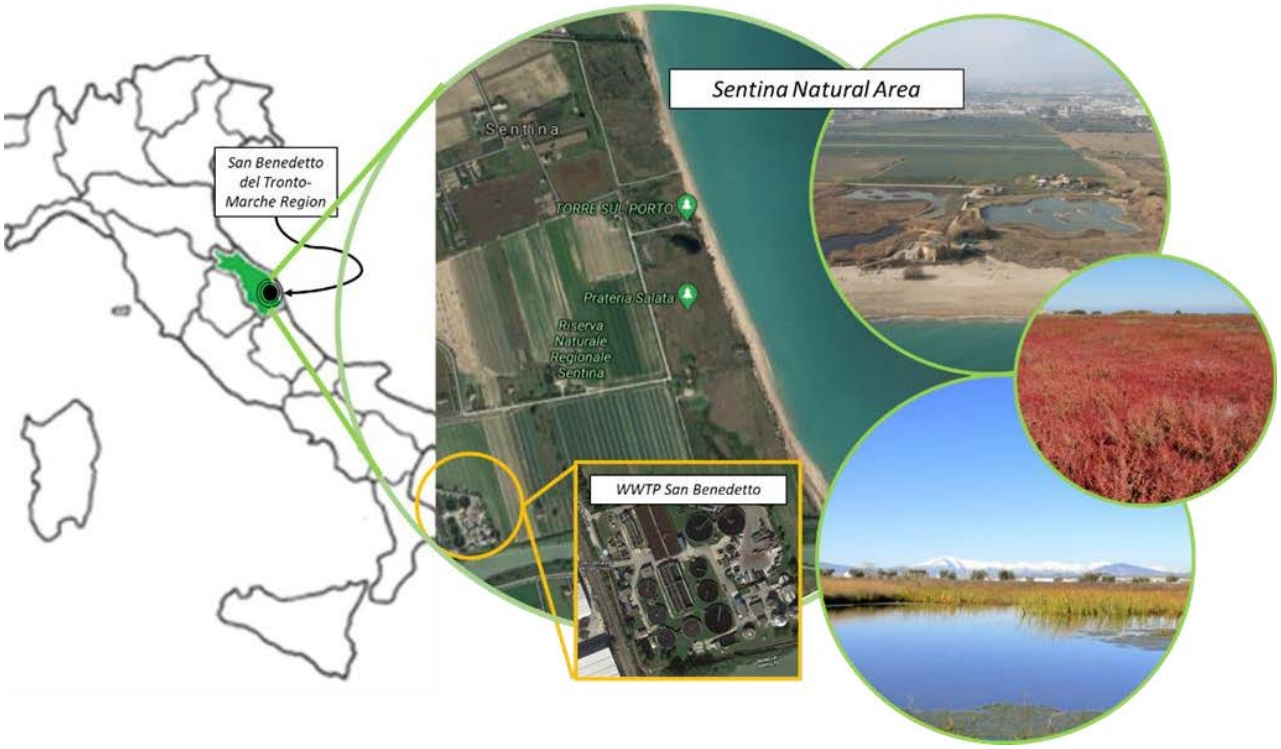


Figure 20: San Benedetto municipality: Sentina Natural Reserve and San Benedetto WWTP

The water supply for the Natural reserve supplied by the irrigation consortia is actually about 39620 m^3/month (corresponding to 277344 m^3/year) which is not sufficient to close water balance of the Natural Reserve (Figure 21). To satisfy the demand of the ecosystem about 883008 m3/year are needed.

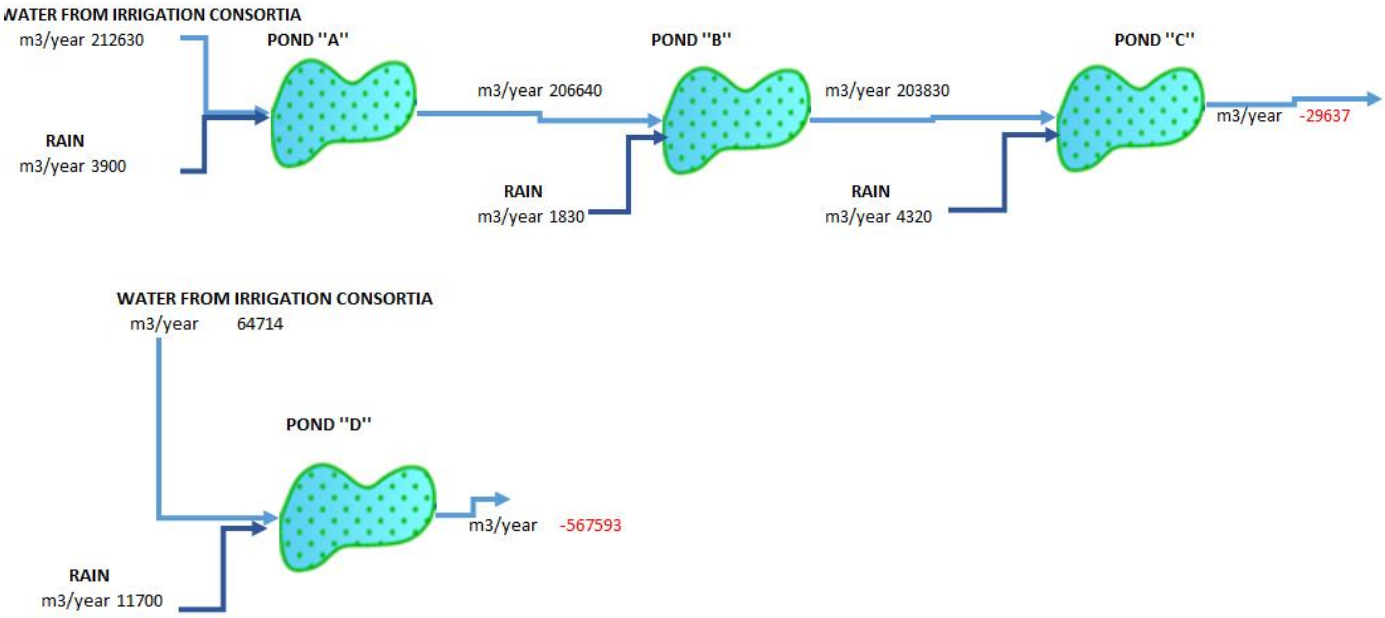


Figure 21: Water mass balance of Sentina Natural Reserve

Close to the natural area is located the Brodolini WWTP of San Benedetto del Tronto (Design capacity of 180000 PE) which could provide wastewater flows with quality suitable for agricultural and environmental objectives also according to the European Reuse Regulation (741/2020). The WWTP has an influent average flow rate of 25000 m^3/d and collects wastewater from the agglomerates of San Benedetto del Tronto and the Lower Tronto valley. It is characterized by consistent seasonal variations of mass load and flow rate mainly linked to the highly touristic interest of the area served. The water line chain is currently composed by pre-treatment, primary sedimentation, secondary biological treatments, secondary sedimentation and final chemical disinfection. Tertiary treatments are being implemented with filtration and UV disinfection for water reuse. Reclaimed water can be used for irrigation or for ecosystem reuse.

In Figure 22 the entire flow scheme of the designed reclamation treatment to allow water reuse is reported. The final effluent, with the implementation of a sand filter unit followed by a UV treatment should reach a quality standard equal to <10 mgTSS/L and <10 UFC/100ml.

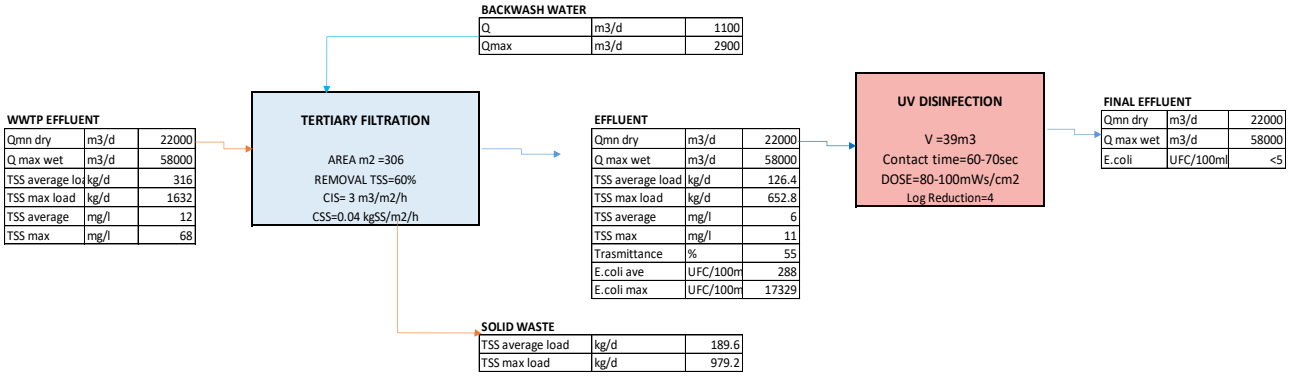


Figure 22: Tertiary treatments implementation in Brodolini WWTP

A summary of the territorial water demand (for both the natural ecosystem and for agriculture purposes) and water availability is reported in Figure 23.

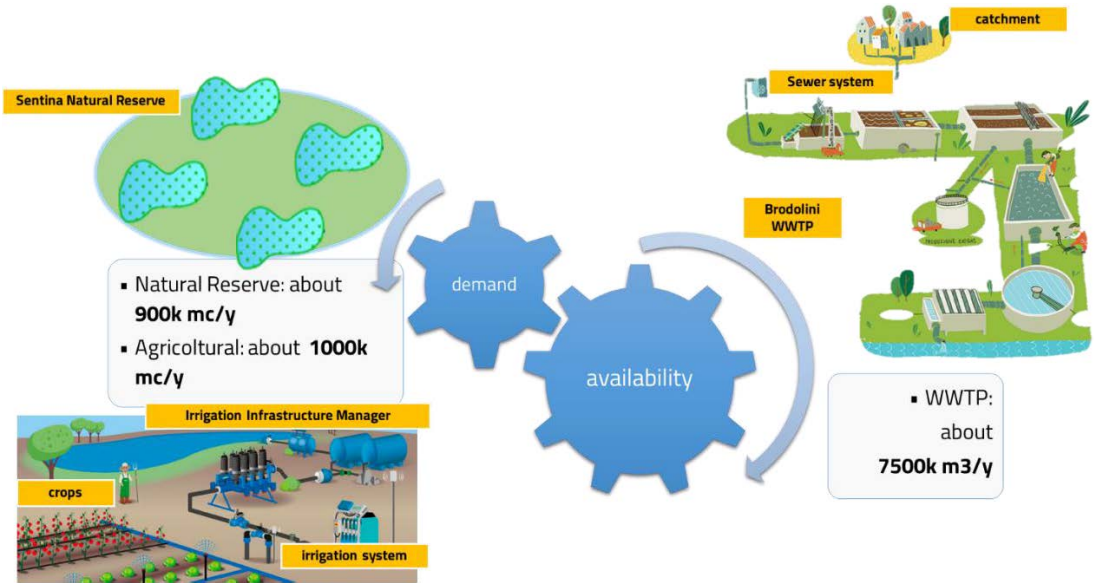


Figure 23: Territorial water balance of San Benedetto municipality: water demand vs water availability

The actual supply of water for irrigation is not sufficient for intensive agriculture (16367 m^3/month) and Brodolini WWTP could support the demand (153429 m^3/month) by contributing, during the watering season, for 137062 m^3/month corresponding to 1074000 m^3/year (Table 11).

Table 11: Water supply and water demand for the intensive agriculture area

Parameter	Units	Value
Agricultural area	m^2	895000
	hectares	89.5
Demand of crops	m^3/hectares	12000
Water Demand	m^3	1074000
Period of cultivation	-	April-October
	months	7
Water demand	m^3/month	153429
Consortia water supply	m^3/month	16367
WWTP water supply	m^3/month	137062

The Municipality of San Benedetto del Tronto is involved with other cities of Adriatic costs in the development of green infrastructures and ecosystem services. In this context, a study was

conducted to apply systemic approach to integrate agriculture and the natural area and the Urban and Peri Urban Territory of San Benedetto also including reclamation activities of non- conventional water sources (wastewater) for environmental need (water balance in coastal natural park) and for agriculture purposes. For this purpose, Brodolini WWTP could supply the necessary volume to Sentina equal to 605664 m^3/year and close the water balance of the ecosystems (Figure 24).

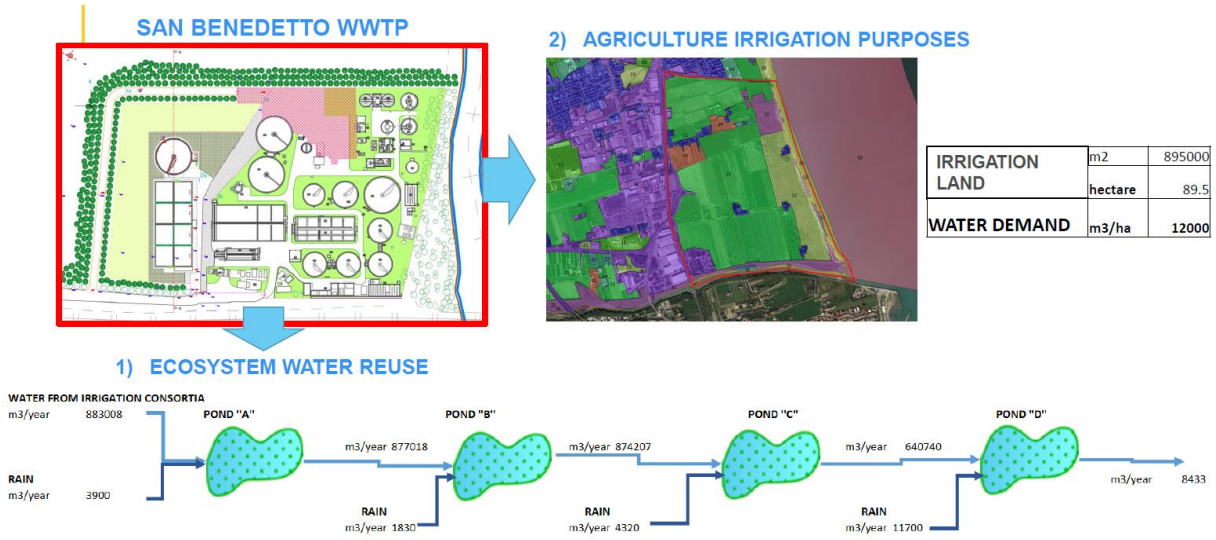


Figure 24: Water mass balance of Sentina Natural Reserve with the supply of the treated wastewater from Brodolini WWTP

Information on the hazards and the hazardous events identified, together with the related populations and environment at risk and their routes of exposures are reported in Table 12.

Table 12: Identification of hazards, route of exposure and populations and environment at risk at the San Benedetto del Tronto water reuse system.

Hazards	Parameters	Populations and environment at risk	Routes of exposure
Health Hazards	Microbial parameters (*E. coli*), according to EU 741/2020 and National Regulation on water reuse DM 185/2003	Local community and by-standers	Ingestion, aerosol and/or direct skin contact with reclaimed water
		WWTP and reclamation facility workers	Ingestion, aerosol and/or direct skin contact with reclaimed water
		Farmers	Ingestion, aerosol and/or direct skin contact with reclaimed water
Environmental Hazards	Physical-Chemical parameters, according to EU 741/2020, National Regulation on water reuse DM 185/2003, as well as Italian D.Lgs n 152/2006. Focus on salinity, Chlorides and metals (Fe, Cu, Zn, Mn, etc.)	Crops	Irrigation (crops uptakes or direct contact with reclaimed water)
		Soil	Infiltration in the soil (salinization and soil structure decline)
		Surface water	Run-off of reclaimed water
		Groundwater	Infiltration of reclaimed water
		Vulnerable and protected areas	Run off or infiltration of reclaimed water

The health risk assessment is being conducted following a semi-quantitative method according to WHO Guidelines for SSP and it will include: i) the characterization of the integrated system, from wastewater catchment to treatment, delivery and reuse in agriculture, also with the support of checklists; ii) the identification and the analysis of possible hazards and hazardous events, and iii) the quantification of risks by the attribution of scores to probability and severity of the detected hazardous events.

Additional requirements were not identified from risk assessment evidence, even if additional monitoring could be set for the environmental hazards that could pose a risk to crops, soil, surface or groundwater, or any of the protected areas (e.g., salinity, chloride, metals).

Preventive measures include treatment units and controls already set up in the WWTP. Multiparametric sensors are installed for continuous online monitoring and control system.

Monitoring programs are performed through quality control system, including operational and routine monitoring.

The WWTP is provided with a network of sensors for continuous monitoring and management of the processes.

Operational monitoring is already applied at WWTP level (KRM8), including periodic standard lab measures (according to D. Lgs 152/2006 and WFD), coupled with real-time monitoring with sensors.

For RMP, different monitoring plans are proposed, considering different levels of detail, frequencies and digital support (Table 13).

Table 13: Monitoring plans proposed for the San Benedetto del Tronto water reuse system

Level	Control point	Parameters	Frequency
Base	Effluent reclaimed water	pH, Conductivity, UV absorption for organic load, TSS/Turbidity	Continuous (sensors)
		TSS, Nitrates, Ammonia, Phosphates, BOD_5, COD, *E. coli*, Salmonella, *Legionella, Intestinal Nematodes*, other parameters from DM 185/2003	From weekly to monthly (lab analyses)
Moderate	Effluent reclaimed water	pH, Conductivity, UV absorbance, TSS/Turbidity, Nitrates, Ammonia, Phosphates	Continuous (sensors)
		TSS, Nitrates, Ammonia, Phosphates, BOD_5, COD, *E. coli* (weekly), *Salmonella, Legionella, Intestinal Nematodes*, other parameters from DM 185/2003	From weekly to monthly (lab analyses)
Advanced	Effluent reclaimed water	pH, Conductivity, UV absorbance, TSS/Turbidity, Nitrates, Ammonia, Phosphates	Continuous (sensors)
		E. coli	Daily
		TSS, Nitrates, Ammonia, Phosphates, BOD_5, COD, *Salmonella, Legionella, Intestinal Nematodes*, other parameters from DM 185/2003	From weekly to monthly (lab analyses)
		CECs e.g., pharmaceuticals, microplastics, pesticides, disinfection by-products, antibiotic resistance	Semestral-annual (lab analyses)
	Distribution	*E. coli*, *Salmonella, Legionella, Intestinal Nematodes* (optional Coliforms and Faecal streptococci)	From weekly to monthly (lab analyses)
	Soil	pH, organic matter, C, N, assimilable P-Na-K, Chlorides, *Coliforms, Streptococci*, heavy metals	Beginning and end of irrigation season
Additional Digital support	WWTP	Alert System sensor for *E. coli* and *Enterococchi* measurements (see Peschiera Borromeo case-study)	Continuous (sensors)
		Early Warning System (see Peschiera Borromeo case-study)	
		TOC sensor	
		Sensors network implementation at the influent	

Coordination and communication are established among the involved parties: Water Utility, Irrigation infrastructure, Natural Park.

2.10 Water reuse risk management in Peschiera Borromeo WWTP in the context of DWC project

The European Horizon 2020 project Digital Water City (DWC) aims to boost the integrated management of waters systems in five major European urban and peri-urban areas representing 30 million inhabitants by leveraging the potential of data and smart digital technologies. The assessment and communication of the benefits provided by digitization aims at raising the awareness of European cities for a necessary digital transformation and at fostering the large-scale market uptake of digital solutions in Europe. The project promotes informed decision support for water management and stakeholder engagement to reduce risks for human health and the environment, with targeted activities in two defined contexts, which consist in bathing sites and water reuse applications. One of the project objectives is to improve the support of digital tools as

control measures to minimise risks and facilitate water reuse, also with a focus on water-energy-carbon-food nexus. Particularly, DWC intends to use the value of a large set of data obtained by employing on-line sensors for monitoring water quality in three selected case studies, which are located in Paris, Berlin and Milan. The case studies of Paris and Berlin present the use of a smart network of real-time sensors for monitoring the impact of sanitation systems on the receiving bathing waters. On the other hand, in the case study of Milan, a smart network of real-time sensors is employed to build up an early warning system (EWS) to inform stakeholders about wastewater quality and related human-health risks, and to prevent microbial and toxic contamination linked to water reuse. Bathing water and wastewater reuse for agricultural practices raise many concerns about the safety of the environment and human health. In these two contexts, a monitoring network represents a strategical control tool to ensure that quality standards for bathing water and treated wastewater are reached, and to minimize risks for human health. Among the project activities, a sensor for monitoring microbiological contamination has been tested in different water matrixes, respectively in surface water and wastewater, to evaluate its usability in support risk management for bathing sites and water reuse.

2.10.1 System description

The *Peschiera Borromeo* water reuse system was developed under the EU H2020 project Digital-Water City. The WWTP of Peschiera Borromeo is managed by CAP Holding, which aims at optimizing the technical, economic and environmental performance of the wastewater treatment-and-reuse physical system (as a whole) by delivering the first Italian sanitation safety plan and water reuse risk management plan. Digital solutions are implemented to minimize risks, optimize performances and overcome social barriers.

The socio-economic context of the surrounding area is typical of the the territory of the Po valley, where agricultural irrigation systems are highly dependent on freshwater diverted mainly from rivers. Particularly, in the plain of the Lombardy region irrigation has been developed since the Middle Age, as witnessed by the extensive network of historical channels, which convey water for irrigating an area of about 550 000 ha. The irrigation network of canals and the related water delivery systems are supervised by the consortium Est Ticino Villoresi, one of Italy's most important irrigation consortia. The irrigated area represents about 85% of the total utilised agricultural area, demonstrating the relevance of irrigation to sustain the agricultural sector of Lombardy, one of the most important in Europe, both in terms of quantity and quality of agricultural and food production. Given its geographical context, agriculture plays a key role in the economy of the region. In this area the most employed agriculture activities are fodder crops, such as maize. The most common irrigation techniques rely on border irrigation, implying a water demand of about 3000-4500 m3/(ha*month) during the irrigation season. Crop quality impacts both the quantity and quality of the required irrigation water because different types of crops need different amounts of water and are sensitive to different water characteristics. Furthermore, the amount of water for irrigation and the frequencies of the irrigation events vary according to the period of the year. Crop quality also implies numerous risk considerations, which depend on harvesting practices, processes accomplished to obtain the final product, and the final use of the product itself (e.g., consumed cooked or raw, consumed by humans or animals).

Irrigation water availability has been traditionally adequate, but competition for water resources access in the area has been increasing in the last years and it is expected to further escalate, mostly

due to a decreasing trend in summer water availability and to a reinforcement of the ecological flow requirements. Therefore, the identification of additional water resources, along with the improvement in irrigation efficiency, represent key issues for the next years. Alternative sources of water, such as reclaimed wastewater, are drawing attention as innovative solutions.

Peschiera Borromeo WWTP is located in the peri-urban area of Milan, in Via Roma - Cascina Brusada, and it is managed by the water utility CAP Holding. The WWTP has a treatment capacity of about 566000 PE, and treats daily an average flow rate of 216000 m^3/d. The plant has two separated treatments lines (i.e., Line 1 and Line 2), which treat the wastewater coming from the two sewer network sectors of the peri-urban region of Milan. Sewages coming from the area managed by Metropolitana Milanese are treated in Line 1, whereas the wastewater coming from the sewer sector controlled by CAP is treated in Line 2. Line 1 includes coarse screening, pumping station, fine screening, grit and oil removal, primary sedimentation, biological treatment for organic carbon removal, tertiary filtration combined with nutrient removal in BIOFOR reactor and chemical disinfection with peracetic acid. Line 2 includes coarse screening, pumping station, fine screening, a compact SEDIPAC unit for grit and oil removal coupled with primary sedimentation, a BIOFOR unit for organic and nutrient loads removal combined with tertiary filtration and a final disinfection treatment with UV.

At Peschiera Borromeo WWTP, a network of sensors and probes (Figure 25) is installed at the plant. It provides on-line and real-time data to monitor the wastewater quality in the influent, during the treatment process, and in the final effluent.

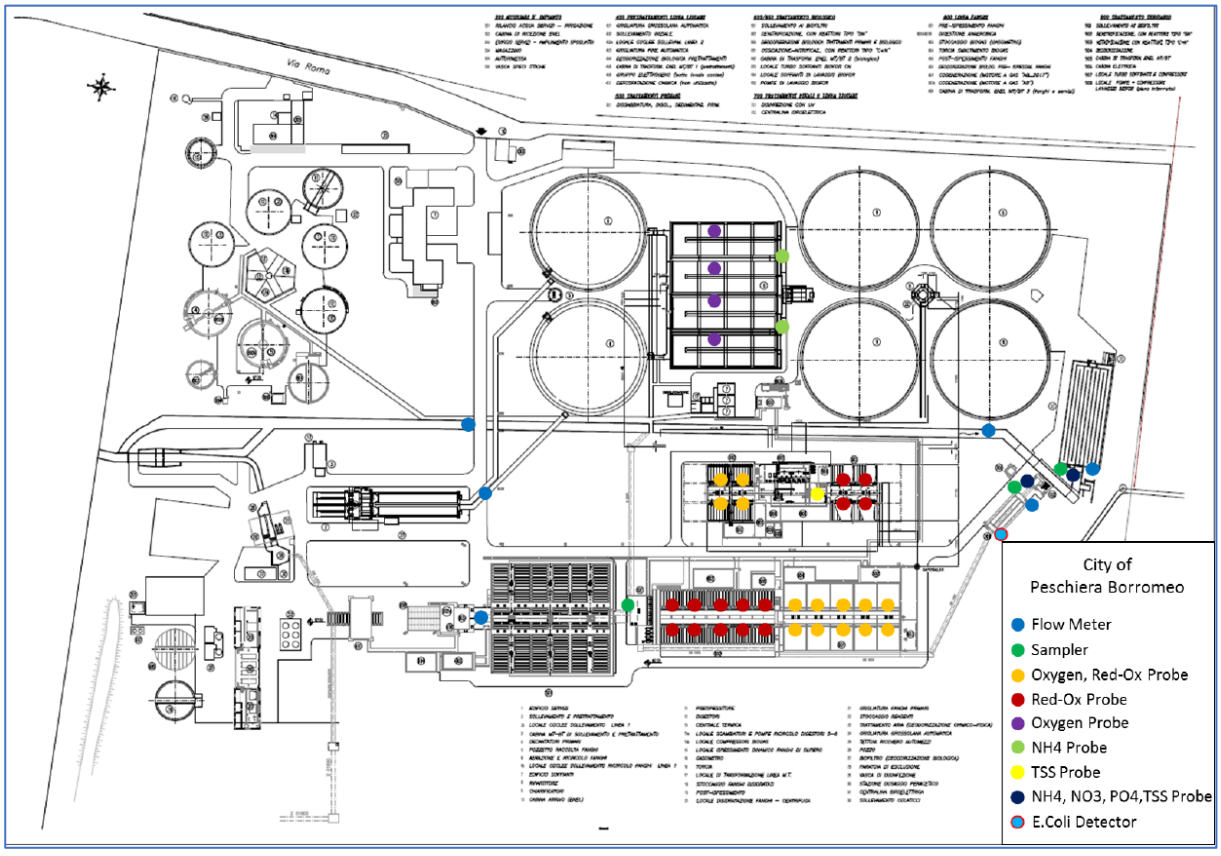


Figure 25: Layout of the treatment trains of Peschiera Borromeo WWTP

The effluent water quality met class C minimum requirements for irrigation of fodder crops and crops for biomass production (mainly maize and mustard) through drip irrigation techniques.

However, as precautionary measure to further reduce health and environmental risks, it was decided to set the compliance with quality standard limits for class B. Two agricultural fields adjacent to the WWTP were selected to be irrigated with reclaimed water. Both fields are cultivated with the same agronomic practices, but one field is irrigated using the most common irrigation method (i.e., border irrigation), while in the second one an attempt to implement a "smart" irrigation practice was performed (Figure 26). The fields are cropped with maize during the summer season, while during autumn and winter mustard is cultivated as a cover crop. In 2021 maize was sown at the beginning of April and harvested at the end of September. In one of the fields a new drip irrigation system was installed for the purpose of the DWC experimentation. On the other hand, the second field has been irrigated using the traditional border irrigation and freshwater source from the canal.

The drip-irrigated field is divided into four different sectors where drip irrigation can be activated autonomously through four remotely controlled electro-valves. Each sector was irrigated for approximately 12 hours every two days during the agricultural season. At the beginning of the season, laterals, connected to the manifold, were installed in the crop inter-row with a spacing of 1.4 m and were partially buried; the emitters' distance and discharge are 30 cm and 1.14 l/h, respectively.



Figure 26: Overview of demo site (left), drip irrigation system installation (centre), electro-valve regulating irrigation in each sector (right)

To obtain information about soil water status, two multilevel humidity probes located at two different points along the drippers line (yellow triangles in Figure 26) and a piezometric well (blue dot in Figure 26) with a sensor to monitor the ground table depth were installed. Sensors were installed to monitor the crop water demand and schedule irrigation accordingly. The same sensor configuration was used in the border irrigated field. In addition, a weather station was installed near the demo site to measure the local weather agrometeorological variables that are required to estimate the crop evapotranspiration; for security reasons, the station was installed inside the WWTP, about 500 m from the field (red star in Figure 26).



Figure 27: Sensors and devices installed: piezometer, water content probe + GSM modem (left); agro-meteorological weather station (right)

2.10.2 Risk assessment – semi-quantitative approach

As a preliminary phase to identify the possible hazards and hazardous events, an analysis of the effluent characteristics and treatments performances was carried out. Lab data were collected between 2018 and 2021, before and after the revamping phase of the plant. Actually, an improvement of the quality of the final effluent was observed after the conclusion of the upgrading of the plant.

To assess the continuous compliance with the EU Regulation of the treated wastewater, an evaluation of the cumulative frequencies of quality requirements achieved in each water quality class for *E. coli*, BOD_5, and TSS, was conducted. In particular, in the case of the quality class B, it resulted that the quality requirement for *E. coli* was achieved for almost 70% of the collected samples. Moreover, after the revamping of the plant, *E. coli* concentrations in the final effluent were often lower than 10 CFU/100ml (59% of the measurements in 2020-2021), and thus in compliance with the limit for the quality class A of EU regulation on water reuse. In general, for the samples collected from 2020 to 2021 after the revamping of the plant, compliance for E. Coli concentration in class B was observed for 87% of samples. As concern the quality requirements for BOD_5 and TSS in class B, they were achieved for almost 100% of the samples.

Since the WWTP resilience is a strategical characteristic of the plant and has to be taken into account during the formulation of the risk matrix, the concentrations over the time of COD, total nitrogen, ammonium nitrogen, total phosphorus, BOD_5 and total suspended solids measured in the raw influent and in the final effluent were compared, to see how much the characteristics of the treated wastewaters were affected by the incoming fluctuations. It can be observed that influent fluctuations (i.e., seasonal variations, occurrence of rain events, etc.) were well faced by treatments, indicating a good resilience of the plant.

Concerning the UV disinfection unit, a specific literature review was conducted to find information about expected pathogens concentrations in the wastewater before and after the treatment along with the observed removal efficiencies (

Table 15). It can be observed that in most of the literature studies, Log removals for pathogens during UV treatment are in the range 2-4.

Table 14: Concentration of microbial contaminant in wastewater

	RAW WASTEWATER	CONCENTRATION		REFERENCE
bacteria	*Total coliform*	CFU/100ml	3.9E+07	(Kay et al., 2008)
		CFU/100ml	1.96e+07 - 4.36E+07	(WERF, 2004)
		CFU/100ml	5.15e+08	(Thwaites et al., 2018)
		MPN/100ml	10^7 - 10^9	(Metcalf & Eddy, 2014)
		MPN/100ml	1.1E+08	(Howard et al., 2004)
	Faecal coliforms	MPN/100ml	10^6 - 10^8	(Metcalf & Eddy, 2014)
		MPN/100ml	8.2E+06	(Howard et al., 2004)
		MPN/100ml	2.1E+06 - 5.3E+06	(Oakley & Mihelcic, 2019)
		CFU/100ml	1.7E+07	(Kay et al., 2008)
		CFU/100ml	2.09e+06 - 5.31e+06	(WERF, 2004)
	E.coli	MPN/100ml	10^5 - 10^7	(Metcalf & Eddy, 2014)
		MPN/100ml	5.37E+03 - 3.47E+07	(Oakley & Mihelcic, 2019)
		logMPN/100 ml	6.42±0.28 (5.99 - 7.28)	(Bailey et al., 2018)
		CFU/100ml	3.6e+06	(Marín et al., 2015)
		CFU/100ml	5.31e+07	(Thwaites et al., 2018)
		N/l	10^5 - 10^10	(NRMMC-EPHC-AHMC, 2006)
	Salmonella	MPN/100ml	10^2 - 10^4	(Metcalf & Eddy, 2014)
		MPN/100ml	266.7	(Howard et al., 2004)
		MPN/L	3-1100	(Lemarchand & Lebaron, 2003)
		logMPN/100 ml	4.08±0.99 (2.88 - 5.88)	(Bailey et al., 2018)
		n/l	10^3 - 10^5	(NRMMC-EPHC-AHMC, 2006)
		n/l	1-10^5	(WHO, 2006)
		N/l	up to 10^5	(US EPA, 2012)
protozoa parasites	*Cryptosporidium*	MPN/100ml	10 - 10^3	(Metcalf & Eddy, 2014)
		n/l	1-10^4	(WHO, 2006)
		n/l	up to 10^4	(US EPA, 2012)
		oocysts/L	0 - 10^4	(NRMMC-EPHC-AHMC, 2006)
		oocysts/L	2.5 - 277	(Hamilton et al., 2018)
		oocysts/L	4.5±0.8	(Carraro et al., 2000)
		oocysts/L	1-87.13	(Lemarchand & Lebaron, 2003)
		oocysts/L	96±105	(Ramo et al., 2017)
		oocysts/L	6–350	Montemayor et al., 2005; Castro-Hermida et al., 2008, 2010; Galván et al., 2014
		oocysts/L	22 - 456	(Oakley & Mihelcic, 2019)
		oocysts/L	6.5 - 37.8	(Oakley & Mihelcic, 2019)
		oocysts/L	6.55 - 37.8	(WERF, 2004)
		oocysts/L	0.3 - 5e+04	(Soller et al., 2017)
		oocysts/L	30±5	(Briancesco & Bonadonna, 2005b)
		oocysts/L	371±24 (64.6 - 955)	(Bailey et al., 2018)
	Giardia	MPN/100ml	10^3 - 10^4	(Metcalf & Eddy, 2014)
		N/L	10^2 - 10^5	(WHO, 2006)
		N/L	up to 10^5	(US EPA, 2012)
		cysts/L	10^2 - 10^5	(NRMMC-EPHC-AHMC, 2006)
		cysts/L	764 - 6606	(Oakley & Mihelcic, 2019)
		cysts/L	3247±2039	(Ramo et al., 2017)
		cysts/L	61.2 - 794	(Oakley & Mihelcic, 2019)

		cysts/L	61.2 - 794	(WERF, 2004)
		cysts/L	89-8305	Montemayor et al., 2005; Castro-Hermida et al., 2008, 2010; Galván et al., 2014
		cysts/L	3.2 - 1.0E+04	(Soller et al., 2017)
		cysts/L	7000 ± 2000	Briancesco et al. 2004
		cysts/L	302 ± 24 (45 - 1122)	(Bailey et al., 2018)
viruses	*Enterovirus*	MPN/100ml	10^3 - 10^4	(Metcalf & Eddy, 2014)
		MPN/100ml	4.64E+02 - 9.37E+03	(WERF, 2004)
		MPN/L	4.6 - 93.7	(Oakley & Mihelcic, 2019)
		N/L	10^5 - 10^6	(WHO, 2006)
		N/L	up to 10^6	(US EPA, 2012)
		PFU/L	10^2 - 10^6	(NRMMC-EPHC-AHMC, 2006)
	Norovirus	PFU/L	10 - 10^4	(NRMMC-EPHC-AHMC, 2006)
		copies/L	10^3.76 ±10^0.93	(Soller et al., 2017)
	Norovirus GI	copies/L	1.12 E+03 - 5.75E+05	(Oakley & Mihelcic, 2019)
	Norovirus GII	copies/L	6.46e+02 - 2.19e+06	(Oakley & Mihelcic, 2019)

Table 15: Pathogens removals observed during UV treatment

	MICRORGANISM		**PRE UV**	**POST UV**	**LOG REMOVAL**	**REF.**
bacteria	*Bacteria*				2 - > 4	(WHO, 2006)
	E. coli				2 - > 4	(NRMMC-EPHC-AHMC, 2006)
					1.8 - 4.7	(DEMOWARE project, 2013)
		CFU/ 100ml	860 - 24000	1 - 2	2.63 - 4.38	(Francy et al., 2012)
		CFU/ml	(31±6)-(82±16)	0 - 14±2		(Anastasi et al., 2013)
	Salmonella	MPN/ 100ml	45			(Howard et al., 2004)
					5.6	(Hijnen et al., 2006)
		CFU/L		6		(Soller et al., 2017)
protozoa parasites	*Cryptosporidium*				> 3	(WHO, 2006)
					> 3	(NRMMC-EPHC-AHMC, 2006)
					3	(Chahal et al., 2016)
		N/l	6 - 23	2 - 8	0.45 - 0.48	(Liberti et al., 2003)
		oocysts/L	-	3.6		(Deng et al., 2019)
					2 - 3.5	(Soller et al., 2017)
virus	*Virus*				1 - > 3	(WHO, 2006)
					2.9 - 4.2	(DEMOWARE project, 2013)
					4	(Chahal et al., 2016)
	Enterovirus				> 3	(NRMMC-EPHC-AHMC, 2006)
		Log copies/L	(3.71±0.43)-(4.13±0.31)	(3.47±0.49) -		(Qiu et al., 2018)

				(3.82±0.32)		
		copies/l	6.8 - 250	36 - 67	-	(Francy et al., 2012)
	Norovirus				0.5 - 1.5	(Soller et al., 2017)
		RT-qPCR (GC/mL)	-	3.30-3.80		(Deng et al., 2019)
	Norovirus GI	Log copies/L	0.84 - 2.4	1.56 - 1.83	-	(Francy et al., 2012)
		Log copies/L	(4.19±0.49)-(4.35±0.53)	(4.02±0.52) - (4.18±0.6)		(Qiu et al., 2018)
	Norovirus GII	Log copies/L	(4.96±0.36)-(5.23±0.29)	(4.86±0.36) - (5.12±0.31)		(Qiu et al., 2018)

After the description of the system, a process flow diagram was drafted and then, for each plant section, a likelihood and a severity score were assigned for specific hazard events. These hazard events were identified by a preliminary checklist analysis (Figure 28) and by several interviews to technical operators and stakeholders. Scores were preliminary assigned to identify the most critical aspects for each unit (column in the right of Figure 28).

			NUMBER OF PARALLEL PROCESS LINES			
PRETREATMENTS	☐	Treatm. NOT present	coarse grilling	☒	several redundant parallel units in the same plant section (specify number)	-1
				☐	bypass present	-1
				☒	automatic cleaning of the grill	-1
				☒	present single with automatic cleaning (specify cleaning method)	-1
				☐	in remote control	-1
	☐	non pres.	initial lift	☒	several redundant parallel units in the same plant section (specify number)	-2
				☐	lifting with electric pumps	-1
				☒	in remote control	-1
	☐	Treatm. NOT present	fine grilling	☒	several redundant parallel units in the same plant section (specify number)	-1
				☐	bypass present	-1
				☒	automatic cleaning of the grill	-1
				☒	present single with automatic cleaning (specify cleaning method)	-1
				☒	in remote control	-1
	☐	Treatm. NOT present	sand separation	☒	sand extraction with pumps	3
				☐	sand extraction with ejector	2
				☒	rectangular tank	
				☐	circular tank	
				☒	più unità in parallelo ridondanti nella stessa sezione di impianto (specificare il numero)	-1
				☒	in remote control	-1
	☐	Treatm. NOT present	oil separation	☐	static	3
				☒	with air blowing	2
				☒	rectangular tank	
				☐	circular tank	
				☒	several redundant parallel units in the same plant section (specify number)	-1
				☒	in remote control	-1
	☐	Treatm. NOT present	primary sedimentation	☒	rectangular tank	
				☐	circular tank	-1
				☐	measurement of the level of sedimented mud	-1
				☒	in remote control	-1
				☒	several redundant parallel units in the same plant section (specify number)	-1

Figure 28: Example of a portion of a typical check list used to acquire information about the main characteristics of each treatment unit, connected equipment, its redundancy level and the related maintenance and monitoring system

The core-phase of risk assessment was conducted, according to WHO guidelines, using first a semi-quantitative approach.

A risk matrix was developed according to the following steps:

- Identification of possible hazards and hazardous events at each step of the system, with the support of preliminary checklists and several interviews to technical operators and stakeholders of the team.
- Quantification of risks by the attribution of scores to probability and severity of the detected hazardous events, based on team expertise and knowledge of the water reuse system.

The risk assessment was carried out in three stages: a first "preliminary risk assessment", a second "residual risk assessment" and a third eventual "re-assessment of the risk".

- In the first stage, a likelihood and a severity score were assigned to each hazardous event to obtain a preliminary risk assessment, without taking into account any control measures already present in the system. The results of this analysis represented the worst-case scenario, and they highlighted the risks to which the reclamation water production chain is potentially exposed.
- In the second stage, a "residual risk assessment" was carried out by identifying all the control measures already in place in the system and by evaluating if these measures are effective at controlling relevant risks.
- In the last third stage, namely the "re-assessment of the risk", in order to reduce residual risk scores of each possible hazardous event, supplementary control and mitigation measures were identified. Particularly, the identified mitigation and control measure are able to act on the occurrence of different hazardous events ensuring at the same time the mitigation of various risks.

The risk score R was calculated through the formula:

$$Risk\ (R) = Likelihood\ (L) * Severity\ (S)$$

The scores used to quantify likelihood and severity were based on the scores proposed by the WHO SSP Manual (2015), while specific descriptions were defined for Peschiera Borromeo WWTP, as reported in Table 16.

Table 16: Risk definitions for semi-quantitative risk assessment of Peschiera Borromeo Water Reuse System

Descriptor		Description
Likelihood (L)		
1	Very Unlikely	Has not happened in the past and it is highly improbable it will happen in the next months (or another reasonable period).
2	Unlikely	Has not happened in the past but may occur in exceptional circumstances in the next 12 months (or another reasonable period).
3	Possible	May have happened in the past and/or may occur under regular circumstances in the next 12 months (or another reasonable period).
4	Likely	Has been observed in the past and/or is likely to occur in the next 12 months (or another reasonable period).
5	Almost Certain	Has often been observed in the past and/or will almost certainly occur in most circumstances in the next 12 months (or another reasonable period).
Severity (S)		
1	Insignificant	Hazard or hazardous event resulting in no or negligible health effects compared to background levels.

2	Minor	Hazard or hazardous event potentially resulting in minor health effects and/or may lead to legal complaints and concern; and/or minimal regulatory non-compliance (downgrading of the quality of the refined water of 1 class, distributed for about 1% of the time).
4	Moderate	Hazard or hazardous event potentially resulting in a self-limiting health effects or minor illness and/or may lead to legal complaints and concern; and/or minor regulatory non-compliance (downgrading of the quality of the refined water of 1 class, distributed for about 10% of the time).
8	Major	Hazard or hazardous event potentially resulting in illness or injury and/or may lead to legal complaints and concern; and/or major regulatory non-compliance (downgrading of the quality of the refined water of 2 classes).
16	Catastrophic	Hazard or hazardous event potentially resulting in serious illness or injury, or even loss of life and/or will lead to major investigation by regulator with prosecution likely.

As a result, a semi-quantitative risk assessment for each identified hazardous event (i.e., microbiological, chemical, physical, radiological and reuse service interruption) was obtained and organized in matrix form.

It should be noted that all the mitigation measures reported in the risk matrix aim at preventing contamination events or service interruptions. Therefore, these measures act by modifying and substantially reducing the probability of occurrence of the hazardous events, with a consequent decrease of the correlated risks.

One of the most important elements of risk in the treatments train of Line 2 is the *"Malfunctioning of UV disinfection system"*. The related rows of the Risk Matrix are reported in Figure 29 and Figure 30.

According to the studies conducted in the preliminary phase of system description, it was observed that the cumulative frequency of quality requirements for *E. coli* in class B was achieved for 87% of the measurements in the period 2020 - 2021. To increase this percentage and reduce the risk to have high concentration of pathogens in the final effluent, further integrative control measures were reported in the Risk Matrix (Figure 29).

LINE 2	RISK MATRIX PESCHIERA-BORROMEO WWTP FOR AGRICULTURAL IRRIGATION WATER REUSE (Class B reclaimed water quality)											
		HAZARDS	PRELIMINARY RISK ASSESSMENT									
NODE P=Process T=Transport	WWTP SECTION	HAZARDOUS EVENT (Potential or found events able to introduce one or more specific hazards)	OBSERVATIONS (Based on bibliographic notes, operative personnel, monitoring data, inspections, users complaints)	MICROBIOLOGICAL	CHEMICAL	FISICAL	RADIOLOGICAL	REUSE SERVICE INTERRUPTION	LIKELIHOOD	SEVERITY	SCORE	RISK RATING
P9 -UV DISINFECTION	**UV DISINFECTION** 2 lines of 6 tanks (3 tanks each line), divided in 114 modules, with a total of 912 lamps with a mono light lenght of 254 nm	Malfunctioning of UV disinfection treatment	• Informations about expected pathogens concentrations in the wastewater before and after the treatment along with the observed removal efficiencies are reported in 3.2.6.1.5 paragraph	x				x	3	16	48	**Very High** serious illness or injury, or even loss of life and/or will lead to major investigation by regulator with prosecution likely

Figure 29: Example of the preliminary risk assessment for an element (row) of the Risk Matrix

RESIDUAL RISK						
EXISTING CONTROL MEASURES	BIBLIOGRAPHIC NOTES - CONSIDERATIONS RELATED TO THE STATISTICAL TREATMENT OF DATA (from D 1.1 e D1.3)	LIKELIHOOD	SEVERITY	SCORE	RISK RATING	INTREGRATIVE CONTROL MEASURES
• P6, P7, P8 Treatments • The number of modules is overdimensioned • Planned maintenance program from the tech-provider • Weekly instant sampling and 24h weekly authomatic sampling for laboratory analysis (frequency 3-6 times/ month) of parameters required for the minimum quality of reclaimed water class B • Daily visual check of the electromechanical state • Check device state and alarms (request for intervention) by remote control • Equipment maintenance and personal training procedures • Non-compliance procedure • Emergency procedure	• In compliance with the EU Regulation, it resulted that the cumulative frequencies of quality requirements for E. coli, regarding the quality class B of our interest, was achieved for 69.5% of the time from 2017 to 2021 and for 87.2% of the time from 2020 to 2021	3	4	12	**Medium** May lead to legal complaints and concern; and/or minor regulatory non-compliance (downgrading of refined water quality by 1 class for about 10% of the time).	• Presence of UV dose working sensor, based on the Q effluent flow • Daily and random monitoring of lamps intensity • Evaluation of the fouling kinetics of lamps for their cleaning • Replacement of wavelenght fixed lamps at 254 nm with multi wavelenght lamps • Integrable Memo Maintenance Program in TLC • In the medium to long term, building of water reservoirs to avoid reuse service interruption • DS 1: Sensors for real-time in-situ E.coli and enterococci measurements at the effluent • DS 1.3 Early warning system for safe water reuse

Figure 30: Example of the residual Risk assessment for an element (row) of the Risk Matrix

The output of the semi-quantitative risk assessment, and particularly of the residual risk assessment stage that takes into account the already existing control measures, was a priority-sorted risks list. This list can be very useful to highlight “high” or “very high" risks that need to be quickly faced by the introduction of new specific control measures.

The numerousness of the hazardous events identified at Peschiera-Borromeo reuse system that belong to different classes of risk (i.e., low risk, medium risk, high risk and very high risk) were considered for risk prioritisation, according to the results of the residual risk assessment. It was observed that most of resultant residual risks were ranked at *Medium Risk* level, no residual risks were ranked as *Very High Risk*, whereas few hazardous events are related to *Low Risk* level (< 6 of risk score R) or *High Risk* level. Hence, to further reduce risks related to the production of water quality of class “B” for agricultural irrigation some integrative control measures could be introduced. According to the results of the semi-quantitative analysis, risks were prioritised to select the most

appropriate integrative control measures to introduce, such as the Early Warning System for safe water reuse.

2.10.3 Risk assessment – quantitative approach

From the semi-quantitative methodology, which resulted in the Risk Matrix of Peschiera Borromeo wastewater treatment plant (WWTP), a quantitative approach was then followed to analyse microbial risk. A Quantitative Microbial Risk Assessment (QMRA) was carried out to quantitatively evaluate microbial risks and compare them with health targets provided by World Health Organization guidelines (WHO, 2016b). This approach, schematised in Figure 31, included the definition of reference pathogens (e.g., Campylobacter, Rotavirus and Cryptosporidium) concentrations and the related removal efficiency along the treatment line. As a microbial contamination indicator, measured *E. coli* concentrations, as well as literature assumptions on indicator/pathogens ratio, were used to evaluate reference pathogens concentrations and removals. Non-technical measures, such as irrigation techniques and harvesting procedures were also considered as further barriers. Probability of infection and illness were calculated considering different exposure scenarios for all the population at risk. Risk was then expressed as Disability-adjusted life years (DALYs) and compared with the health target of 10^{-6} DALYs proposed by WHO guidelines (2016).

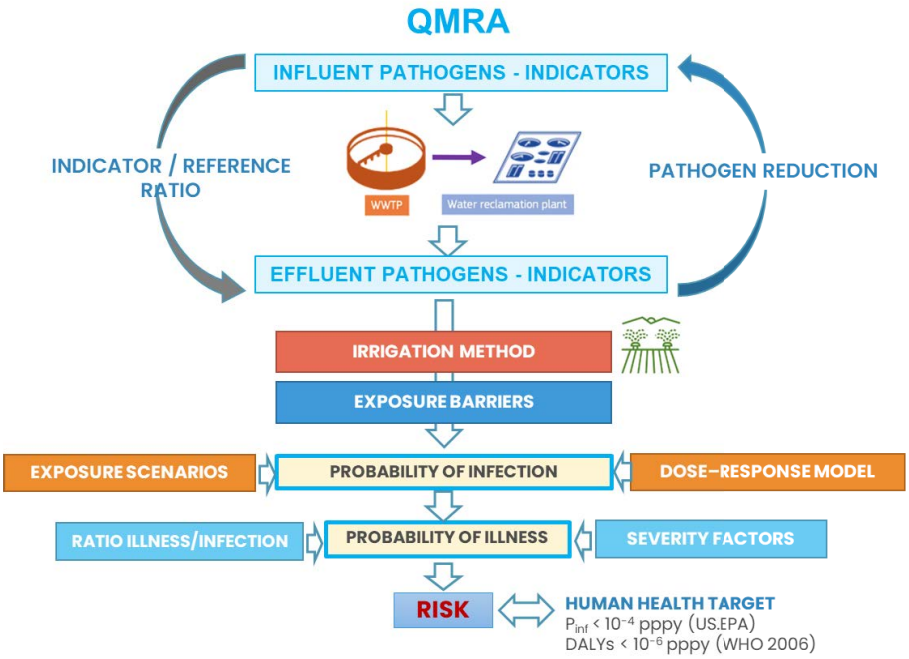


Figure 31: Proposed methodology for QMRA

Moreover, in order to identify the physical and chemical hazards to evaluate the feasibility of the implementation of a Quantitative Chemical Risk Assessment (QCRA), FMEA (Failure Modes-Effects Analysis) and PCA (Principal Components Analysis) models were applied to the following parameters monitored with lab analyses: Conductivity, BOD_5, COD, Total Suspended Solids, Total Nitrogen, Ammonium, Nitrate, Total Phosphorous, Phosphates, Chlorides, Sulphates, Sulphites, Sulphides, Cyanides, heavy metals (e.g., Cadmium, Chromium, Iron, Mercury, Nickel, Lead, Copper, Zinc) and organic surfactants. As confirmed by PCA analysis, results of the FMEA model, using limits set by ISO

16075-1:2020 for the most significant parameters and by national regulation for water reuse DM 185/2003, proved that the accomplishment of a QCRA was not justified and health and environment protection were confirmed.

The QMRA was conducted according to the guidelines proposed by WHO and was structured as follows:

- Hazard identification: This step involves deciding which microorganisms are of interest in the study and finding out what diseases these microorganisms cause. It includes general information about the microbial agent (pathogens) and the adverse consequences to the host from infection.
- Hazard characterization: The scope of this step is to have a detailed description of the mechanisms and the cause of the actual adverse health effects. It includes information on the required level of detail, hazards (pathogens) and health outcomes to be considered and exposure pathways and hazardous events to be included.
- Exposure assessment: The purpose of exposure assessment is to predict the fate of a hazard from its source to the endpoint and quantity what this endpoint is exposed to. Different groups of people could be exposed to hazards through different pathways. In this step the frequency and magnitude of exposure to pathogens via the pathways and hazardous events are defined. It is important to determine dose-response relations.
- Risk characterization: The final step of the risk assessment combines the information from the previous steps to estimate the likelihood of an adverse consequence (Haas et al., 2014). The health impact data for the identified hazards and the specific study population are reported. They include: the type of health effects, the severity and duration of a disease or illness that may occur after ingestion of the pathogen and the relationship between ingested dose and the probability that health effects (infection, illness, sequelae) occur (WHO, 2016b). Risk characterizations range from a "point estimate" of risk to more sophisticated methods, also known as probabilistic risk assessment, that consider uncertainty in model input parameters and variability across individuals and subpopulations.

Risk assessment includes a certain level of uncertainties within its estimation. The terms variability and uncertainty refer to imprecise or not reliable data, assumptions or lack of information, which might lead to errors in the overall result (Wolfgang Seis, 2012).

Early approaches of quantitative microbial risk assessment (QRMA) were based on point estimates and resulted in a single value of risk. Point estimates means that one value is chosen to represent each variable and the risk is calculated considering that value. Mean values of variables are chosen to calculate the average risk while extreme values, such as the 95-percentile, could give an idea of the worst-case scenario. Such an approach does not give a comprehensive picture nor appropriate weight of all combinations. On the other side, stochastic modelling could be used to have a more realistic representation of the distribution of data. Monte Carlo method can be used to obtain the output risk distribution using random samples of each distribution. This latter approach was used in this study.

In this study, QMRA was performed through the web tool QMRA.org (Wolfgang Seis 2022- QMRA (Version 0.1.3) [Computer software] https://doi.org/10.5281/zenodo.6457511). The tool includes a complete database of typical pathogens concentrations, treatment log removals, exposure scenarios, dose-response and health parameters. Moreover, it can be customised to allow the user

to consider site-specific data and/or conditions. The tool QMRA.org runs Monte Carlo simulations and it is able to evaluate the probability of infection and the DALYs associated with defined scenarios of microbiological contamination of reused wastewater.

2.10.3.1 Hazard identification

The first step of a QMRA procedure is to identify the possible hazardous pathogens that may be reason of illness for humans due to wastewater reuse practices.

Risk assessment is generally conducted for relevant reference pathogens, which should be representative of each the major groups of pathogenic organisms (i.e., bacteria, viruses, protozoa and helminths), since different groups of pathogens may have different behaviours and susceptibilities during wastewater treatment processes.

Reference pathogens were selected considering suggestions by WHO guidelines and by the Australian Guideline for water recycling (NRMMC-EPHC-AHMC, 2006). Hence, *Campylobacter*, *Cryptosporidium* and *Rotavirus* were selected to be representative organisms of bacteria, protozoa and virus, respectively.

Campylobacter infectious are currently the second most frequently reported bacterial infections causing gastroenteritis (Seis et al., 2012). *Campylobacter* bacteria are able to survive in the environment for a certain time, and infections can occur due to the consumption of and bathing in contaminated water. Hence, they can be considered suitable representative of bacteria group to conduct risk analysis.

Rotavirus is a good candidate to be representative of viruses during risk analysis. Indeed, *Rotavirus* poses a major threat of viral gastroenteritis worldwide, has a relatively high infectivity compared with other waterborne viruses and a dose–response model is available in the literature (A.H. Havelaar, J.M.Melse, 2003)

Cryptosporidium parvum is a good candidate to be the reference organism for protozoa. It is reasonably infective, resistant to chlorination, and it is regarded as one of the most significant waterborne human pathogens in developed countries since it is cause of bacterial gastroenteritis (NRMMC-EPHC-AHMC, 2006).

2.10.3.2 Hazard characterization

Pathogens concentrations available at Peschiera Borromeo WWTP are related to the concentration of the indicator organisms *E. Coli* in the raw influent to the plant, in the secondary effluent and in the final effluent after UV disinfection, which are measured by periodical standard routine analysis.

To define the expected concentrations of *Campylobacter*, *Cryptosporidium* and *Rotavirus*, typical ratios between *E. coli* and reference pathogens in raw wastewater were used (Mara, 2006), as reported in Table 17.

Table 17: Reference ratios between *E. Coli* and pathogens concentration in raw wastewater

	Campylobacter	***Cryptosporidium***	***Rotavirus***
min	1.00E-06	1.00E-07	1.00E-06
max	1.00E-05	1.00E-06	1.00E-05

Particularly, for Cryptosporidium and Rotavirus the literature ratios shown in Table 17 were applied to the concentration of *E. coli* in the influent. Literature data on log removals at each treatment unit were then applied to define the pathogens concentrations in the effluent.

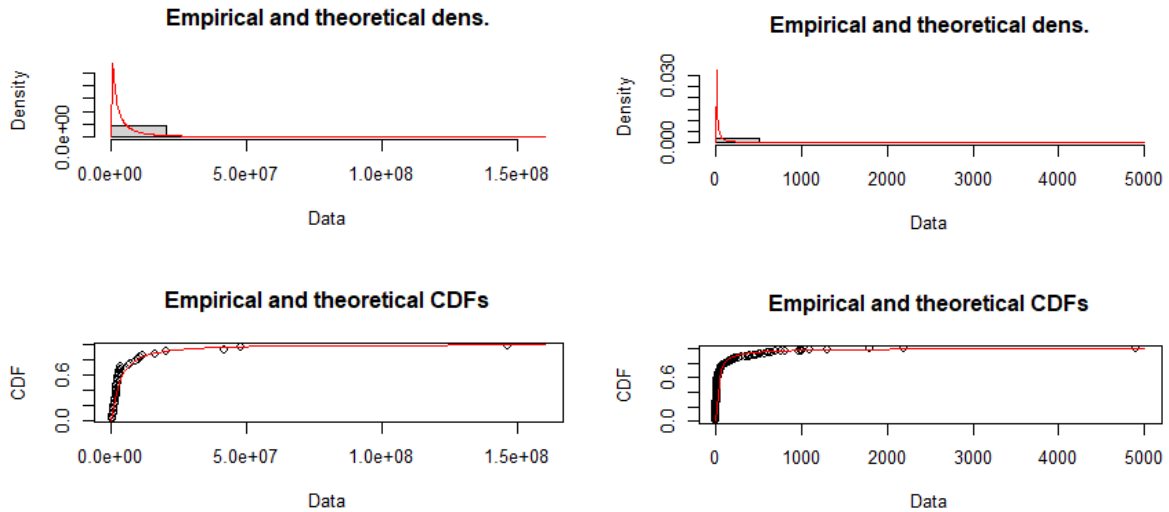
Reference values (NRMMC-EPHC-AHMC, 2006; Wolfgang Seis & Remy, 2013; WHO, 2006) for the applied log reductions are reported in Table 18.

Table 18: Indicative log-removals for *Cryptosporidium* and *Rotavirus* in different wastewater treatment units

Treatment unit	***Distribution***	***Cryptosporidium***		***Rotavirus***	
		min	**max**	**min**	**max**
Primary Treatment	uniform	0	1	0	0.1
Secondary Treatment	uniform	0.5	1.5	0.5	2
UV disinfection	uniform	3	3	1	3

In the case of *Campylobacter*, the literature ratios shown in Table 17 were applied to the concentrations of *E. Coli* in the final effluent of Peschiera Borromeo WWTP. Indeed, *Campylobacter* and *E. Coli* are both bacteria and, thus, same log removals are applicable in each treatment unit for the two organisms (NRMMC-EPHC-AHMC, 2006; Wolfgang Seis & Remy, 2013; WHO, 2006).

E. Coli concentrations in both the influent and effluent of the WWTP were fitted by a log-normal distribution (Figure 32). Then, uniform distributions were utilized to apply the ratios in Table 17 and obtain the needed distribution of *Cryptosporidium*, *Rotavirus* and *Campylobacter*.



a) b)

Figure 32: Fitting of *E. coli* trends in the a) influent, and b) effluent with lognormal distributions

2.10.3.3 Exposure Assessment

In this step, health impact data for the identified hazards and the target population are specified. This includes the type of health effects, the severity and duration of a disease or illness that might occur after ingestion of the pathogen, and available information on the relationship between ingested dose and the probability that health effects occur (dose–response relationship). In addition, the fraction and vulnerability of the population exposed might need to be considered.

Fieldworkers and local communities might be exposed through direct contact with wastewater or contaminated soil or crops. These groups might inhale or ingest wastewater used for irrigation. Each group could enter in contact with contaminated wastewater or products in several ways or routes of exposure. Different barriers or log removals could be applied at each route of exposure to reduce the risk.

Other possible exposure routes of humans to pathogens due to wastewater irrigation could be considered, but not all of them are relevant for the case-study in object. As an example, the exposure via drinking water can be neglected, since there are no contacts with water catchment areas for providing drinking water.

Moreover, in Peschiera Borromeo reuse system, no crops are grown to be consumed without further processing. Thus, the pathways through the consumption of animals and animal products as well as direct consumption of crops grown on wastewater irrigated areas are neglected, too.

In conclusion, both fieldworkers and local community were considered as exposure groups through ingestion and inhalation. No pathogen die-off was assumed after irrigation. Dose-response models proposed by Haas were adopted to evaluate the risk of infection per person per year (pppy).

Routes of exposure

The routes of exposures are the activities through which people enter in contact with pathogens. A volume (mL) and a frequency per person per year (pppy) of exposure must be associated to each route. Due to lack of data and impossibility to obtain site-specific information, suggestions provided by Australian Guidelines were considered. The main route of exposure to microbial hazards from recycled wastewater is ingestion, including ingestion of droplets produced by sprays (inhalation). Dermal exposure is also possible, but there is a lack of evidence of health impacts through this route and it is considered unlikely to cause significant levels of infection or illness in the normal population (NRMMC-EPHC-AHMC, 2006). Data on exposure volumes and frequencies per person, utilized in this study, are provided in Table 19 according to the indications of the Australian water recycling Guideline (NRMMC-EPHC-AHMC, 2006) and Seis et al. (2012).

Table 19: Associated exposures for recycled water during irrigation

Activity	Exposed group	Rout of exposure	Volume (mL)	Frequency/person/year
Agriculture irrigation	Fieldworker	Indirect ingestion	0.01	100
	Nearby community	Inhalation (min)	0.0045	180
		Inhalation (max)	0.0069	180
	Children in the nearby community	Ingestion (min)	0.02	10
		Ingestion (max)	0.1	10

For highly mechanized agriculture, a daily intake of 1-10 mg contaminated soil, or 1-10 µl treated wastewater can be assumed for fieldworkers with a number of exposure events per year of 100 days per person per year (Seis et al., 2012). In this case, it is assumed that number of 100 ml water ≙ number of 100 g soil (Seis et al., 2012).

For assessment of the exposure of nearby residents, the dose of liquid aerosol particles people are exposed to must be estimated. Due to lack of local data, it can be considered that, due to wind

presence, nearby resident could inhale 4.5 - 6.9*10^{-3} mL of water, in the case of spray irrigation, for each irrigation event (Seis et al., 2021). The number of exposure events per year is equal to the number of irrigation events in one year. In the peri-urban area of Milan, agricultural fields cultivated with corns are irrigated once per day for six months. Hence, an exposure of 180 events per person per year can be assumed. However, spray irrigation is not applied in the investigated area and this exposure scenario does not result in a high risk for the local community.

On the contrary, children of the nearby community may play on agricultural areas or may accompany adults while they go for a walk. Especially young children tend to ingest higher amounts of soil. To account for this kind of risk an annual number of exposure events of 10 is applied. The amount of soil ingested is set to 20-100 mg (i.e., 0.02 – 0.1 mL of water) per exposure event according to Seis et al. (2012).

Reassuming, in this study the (indirect) ingestion of wastewater by fieldworkers and by children of the local community were considered as the most relevant exposure routes for microbial risk calculation.

Preventive measures

Preventive measures, or barriers, are strategies to reduce the exposure to hazard, which include actions to reduce the pathogens concentration in the wastewater (e.g., wastewater treatment processes) or actions to reduce the volume of water target people are exposed to.

Each preventive measure can be associated with a log reduction value. Characteristic values of log reduction have been taken from (NRMMC-EPHC-AHMC, 2006) and *WHO Guidelines (2006)* and are summarized in Table 20.

Table 20: Log reductions applied to each barrier

Reference	***NRMMC-EPHC-AHMC, 2006***		**WHO guidelines for safe use of wastewater, excreta and greywater**	
	min	**max**	***min***	**max**
Drip irrigation of crops	2			
Drip irrigation of crops with limited to no ground contact (e.g., tomatoes, capsicums)	3			
Drip irrigation of raised crops with no ground contact (e.g., apples, apricots, grapes)	5			
Drip irrigation of plants/shrubs	4			
No public access during irrigation	2			
No public access during irrigation and limited contact after (until dry 1 – 4 hours) (e.g., food crop irrigation)	3			
Buffer zones (25–30 m)	1		1	
Natural die-off			0.5	2

In the present study, a log removal value of 2 was considered to evaluate the exposure when drip irrigation is utilized. Indeed, the use of drip irrigation significantly reduces the volume of utilized wastewater and the production of aerosol compared to other irrigation technologies (i.e., spray irrigation or surface irrigation).

For the selected exposed groups, two microbial risk scenarios were analysed, which were related to the application or not of drip irrigation.

Dose-response relationship

The dose of pathogens with which the exposed group enter in contact can be calculated with the following equation:

$$d = \frac{c * exposure/event}{\log reduction}$$

Where:

- C = concentration of pathogens
- log reduction = reductions required to achieve a residual risk coming from preventive measures or barriers
- exposure/event= volume (mL) with which people enter in contact in a single event of exposure during a certain activity

Next step consists in the identification of dose-response models, which are mathematical functional relationships between the number of pathogens someone is exposed to and the probability of occurrence of the related adverse effect. A fraction of infected people may develop different health outcomes. The simplest dose-response model is an exponential relationship (Haas et al., 2014):

$$P_{inf} = 1 - e^{-r*d}$$

- P_{inf} =probability of infection
- r= infectivity constant
- d= dose

The exponential model assumes that the probability of infection is constant for similar kind of pathogens (C.N. Haas et al, 1999), while actually not all pathogens of the same species are equally infective and not all humans have the same health outcomes. For that reason, the Beta Poisson-model is also used (Haas et al., 2014):

$$P_{inf} = 1 - (1 - \frac{d}{\beta})^{-\alpha}$$

(3.3)

Where:

- d = dose per event
- α, β = dose response constants

A Dose-response model is used to calculate the Probability of infection (P_{inf}) related to each event of exposure to the target pathogen. In this study, according to what suggested by (Haas et al., 2014), a Beta-Poisson model has been used to calculate P_{inf} in the case of *Campylobacter* and *Rotavirus*, while an exponential model was used for *Cryptosporidium*.

$P_{inf} = 1 - e^{-r*d}$ (used for *Campylobacter* and *Rotavirus*)

$$P_{inf} = 1 - (1 - \frac{d}{\beta})^{-\alpha}$$ (used for *Cryptosporidium*)

Constant values "α" and "β" for the Beta-Poisson model and "r" for the Exponential equation were obtained from literature (Table 21). Particularly, reference values were obtained by Australian guidelines, QMRA Wiki community portal (a data repository website created by researchers of the Center for Advancing Microbial Risk Assessment (CAMRA) OF Michigan State University), and Aquanes tool website.

Table 21: Dose-response constants for selected pathogens

		Campylobacter	*Cryptosporidium*	*Rotavirus*
Dose-response constants	**α**	1.44E-01		2.53E-01
	β	7.58		4.26E-01
	r		5.72E-02	

2.10.3.4 Risk characterization

Once calculated Pinf, the total probability of infection in one year is obtained by the following equation:

$$\mathrm{P_{inf}\ combined\ final} = 1 - \prod_1^n (1 - \mathrm{P_{inf\,i}})^{\mathrm{Ni}}$$

Where:

- N = number of activities
- Pinf i = probability of infection of the i^{th} activity
- Ni = frequency/person/year of i^{th} activity

The final probability of infection of each pathogen calculated by eq. (3.7) should be compared with the limit value established by US EPA of $1*10^{-4}$. If the calculated probability of infection is lower than this health-target value, the microbial risk can be considered acceptable.

Once the value "$\mathrm{P_{inf}\ combined\ final}$" has been calculated, the probability of illness (P_{ill}) is obtained multiplying "$\mathrm{P_{inf}\ combined\ final}$" with the ratio illness/infection, which is provided by literature (Table 22) (NRMMC-EPHC-AHMC, 2006)

$$\mathrm{Pill} = \mathrm{P_{inf}\ combined\ final} * \mathrm{ratio\ illness/infection}$$

Table 22: Ratios illness/infection for the selected pathogens

Campylobacter	*Cryptosporidium*	*Rotavirus*
0.3	0.7	0.5

WHO guidelines consider Disability-adjusted life years (DALYs), as a metric for expressing the burden of disease within a population. The DALY is a health gap indicator for the status of health of a population expressed as burden of disease due to a specific disease or risk factor, and it takes into account both the morbidity and the mortality caused by a specific disease. A health-target of 10^{-6} DALYs was set by WHO as tolerable health risk.

The DALY value can be calculated by the following equation:

$$\text{DALY per year} = \text{Pill} * \text{DALYd} * \text{susceptibily fraction} \quad (3.9)$$

Where:

- Pill= probability of illness per year
- DALYd= DALY per case

Daly per case of reference pathogens were obtained by WHO guidelines (2011), as shown in Table 23.

Table 23: DALY per case for the selected pathogens

	Campylobacter	***Cryptosporidium***	***Rotavirus***
DALY per case	0.0046	0.0015	0.014

Once values of DALYs per year for each pathogen have been calculated, the obtained values can be compared with the tolerable level of risk ($1*10^{-6}$) set by WHO in order to understand if the risk is acceptable or not.

2.10.3.5 Results

Results are summarised in the following paragraphs, both for workers and for children of the local community.

Graphs (Figure 33 – Figure 38) show the calculated Probability of infection that were compared with the health-target limit suggested by US EPA (10^{-4}) and the calculated DALYs. In this case, the tolerable value of 10^{-6} suggested by WHO Guidelines was considered as the threshold value.

In each graph, results of the expected microbial risk are shown considering minimum and maximum log removals that can be achieved during wastewater treatment. Indeed, log removals for viruses and protozoa during the different treatment stages were taken from literature and are highly variable since are referred to WWTPs that work under very different operational conditions (Table 18). Particularly, low log removals are often related to small WWTPs that work under non-optimised conditions and that reach low treatment performances. On the contrary, high values of log removals comes from large WWTPs, which are generally operated under better conditions. Showing results for minimum and maximum removal of pathogens gives indication of the uncertainty of the assumptions done to evaluate the risk. In these terms, the variability of the obtained results (i.e., difference between minimum and maximum log removal scenarios) represents the uncertainty related to the use of literature data and assumptions done to evaluate the risk. Only site-specific measurement campaigns could confirm or deny the assumed hypothesis, allowing to utilize site specific values for expected log removals of pathogens at Peschiera Borromeo WWTP.

A decision support tools such as the Early Warning System will be then very useful to lower the expected microbial risk. Indeed, the EWS can detect plant malfunctions or low treatment efficiencies and rapidly send alarms. Hence, the presence of the EWS can ensure that when wastewater is provided for irrigation, the WWTP is working properly and that treatment performances met the required levels. In this case, risk scenarios related to high log removals of pathogens will result more plausible.

Risk for workers

Risk was evaluated for fieldworkers, considering or not drip irrigation as a barrier. Indeed, wastewater treated at Peschiera Borromeo WWTP is reused in agriculture for corn cultivation by surface irrigation and by drip irrigation. Figure 33 and Figure 34 show the calculated microbial risk in the case of use of drip irrigation and conventional irrigation, respectively. Results are similar for both the scenarios. Particularly, the probability of infection and DALY values are higher than related health-target limits only for the pathogen *Rotavirus* in the case of low performance of wastewater treatment operations and low log removals for pathogens. Hence, the uncertainty related to literature values for log removals (Table 18) does not ensure that an acceptable level of risk is reached during wastewater irrigation. A strategy to reduce uncertainties about treatment performances could be represented by the implementation of an Early Warning System. If the tool is operated, it can provide information about the status of the wastewater treatment units and thus guarantee that they are working with high treatment efficiency. The presence of the EWS can ensure the good operation of treatment units and that the required performance levels are met, allowing to calculate microbial risk using high log removal for pathogens (Figure 33 and Figure 34).

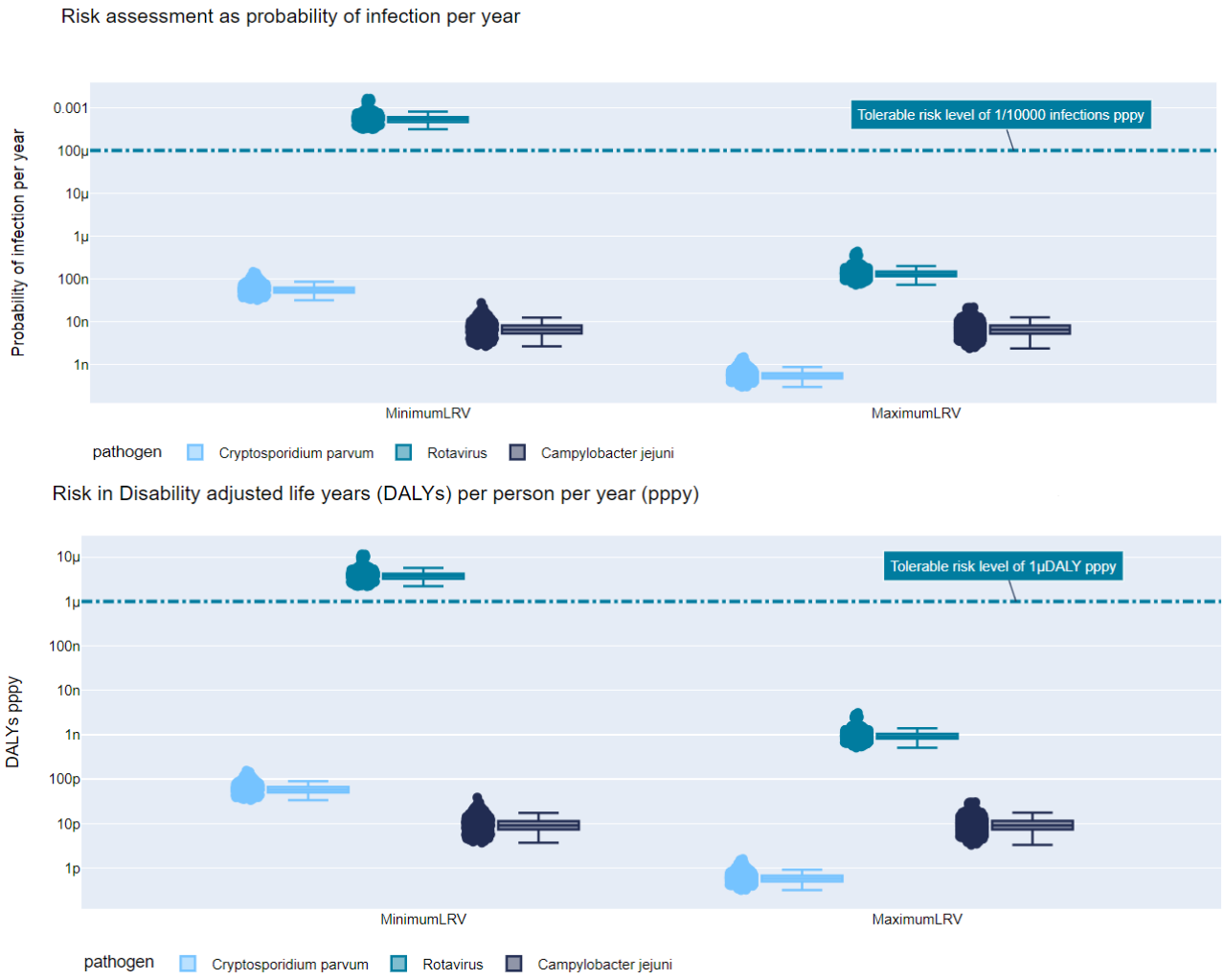


Figure 33: Risk expressed in Probability of Infection (up) and DALYS (down) for workers with drip irrigation in the case of maximum log removals (MaximumLMV) and minimum log removals (MinimumLMV) of pathogens.

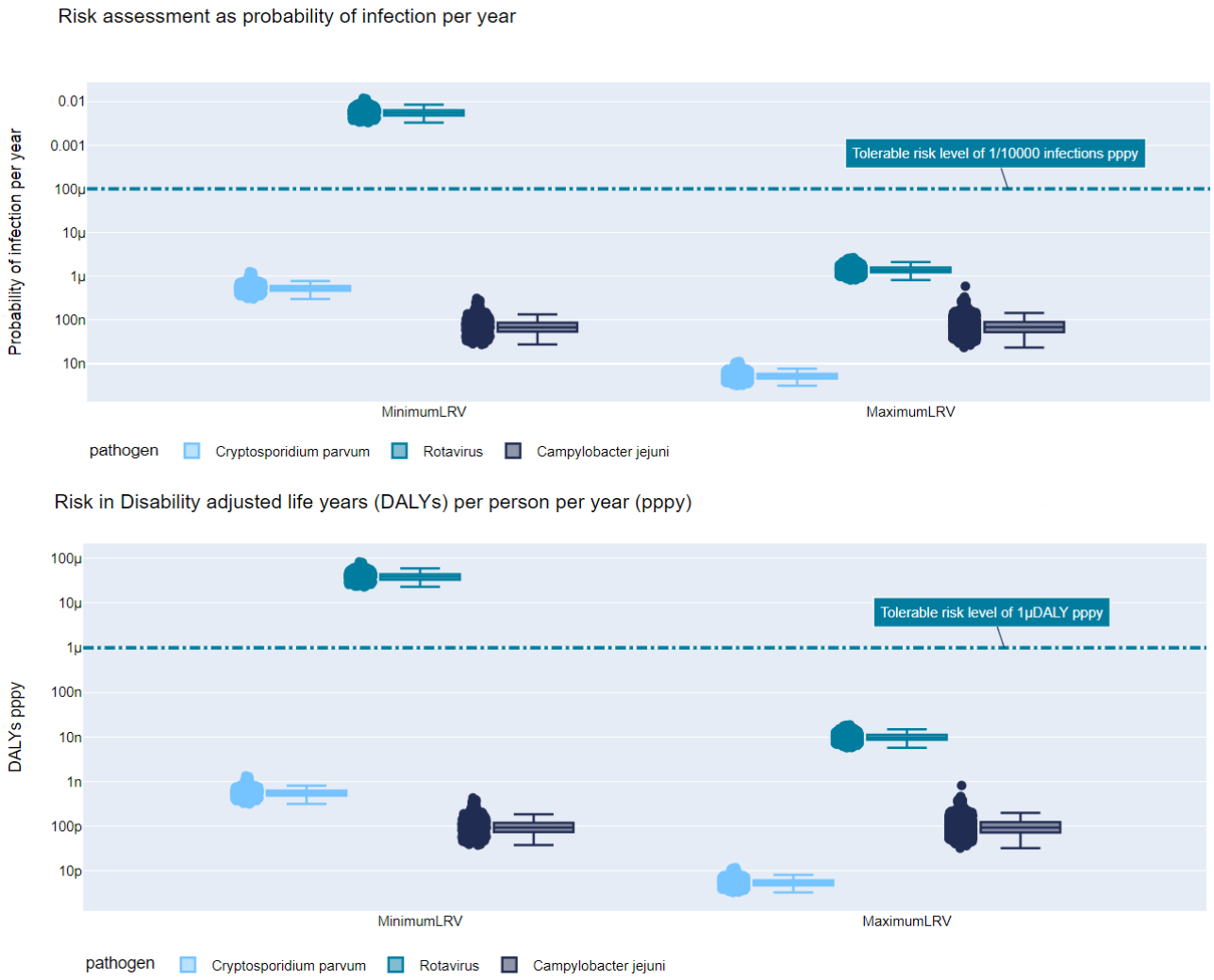


Figure 34: Risk expressed in Probability of Infection (up) and DALYS (down) for workers without drip irrigation in the case of maximum log removals (MaximumLMV) and minimum log removals (MinimumLMV) of pathogens.

Even though for *Rotavirus* the use of drip irrigation was not able to assure an acceptable level of risk for wastewater reuse, this barrier results in any case highly effective to reduce the risk for fieldworkers as it was better highlighted in Figure 35.

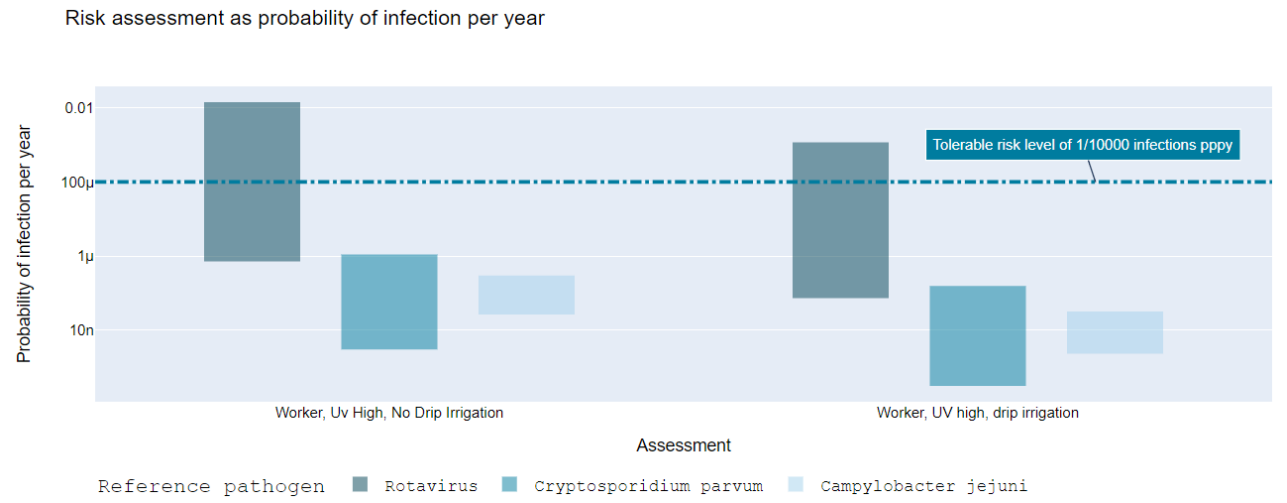


Figure 35: Comparison of risk calculation for workers related to scenarios without and with the use of drip irrigation as barrier

Risk for local community

Outcomes of risk calculations for children living in the nearby community are reported in Figure 36 and Figure 37. However, in this case, when drip irrigation is utilized for irrigation, the risk levels is always below the health-target threshold limits (Figure 36). On the contrary, if drip irrigation is not used, the risk is not acceptable for *Rotavirus* in the case of low performance of the wastewater treatment processes (MinimumLRV in Figure 37). This latter result highlights again the importance of an EWS able to monitor continuously the quality of the reused wastewater to assure a safe irrigation reuse.

Risk for local community is performed using the same hypothesis as the ones expressed for fieldworkers but considering a lower number of exposure events.

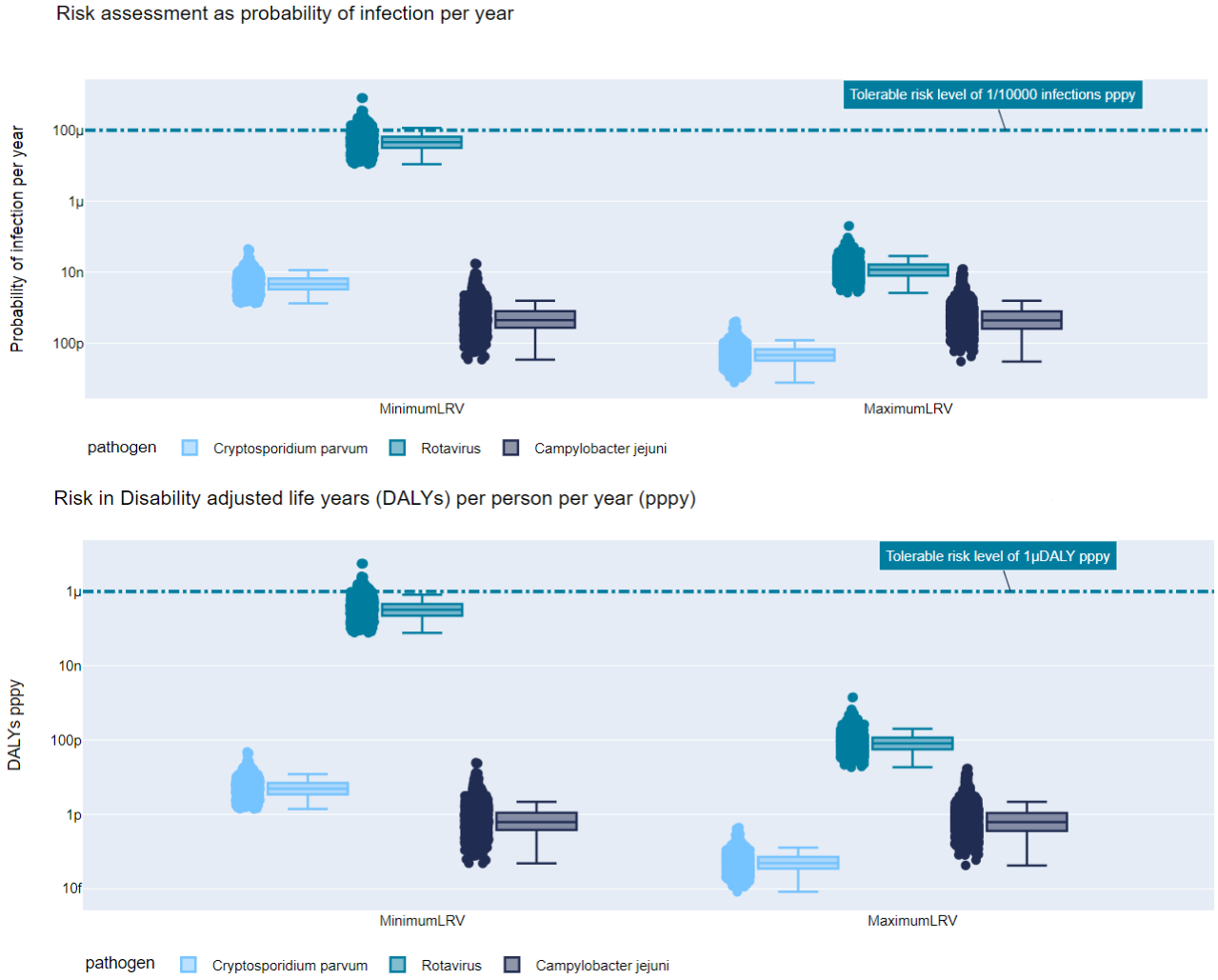


Figure 36: Risk expressed in Probability of Infection (up) and DALYS (down) for local community with drip irrigation in the case of maximum log removals (MaximumLMV) and minimum log removals (MinimumLMV) of pathogens.

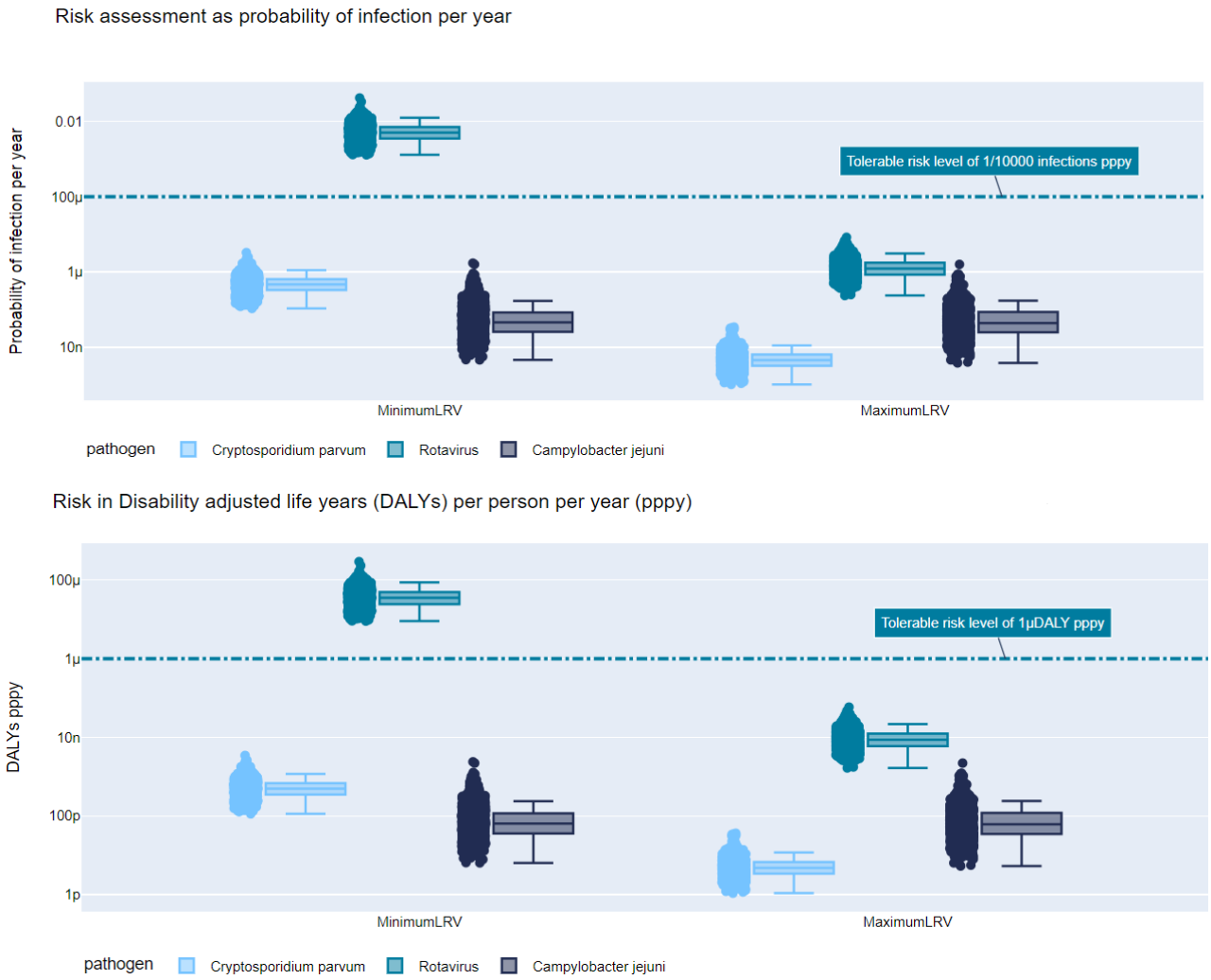


Figure 37: Risk expressed in Probability of Infection (up) and DALYS (down) for local community without drip irrigation in the case of maximum log removals (MaximumLMV) and minimum log removals (MinimumLMV) of pathogens.

The efficiency of drip irrigation as a barrier to reduce risk for local community is shown in Figure 38.

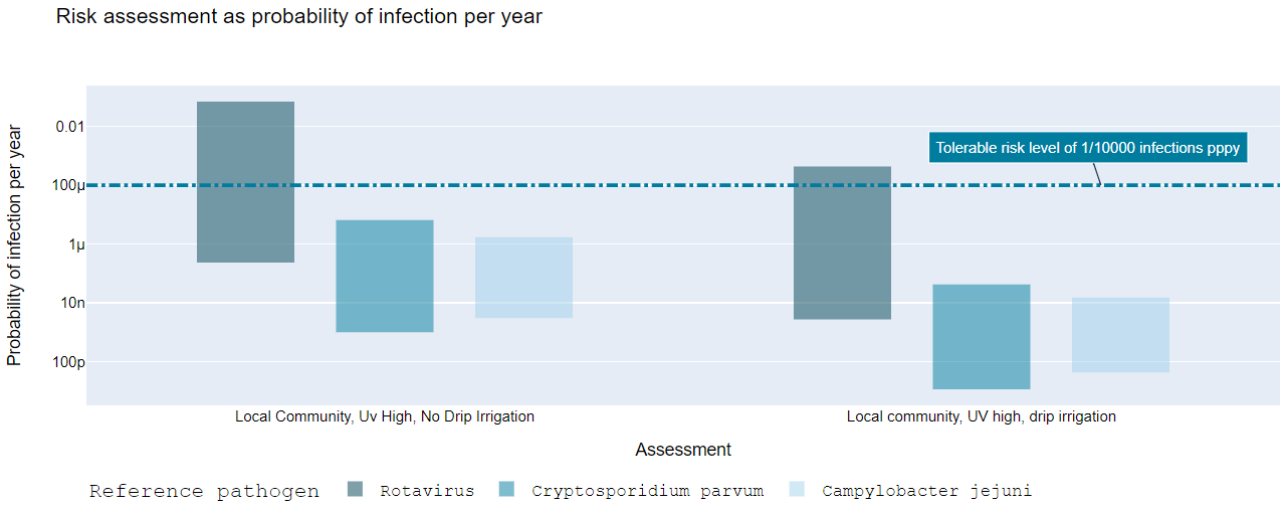


Figure 38: Comparison of risk calculation for local community related to scenarios without and with the use of drip irrigation as barrier

2.10.4 Preventive measures

Other than the treatment units and controls already set up in the WWTP, additional preventive measures were implemented in Peschiera Borromeo Water Reuse System.

Early warning systems (EWS) are tools that use real-time signals and other WWTP data to detect system malfunctions or anomalous events occurrence, allowing rapid interventions in case possible hazardous outcomes are identified. Those tools can overcome technical barriers related to the delay in data acquisition from grab samples due to the lag time between sampling, measuring and lab analysis. The EWS developed for the Peschiera Borromeo Water Reuse System aims to manage microbial and chemical risks by using machine learning tools to perform analyses on the signals acquired from the multi-parameter network of sensors installed at the WWTP, as well as data from lab analysis and open-source models and tools, to provide rapid alert in case of failures detection. The EWS is designed to predict effluent TSS concentrations, since it was observed that solid content could be related to E. coli contamination, as well as COD and BOD_5 in the effluent. The EWS uses Artificial Neural Network (ANN) techniques to provide predictions on effluent values of TSS, COD or BOD values, using as input the parameters detected along the plant (flowrate, pH, ammonia, phosphates, temperature and dissolved oxygen in biological tank and effluent nitrates).

2.10.5 Monitoring

At Peschiera Borromeo WWTP, monitoring of wastewater quality is performed by sensors installed at the plant and by laboratory analyses. The digital monitoring of wastewater quality at Peschiera Borromeo WWTP is performed by a remote control and a SCADA system for the continuous acquisition of online data measured at the WWTP by sensors. Laboratory analyses are also performed periodically for influent and effluent characterization to control specific processes. Equipment status and related alarms on electro-mechanical units are continuously monitored. Offline data about cumulative energy consumptions, chemicals supply, sludge and waste production and disposal are stored in internal management systems. Maintenance operations, internal report and emergency procedures follow specific and documented protocols.

The integrated Reuse System of Peschiera Borromeo is equipped with a series of sensors for continuous monitoring of raw wastewater, effluent and reclaimed water reused in agriculture.

Correct and continuous maintenance is essential for the affordability of sensors signals. Moreover, a data-cleaning procedure based on the integration of statistical methods (moving standard absolute deviation M-SAD and T-squared) was applied to remove sensors faults and outliers.

The WWTP is provided with a Supervisory Control And Data Acquisition (SCADA) system for remote control and continuous acquisition of online sensors. Equipment status and related alarms on electro-mechanical units are also continuously monitored. Energy meters are installed to monitor the most consuming treatment units, such as biological treatments and UV disinfection.

A specific software (WaterLims) is used to upload and update laboratory analyses results. Other offline data about cumulative energy consumptions, chemicals supply, sludge and waste production are stored in internal management systems as well.

Operational monitoring includes laboratory analyses, which are periodically performed for influent and effluent characterization to verify compliances with D. Lgs 152/2006 (according to WFD).

The Early warning system (see Module II – Preventive measures) is going to be implemented as a support tool to forecast the TSS values in the effluent in order to indirectly determine microbial contamination by using the other sensors data installed in the WWTP (e.g., flowrate, pH, ammonia, phosphates, temperature and dissolved oxygen in biological tank and effluent nitrates). Moreover, COD and BOD_5 effluent values could be also predicted, according to the specific operative monitoring needs.

Table 24 schematised the monitoring system set out at Peschiera Borromeo WWTP, distinguishing between the measures applied to monitor the different treatment stages.

Table 24: Monitoring system set out at the *Peschiera Borromeo* Water Reuse System

Control point	Parameters	Source of data	Communication
Influent	Ammonium, pH, Conductivity, ORP, Phosphates, TSS	Online sensors (5-15 min response time)	Connected to SCADA
	Parameters foreseen in D. Lgs 152/2006 (according to WFD).	Weekly lab analyses	Connected to WaterLims
Effluent	Ammonium, pH, Conductivity, Phosphates, TSS, Nitrates, UV - 254 nm, ORP, TOC.	Online sensors (5-15 min response time)	Connected to SCADA
	Parameters foreseen in D. Lgs 152/2006 (according to WFD).	Weekly lab analyses	Connected to WaterLims
Biologic treatment	DO, T, Nitrates, REDOX	Online sensors (5-15 min response time)	Connected to SCADA
UV treatment	UV intensity/transmittance	Online sensor	Connected to SCADA
Energy consuming units (e.g., UV lamps, aeration system)	Energy meters	Online sensors (15 min response time)	Connected to SCADA
Electromechanical equipment	Status (on/off) and alarms	Online sensors	Connected to SCADA
All WWTP	TSS predictions	Early Warning System	Implementing

Analytical measures are planned for contaminants accumulation in soil, plants and irrigated environment. Moreover, ground sensors, satellite data and active unmanned aerial vehicle (drone) are used to monitor water stress and other relevant parameters in the soil-plant-atmosphere system. Those data are then acquired by the Match making tool, which is a tool to manage communication and information between water utility and final users. It consists in a web-app which

provides information on reclaimed water quality and available quantity, as well as data on field and crops. The match-making tool (MMT) allows to share information with farmers and promote smart irrigation techniques. The water utility will ensure tracking of water quality and available quantities, while farmers will share site-specific information about crops, sowing date, soil and irrigation method.

2.11 Support of innovative sensors for pathogens detection: reliability and use

Implementation of water reuse practices is complex and there are still many of factors that hinder it, from technical treatment performances to socio-political aspects. Consequently, apart from managing proficient reclamation facilities and developing WRRMPs that assure the safety use of reclaimed water, other activities, methodologies, tools, etc. are needed to overcome those obstacles.

Water management sector usually relies on treatment processes and removal efficiencies, using a large number of sensors and meters, alarms and automatic control tools. In recent years, technological progresses allowed the digitalization of the water sector providing new sensors, always more precise and reliable, and tools for decision support, implementing monitoring and control systems, together with tools to clean, process and analyse the data (Newhart et al., 2019). Digital tools can be also used to implement water reuse practices (Goodwin et al., 2019; Radini et al., 2021), other than for treatment optimisation and energy savings, by enabling rapid responses through real-time monitoring. On the other hand, health authorities need to rely only on standardized methodologies and certificated data. Indeed, one of the main lacks on risk management approach is the absence of common procedure on how to treat non-standardized data, such as real-time online data from sensors or model simulations results. The main difficulty consists in the validation and reliability of non-standard data for their utilization to evaluate risks.

When talking about innovative sensors for microbial detection, the definition of the parameters to evaluate sensor performances are even more complicated. In fact, microbiological measures are for their nature not robust (ISO/TR 13843:2000), since they are strictly depending on sample characteristics, variability and heterogeneity, environmental conditions, cultivation method and are affected by personnel operations. Existing guidelines for microbiologic measures are limited to the comparison between analytical lab procedures and aims to validate alternative methods against reference procedures.

Data from innovative sensors need to be compared with parallel lab measures for their validation, possibly using standard methods. It has to be noticed that sensors data need to be validated, but online measures are not supposed to substitute lab data, which are the only standard references for health authorities and regulatory requirements.

2.11.1 Fluidion sensors for surface water and wastewater

The ALERT technology provided by Fluidion for bacteria measurement allows a rapid detection of pathogens, decreasing the response time from the 24 – 48 hours needed for laboratory analysis to 6 – 12 hours. Moreover, sampling can be automatized in order to perform periodic measures, even without the presence of operative personnel. The ALERT line of instrumentations utilizes a modified substrate method for bacterial enumeration and allows in-situ quantification of *E. coli* and total coliforms. Alternatively, ALERT technology can allow the quantification of the intestinal enterococci

concentration or faecal coliform concentrations in both fresh water and seawater environments. The limit of detection corresponds to 1 target bacterium in the sample volume of 25mL (i.e., a limit of detection of 4 bacteria/100mL in fresh water), while the upper limit of measurement is 5×10^5 bacteria/100mL.

The bioreagent used in ALERT instruments contains a mixture of selective growth medium and 4-methylumbelliferyl-β-D-glucuronide (MUG), which is the standard substrate used in approved *E. coli* testing methods and can be hydrolysed into fluorescent 4-methylumbelliferyl (MUF) by the β-glucuronidase enzyme present in *E. coli* bacteria (MUG). The bioreagent used also contains ortho-nitrophenyl-β-galactoside (ONPG), another bacterial indicator that is metabolized by all types of coliforms in the sample and transformed into ortho-nitrophenol (ONP), resulting in development of yellow coloration. During the selective culture step (involving incubation at 37.0°C) bacterial metabolism progressively transforms MUG into MUF, generating broad fluorescence when excited at 385nm, with emission peaking around 460nm. Growth of non-target organisms is not promoted during the selective culture step, which makes the method highly selective to culturable E. coli, unlike rapid tests based solely on enzymatic activity without culture. All ALERT instruments contain multiple individual bioreactors (six for the portable ALERT Lab, and seven for the in-situ ALERT System), each capable of independently incubating a sample and performing optical measurements using an optical sensor ring. The sensor ring contains three LEDs arranged to excite MUF fluorescence (385 nm excitation), measure ONP absorbance (430 nm) and compensate for sample turbidity (610 nm), as well as a photodiode coupled to a low-pass optical filter that blocks the UV excitation light. The fluorescence signal is measured at periodic intervals (every 5 minutes), with data transmitted in real time through the mobile phone network, to a remote cloud-based data server. The resulting curve (Figure 39) consists of an initial plateau, followed by sharp increase in fluorescence starting a few hours into the measurement. The fluorescence detection time is automatically interpreted by the cloud server and used for providing E. coli quantification. The curve is automatically analysed by the data server to establish the fluorescence detection time, which is then used, after applying a specific calibration, to calculate the number of bacteria present in the original sample.

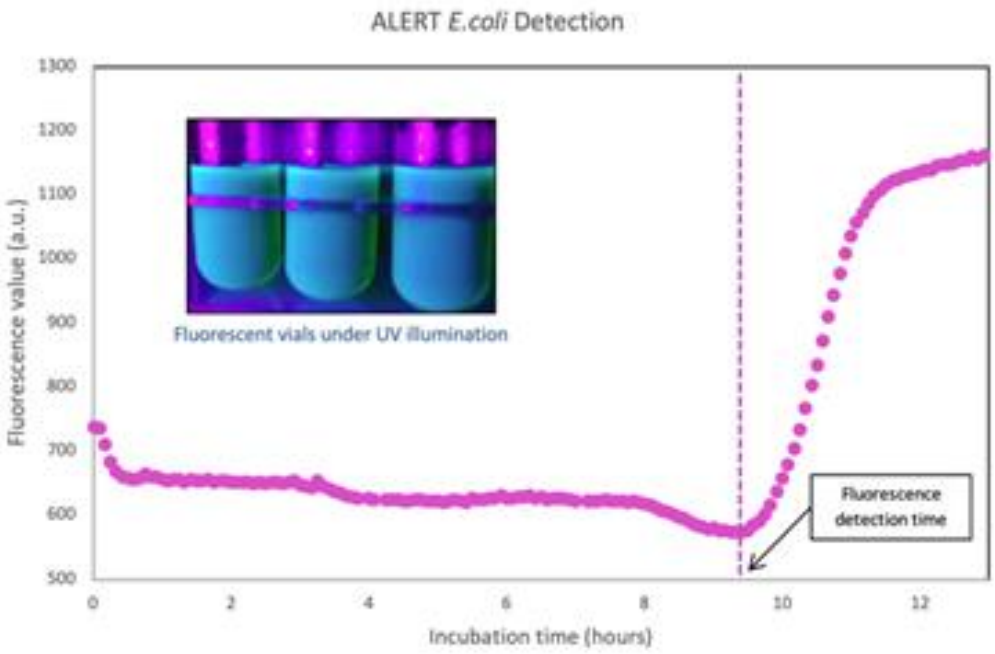


Figure 39: Typical fluorescence signal obtained from the ALERT device. Inset: Photo of sample vials illuminated by UV light, after incubation.

ALERT technology has been employed in numerous applications worldwide, which includes seawater monitoring, in-situ environmental monitoring of highly-polluted streams (Angelescu et al., 2018), pollution-source identification performed by regulatory agencies (Angelescu and Saison, 2020; Cronin et al., 2018; Loewenthal et al., 2018), monitoring campaigns accomplished in conjunction with remotely-controlled aquatic drones (Angelescu and Hausot, 2019). Recently, the ALERT technology has been applied to monitor E.coli concentration at different treatment points of a WWTP (Angelescu et al., 2020). ALERT instruments can automate the full range of operations: sampling, reagent mixing, incubation, real-time multispectral optical analysis (absorbance/fluorescence), turbidity correction, signal analysis, bacterial quantification, wireless data transmission and automatic generation of notifications. No sample transport, conditioning, or preparation is necessary, leading to logistic advantages, and reducing the risk of sample degradation and human error. In addition, this technology produces rapid and reliable information on water quality to enable effective decision making in real-time.

ALERT technology can be employed in multiple configurations: ALERT System for performing automated in-situ bacterial enumeration to obtain time-series data at a target location; ALERT Lab as a portable device for rapid mapping of bacterial contamination at multiple sites, or as a bench-top device for rapid laboratory analysis (Figure 40). The system can be remotely controlled from a cell phone or web interface and supplies data to the operator wirelessly. Both ALERT System and ALERT Lab devices were employed in DWC.



Figure 40: In situ ALERT System; a – ALERT Lab portable/bench-top instrument; c – View of the ALERT System during field maintenance operations

Recently, FLUIDION has developed a new version of the Alert System, which is named as ALERT System V2 (ALERT V2) and that has been also tested in DWC case-studies. The ALERT V2 is a completely redesigned instrument, implementing a disposable cartridge system for measurements, reducing maintenance due to cleaning and disinfecting all the hydraulic parts that have been in contact with the samples (Figure 41).



Figure 41: The ALERT V2 instrument, including a zoom on the sampling cartridge, at left.

Moreover, the ALERT V2 contains an external sample temperature sensor, which allows it to modulate the incubation program accordingly, in order to ensure rapid heating to the incubation setpoint. It also contains an external data port that allows the ALERT V2 to be connected to an external water quality probe or sensor, in order to monitor and log different parameters in real time, and perform adaptive sampling based on the measured values. The system can be installed either in rail configuration, attached to a fixed structure, or in floating configuration, as shown in Figure 42.

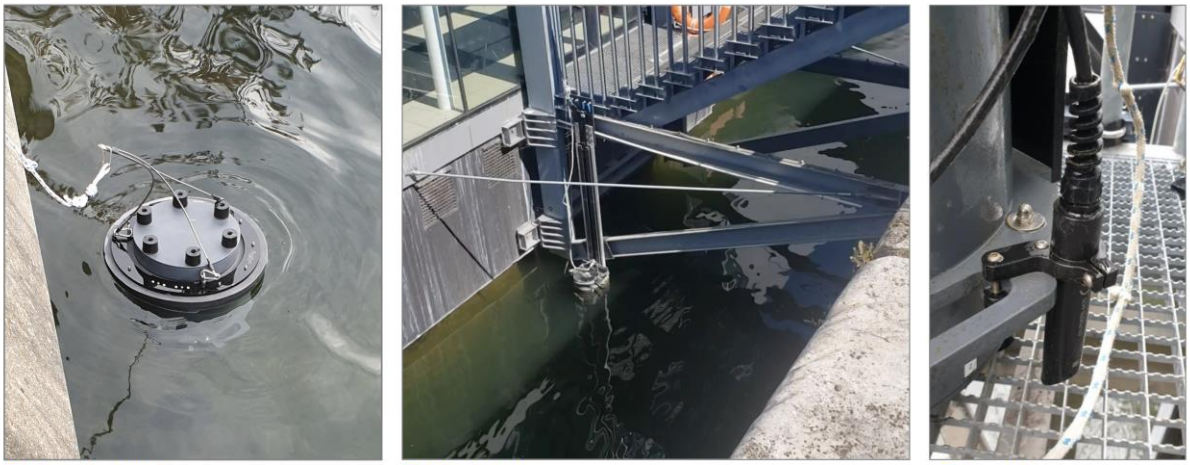


Figure 42: The different installation configurations for the ALERT V2: free-floating configuration (left), rail mount attached to fixed structure (middle), connected to an external sensor (right).

2.11.2 Tests with Alert instrumentations

Alert instrumentations were tested in Line 2 of Peschiera Borromeo WWTP, along the main WWTP treatment units (after primary treatments, after biologic and after UV), while the Alert Systems V1 and V2 were installed before the UV disinfection unit, after that efforts were accomplished to identify the most suitable location (Figure 43). For the Alert Systems, an installation point before the discharge would have been more suitable to guarantee a direct measurement of faecal contamination in the final effluent. However, the high turbulence in this point might hinder the good performance of the on-line analyser. On the contrary, a sampling location placed before the UV disinfection unit showed suitable hydraulic condition for the on-line monitoring, although the measurements obtained in this point were not representative of the bacteriological quality of the discharged effluent, but only to the one upstream of the UV lamps. However, by knowing the bacteria removal efficiency of the disinfection process, it was still possible to estimate the bacteria concentration in the final effluent.

The ALERT Lab needed manual operation for sample collection, vials sterilization, sterilization of deionized water, sample dilution and reagent dosing. Cleaning procedure was necessary after every measurement, and it took approximately 15 minutes of manual washing with bleach followed by 20 minutes of sterilization with autoclave. The ALERT Systems tested in situ enhanced the degree of automation of the measurement performing bacterial quantification simultaneously in seven collected samples. However, some manual procedures, such as vial sterilization, deionized water sterilization, and reagent dosing were still required for the Alert V1. Hence, the cleaning procedure was more onerous than expected and lasted longer than the cleaning procedure of the lab portable version. Finally, it is worth highlighting that the reagents had to be kept at low temperatures for long-term storage. It was a critical point to take into account during the warm seasons.



Figure 43: installation of the ALERT System at Peschiera-Borromeo WWTP

As concern Alert V2 testing, two monitoring campaigns were performed with the same operating conditions but using different types of cartridges, without and with an additional filter, to simulate the filtering procedure performed in the standard lab analysis.

Bacteriological analyses of wastewater samples were performed in parallel by the Alert devices and in the microbiological laboratory of the water utility CAP Holding, using membrane filtration and plating technique. The results elaborated by ALERT were compared with those obtained by standard analyses in the laboratory to evaluate the ALERT performances.

2.11.3 Methods to evaluate sensors performances

Sensors' data should be evaluated using simple and standard indicators in order to assess their reliability. Some characteristics of the sensors, such as the resolution and the working range, are available from the technical sheets. Standard indicators for sensors should be used not only to evaluate the "goodness" of the data provided, but also to make some considerations about their costs, their lifetime, their usability and the efforts required for their management. The most common parameters used to evaluate the quality of the measurements are the resolution, defined as the minimum unit that the sensor can measure, the range, which includes the upper and the lower values that can be measured, and the accuracy. From a functional point of view, external factors, and environmental and operative conditions, such as temperature, pressure and humidity, may affect sensors' reliability. The width of the ideal working ranges and the sensitivity to the variability of those operative parameters could be used as indicators of the robustness of the probe outputs and must be compared with the effective conditions that usually occur in the field. Thus, a standard procedure to detect sensors malfunction or outliers in the measurements need to be defined.

Standard procedures for the comparison of microbiological methods are briefly described in the following sections, in order to get a clear overview of the main methodologies currently used to compare two different methods. Finally, a method is proposed to assess the accuracy of the microbiological measurements performed by Alert devices.

2.11.3.1 Standard for methods comparison ISO 17994:2014

The International Standard ISO 17994:2014 "Water quality – Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods" reports a procedure to compare the results of two different microbiological analytical methods and can be applied for both the colony counts-based method and the MPN-based methods. The preliminary phase of the comparison procedure consists in the establishment of a confidence interval. This interval is defined as a predetermined limit and its extension ranges from *-2L* to *+2L*. Concerning environmental waters, such as bathing waters, the ISO 17994:2014 proposes a predetermined limit of *2L* = 20%. Here, *L* is defined as the smallest microbiologically significant mean relative difference. Collected samples should be representative of the characteristics of the water source of the geographical and environmental area under investigation. Natural samples are preferred. However, appropriate samples may also be prepared by dilution, spiking, or mixing of different kinds of water to achieve the desired population in a suitable density. Spiking with pure cultures should be considered the last resort. The most important basic requirement of comparison studies is a wide range of samples. Actually, there is no way to determine a priori the minimum number of samples required, since it depends on the differences measured, their standard variation and the chosen significance level. In general, it has been observed that the standard deviation of the mean relative difference should be less than 100. When it is used a 95% confidence limit, the number of samples required can be estimated as:

$$n = \frac{4s^2}{L^2}$$

- *n*: number of samples required for the detection of a difference *L*;
- *L*: smallest microbiologically significant mean relative difference;
- *s*: experimental standard deviation.

Samples shall be excluded if both results are zero (0,0) or either method gives a non-countable result (e.g., TNTC, larger than, …). Outliers can be detected by plotting $\ln(a_i)$ against $\ln(b_i)$. If data are excluded, motivation should be explained.

The first step is to calculate the logarithmic values of the test results, in order to reduce the influence of concentration in the evaluation. After that, the difference between the logarithmic values is calculated. In this way the test variable *x* is obtained, representing the relative performance of two methods:

$$x_i = [ln(a_i) - \ln(b_i)] \times 100\%$$

When the results of one of the methods are zeroes, the relative differences are calculated, respectively in the case of pairs $(a_j; 0)$ and $(0;\ b_k)$:

$$x_j = ln(a_j + 1) \times 100\%$$

$$x_k = -ln(b_k + 1) \times 100\%$$

However, it should be preferred to obtain at least 75% of the samples containing regular count data from both methods.

The average relative performance $\overline{x}$ is estimated, according to the following formula:

$$\overline{x} = \frac{\sum x_i}{n}$$

- n: number of samples.
- x_i: relative difference in sample *i*.

The standard deviation of the mean relative difference (uncertainty) is calculated as:

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

The standard uncertainty of the mean (formerly standard error) is expressed as:

$$s_{\bar{x}} = \frac{s}{\sqrt{n}}$$

The half-with of the confidence interval W is defined as:

$$W = \frac{2s}{\sqrt{n}}$$

Once all the parameters have been defined, the next step consists in the calculation of the limits of the confidence interval around the mean:

- Lower limit: $x_L = \bar{x} - W$
- Upper limit: $x_U = \bar{x} + W$

Then, the comparison of the two methods can be performed.

a) The data are insufficient for a decision when: $x_L < -2L$ and $x_U > 0$ *or* $x_L < 0$ and $x_U > +2L$

In this case, additional samples are required and their number can be calculated using the following expression:

$$n = 4\left(\frac{s}{y}\right)^2$$

- *n*: number of samples required
- *s*: standard deviation of the relative difference
- *y*: max{ $\overline{x}$; | $\overline{x}$ | - | 2L |}
- *2L*: predetermined stipulated limit from 0 in the case that the methods are not different in %;
- $\bar{x}$: arithmetic mean of the relative difference in %.

b) Two methods are considered quantitatively not different if: -2L ≤ xL ≤ -2L and 0 ≤ xU ≤+2L

This happens when the mean relative of the paired confirmed counts does not differ significantly from zero and the confidence interval does not extend beyond the level of the predetermined stipulated limit.

c) The methods are statistically different, but the difference is too small to be microbiologically significant when: $x_L > -2L$ and $x_U < 0$ *or* $x_L > 0$ and $x_U < +2L$

d) The methods are different when: $x_L > 0$ *or* $x_U < 0$

2.11.3.2 Standard for methods comparison from US EPA Site-Specific Alternative Recreational Criteria Technical Support Materials for Alternative Indicators and Methods

The US EPA Site-Specific Alternative Recreational Criteria Technical Support Materials For Alternative Indicators and Methods (USEPA, 2014) can be used to compare two methods through the definition of specific indexes or coefficients. This procedure is specifically referred to ambient water and not to wastewater, but provide a simple methodology to evaluate if an alternative method can be used as a substitute of a standard one, for a specific site. Environmental samples, representative of local conditions where the method will be applied, should be used, and at least 30 paired data are required. Instructions for samples selection are specifically provided in the US EPA Site-Specific Alternative Recreational Criteria Technical Support Materials For Alternative Indicators and Methods (USEPA, 2014) documentation. If one of the paired measurements is below the limit of quantification or above the maximum level of detection, the coupled data should be removed. Outliers can be eliminated but must be justified.

Data a_i and b_i from the two methods are first transformed in their respective logarithmic values x_i and y_i:

$$x_i = [log_{10}(a_i)]$$

$$y_i = [log_{10}(b_i)]$$

Then the Index of Agreement (IA) is calculated, varying from 0 to 1:

$$IA = 1 - \frac{\frac{1}{N}\sum_{i=1}^{N}(x_i - y_i)^2}{\frac{1}{N}\sum_{i=1}^{N}(|x_i - \bar{x}| + |y_i - \bar{y}|)^2}$$

Where:

- N is the total number of data points in the data set;
- $\bar{x}$ and $\bar{y}$ are, respectively, the averages of the *x* and *y* data set.

The agreement of two methods is considered sufficient when IA ≥ 0.7.

If IA < 0.7, then the Pearson's correlation coefficient squared, R-squared, should be calculated. If the R-squared value is higher than 0.6, the alternative method can still be used, deriving new numerical limits through linear regression of the log-transformed data, in order to evaluate the geometric mean and the statistical threshold value.

2.11.3.3 Methods to assess the accuracy of ALERT data

For estimating the accuracy of measurements performed by ALERT devices, in this work were used the indications reported by the ISO 5725-1. The accuracy describes the combination of random and systematic errors. So high accuracy requires both high precision and high trueness (i.e., low bias).

Bias

Bias checks whether two measurement methods agree on average or whether there exists a systematic bias. If both methods agree, the average difference between paired samples should be zero. In microbiology, a log-normal distribution is a common assumption, so that the difference between a single pair *i* of measurements of ALERT and laboratory samples is expressed on a log-scale, by:

$$x_i = \log ALERT_i - \log LAB_i$$

The point estimate for the bias is calculated then by:

$$\bar{x} = \frac{\sum x_i}{n}$$

The calculated point estimate is subject to uncertainty. The 95 % confidence interval for estimating the bias can be readily calculated by:

$$confidence\ interval\ (x_{Lower}, x_{Upper}) = \bar{x} \pm 1.96 * se$$

with *se*, which estimates the error on the bias, is calculated as:

$$se = \frac{sd}{\sqrt{n}}$$

Where the standard deviation, *sd*, is calculated as:

$$sd = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

This procedure follows a classical paired t-test.

It is important to note that the bias is normally calculated for repeated measurements of the same reference quantity, which is precisely known. In the case of microbiology, there are no concentration standards. Therefore, a measurement of bias can be performed by performing repeated measurements of *E.coli* concentrations in the same sample using two methods, one of which is considered the reference. The bias may depend on actual concentration (i.e., may not be a

unique value across the full range of measurements), and should be evaluated at each relevant concentration. Bias measures the systematic errors produced by a measurement, method or instrument.

Precision

Precision in metrology refers to the variability between different measurements of the same specimen. Many different factors may contribute to the variability of results from a measurement method (ISO 5725-1:1994), including, the operator, the equipment used and its calibration, the environment or the time elapsed between measurements. The general term for variability between repeated measurements is precision. Two conditions of precision, termed repeatability and reproducibility conditions, have been found necessary for describing the variability of a measurement method. Under repeatability conditions, factors listed above are considered constants and do not contribute to the variability, while under reproducibility conditions they vary and do contribute to the variability of the test results. Thus repeatability and reproducibility are the two extremes of precision, the first describing the minimum and the second the maximum variability in results. Other intermediate conditions between these two extreme conditions of precision are also conceivable, when one or more of factors may vary, and are used in certain specified circumstances. Precision is normally expressed in terms of standard deviations.

In statistics, the precision is assimilated to the standard deviation of repeated measurements of the same sample.

$$precision = \frac{1}{\sigma^2}$$

In DWC precision was addressed in the replication study in Berlin:

a. within - ALERT - repeatability based on repeated measurements

- Repeated measurements of same sample with ALERT-Lab and ALERT-System at seven concentration levels spiked with WWTP secondary effluent.

$$sd_{ALERT} = sd(\log ALERT_i))$$

b. within - laboratory - repeatability

- Repeated measurements of same sample at 2 difference laboratories at seven concentration levels spiked with WWTP secondary effluent. σ, σ^2 of obtained measurement at each concentration are calculated.

$$sd_{Lab,i} = sd(LAB_i)$$

2.11.4 Results and data analysis of ALERT systems

Alert V1 data versus lab data were plotted in log scale (Figure 44), in order to evaluate the linear correlation between values and visually identify possible outliers.

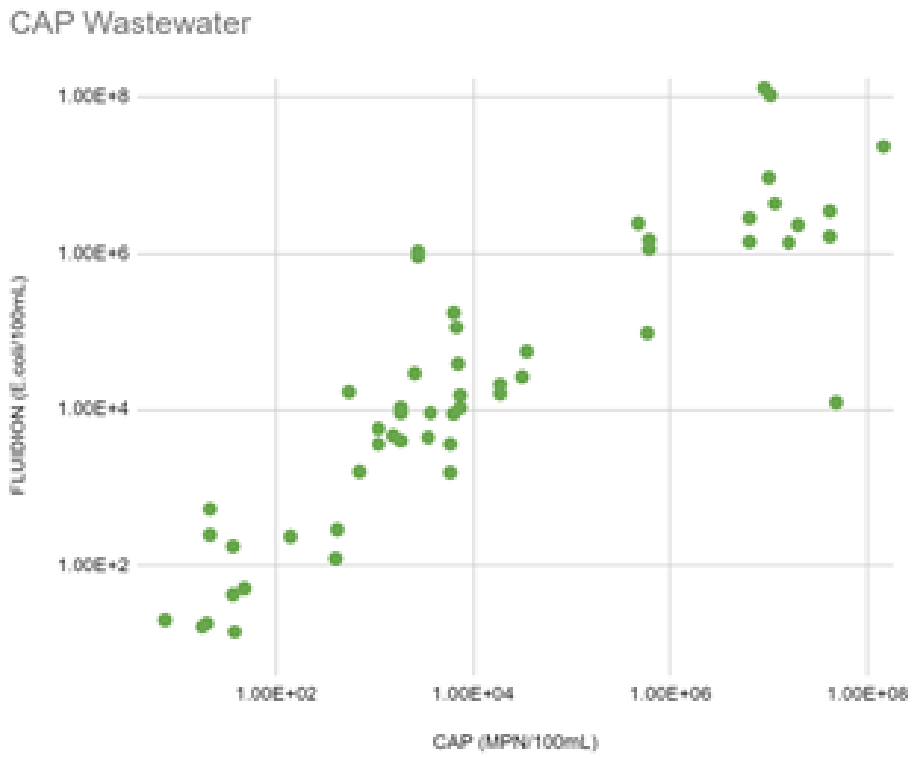


Figure 44: Lab vs Alert data in Milan sampling campaign

The correlation coefficient (R) for data in Figure 44 is 0.8, the bias was calculated to be -0.35 $\log_{10}$ units, while the precision (calculated as standard deviation of the log differences) is 1.81 $\log_{10}$ units.

Data from the in situ measuring campaign were elaborated to be compared with lab measures. According to the ISO 17994:2014, for the Milan case study, the following parameters were calculated:

$\bar{x}$= -81.72%

s = 417.68%

$\bar{s}$= 50.65%

$$W = 101.30\%$$

$$x_L = -183.03\%$$

$$x_U = 19.58\%$$

Since x_L < -2L and x_U > 0, the result of the comparison is inconclusive and further measures are required.

Considering the US EPA comparison method by calculating the Index of Agreement, it was obtained:

$$IA = 0.88 > 0.7$$

In this case, the comparison between the two set of data can be considered satisfied.

For the ALERT V2 campaign, the results with the two different cartridges used were analysed. A first sampling campaign was carried out with the conventional cartridges without filters, as shown in Figure 45. Lab and Alert time-series indicated very similar relative trends, but with an almost constant offset of 0.98 $\log_{10}$ units between the two curves. The laboratory average also seemed to be about an order of magnitude too low for untreated effluent, when compared to similar samples measured at other WWTP with similar treatment stages (S. Azimi & V. Rocher, 2021).

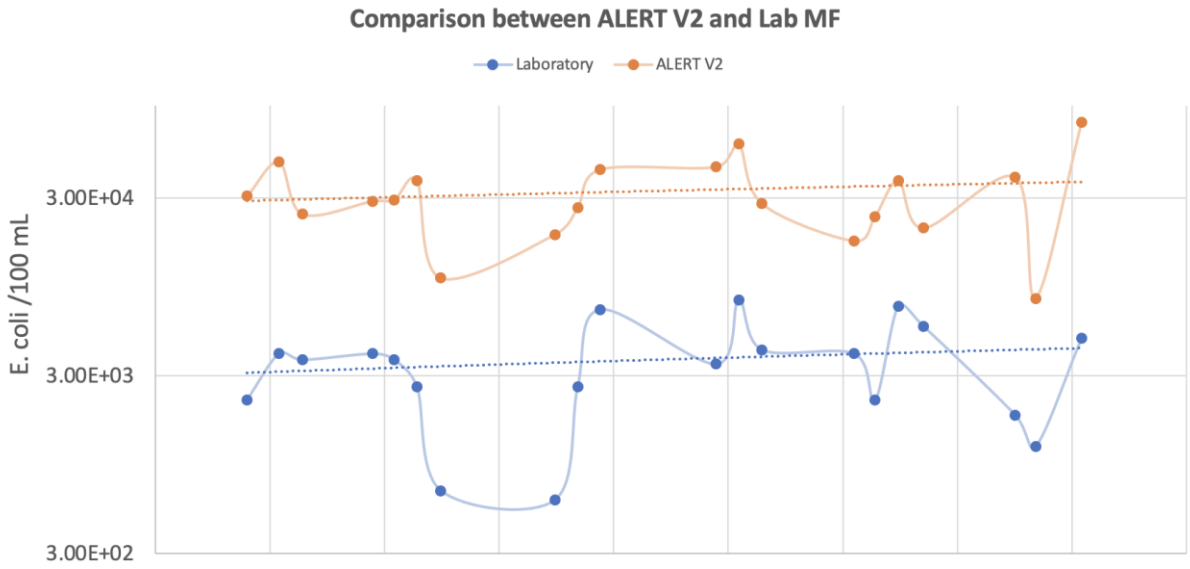


Figure 45: The side-by-side timeseries obtained in the Milan WWTP installation in the first half of the 2021 campaign.

A hypothesis to understand the offset could be that at the installation site there was a significant presence of bacteria attached to particles. It is known from TSS measurements that a large quantity of solids was present in the effluent. Faecal particles can create large discrepancies between the standard laboratory method and the ALERT V2 method, which originates from the fact that the standard methods are unable to quantify attached bacteria, since the procedures require a filtration preliminary phase. Indeed, a faecal particle is counted as a single bacterium by the standard laboratory methods, although it could contain thousands of bacteria in reality. ALERT V2 does measure all the bacteria present in the sample, including all the bacteria attached to particles, since they all contribute to the substrate metabolization which generates the fluorescent signal. This difference is fundamental and leads to a number of interesting questions that will be outlined later.

In order to test this hypothesis, a second campaign was initiated in 2021. This time, at every sampling time, two samples were analysed in the ALERT V2, one not filtered (similar to first campaign), and one which was automatically filtered at 5µm at sampling time. It must be noticed that the 5µm filtration size is larger than a bacterium (0.5µm diameter, 2µm length) so this filter will remove large particles but may still allow smaller bacterial aggregates to pass through.

A total of 11 laboratory samplings were performed, with results from the laboratory, ALERT V2 without filter, and ALERT V2 filtered. Figure 46 shows the comparative time series. Every filtered ALERT V2 measurement showed significantly lower bacterial charge than its unfiltered counterpart, sometimes by as much as 1.83 $\log_{10}$ units (0.62 $\log_{10}$ units on average), confirming the hypothesis that particles play a major role in the observed phenomenon, and that the large majority of bacteria was attached to particles larger than 5µm.

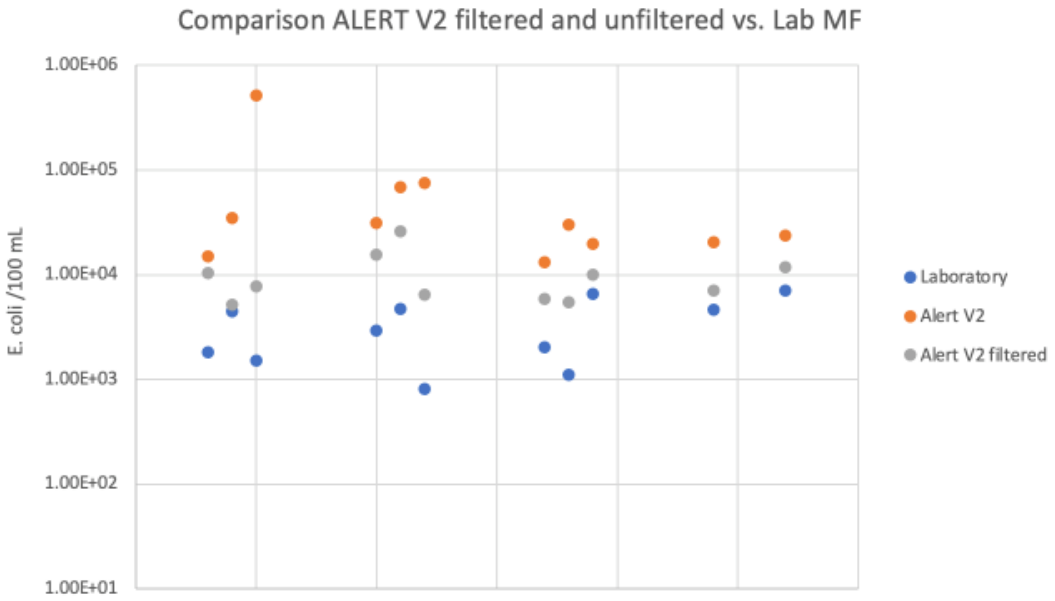


Figure 46: Comparative time series obtained during the second 2021 ALERT V2 campaign at the Peschiera Borromeo WWTP. The laboratory data (in blue) is compared to the ALERT V2 unfiltered sample (orange) and, respectively, with the ALERT V2 sample filtered at 5µm (in light gray)

It is also observed in Figure 46 that the ALERT V2 filtered measurement values were significantly closer to the laboratory value but still slightly higher, which suggested that particles smaller than 5µm which might pass through the filter were also present. For some of the data points the ALERT V2 and laboratory measurements were very close, within 0.20 $\log_{10}$ units.

2.11.5 Benchmarking and comparison of ALERT data in the three case studies

To obtain a benchmarking of ALERT devices performance, results were evaluated considering different indicators, such as correlation coefficient, bias, precision and IA, and including the three case studies where the ALERT devices were tested in DWC. Results of all the three case studies were presented, in order to compare the ALERT performances evaluated in Milan with wastewater matrix with the ones obtained in Paris with surface water and in Berlin, where additional tests were performed to evaluate the precision.

It is important to note that in 2019 Paris and Milan cases, the results were aggregated over many different types of samples, sometimes using different protocols. The benchmarking factors therefore are not uniform, do not reflect the expected performance in a fixed environment or water matrix, and should be used with caution.

In the Berlin case, the two studies conducted using different methodologies were both reported. The 2019 study used an ALERT System in the Spree river, compared to side-by-side lab measurements. In 2019 no assessment was done of inter laboratory variability, or of repeatability. ALERT LAB was not used in 2019. In 2020, the Berlin study used a different methodology. Both ALERT System V1 and ALERT LAB were assessed, and two laboratory measurements were systematically performed for each determination, to allow an evaluation of the current capabilities using the regulatory approved methods (i.e., benchmarking of the approved laboratory 1 data against approved laboratory 2 data).

It is also important to note that the ALERT SYSTEM used in all the pre-2020 studies was the V1, which requires manual disinfection of the tubes between two measurements. It was apparent that the poorer precision of the ALERT V1 (visible only at lower concentrations) was due to residual disinfectant leftover from the maintenance killing a portion of the bacterial load.

Table 25: Summary of parameters that can be used as benchmarking of ALERT system in the three demo-cases

	CORRELATION COEFFICIENT (R)	BIAS	PRECISION	ISO 17994:2014	IA (US-EPA method)
PARIS 2019 ALERT V1, LAB	0.9	-0.029 log_{10} units	0.34 log_{10} units	Inconclusive	0.96 > 0.7
PARIS 2021 ALERT V2	N/A	0.06 log_{10} units	0.10 log_{10} units	N/A	N/A
BERLIN 2021 ALERT V2	0.99	-0.24 log_{10} removable with single point offset correction	From 0.03 log_{10} (@1E4 MPN/100mL) to 0.15 log_{10} (@1E2 MPN/100mL)	N/A	N/A
BERLIN 2020 ALERT LAB SPREE	N/A	0.12 log_{10} units	0.28 log_{10} units	N/A	N/A
BERLIN 2020 ALERT V1 SPREE	N/A	0.03 log_{10} units	0.47 log_{10} units	N/A	N/A
BERLIN 2020 LAB SPREE	N/A	0.02 log_{10} units	0.20 log_{10} units	N/A	N/A
BERLIN 2019 ALERT V1 SPREE	0.8	-0.10 log_{10} units	0.58 log_{10} units	Inconclusive	0.90 > 0.7
MILAN 2019 ALERT V1 ALERT LAB	0.8	-0.355 log_{10} units	1.81 log_{10} units	Inconclusive	0.88 > 0.7
MILAN 2021 ALERT V2	N/A	In the Milan 2021 study the difference between the ALERT V2 and the laboratory is probably due to a major portion of the bacterial load not being detected by the laboratory, due to the presence of significant amounts of bacteria attached to particles.			

Overall, the different campaigns performed in 2019, 2020 and 2021 capture the limitations of the previous version of the ALERT System technology, and demonstrate the major improvements obtained in the ALERT V2 through the use of disposable cartridges.

Comparing sensor results with lab measurements, it emerged that sensors could reach good performances, with a correlation coefficient of 0.8, bias of -0.35 log_{10} units and a precision of 1.81 log_{10} units on wastewater matrix.

The data from the Milan 2021 campaign investigated the bias observed at this location, probably due to the presence of large bacterial charges on particles that couldn't be measured using standard laboratory methods, that required a pre-filtering process, but was detected by the ALERT systems. It was also shown that the ALERT V2 could be installed with a prefilter to eliminate the larger particles, obtaining results considerably closer to those measured by the laboratory.

The 2021 campaigns in Paris demonstrated the successful operational deployment in both outdoor riverine settings, and within a wastewater treatment plant. The Paris 2021 side-by-side data, although insufficient for applying ISO or EPA methodology for method comparison, demonstrates the excellent agreement of ALERT V2 and laboratory data, both in terms of bias (0.058 $\log_{10}$ units) and precision (0.099 $\log_{10}$ units, and coherent with the one measured in the repeatability study).

In 2021 only the repeatability study in Berlin provides a quantitative basis for comparing the performance of ALERT SYSTEM V2 *vs.* the previous version of the product, ALERT SYSTEM V1. The major improvements in metrology performance measured for the ALERT V2, both in terms of linearity and precision, demonstrate that the single-use cartridge concept does eliminate the errors associated with residual disinfectant left in the sampling tubes after the maintenance procedures, which affected the measurements of the ALERT V1, particularly at the low end of the concentration range. The excellent linearity allows correcting the observed offset which was constant over the full concentration range using a single-point correction. It is recommended that this correction be applied at a concentration around 1.0E+4 *E.coli*/100 mL, where the ALERT V2 shows the highest level of precision. With such simple single-point correction, the ALERT V2 data becomes of similar quality to the laboratory data, both in terms of accuracy and precision (the laboratory shows slightly better precision at concentrations below 1.0E+3, whereas ALERT V2 is more precise than the laboratory at higher concentrations).

Through the different series of operational deployments and in-lab repeatability studies performed independently within DWC, between 2019 and 2021, by industry professionals, and analysed using approved laboratories, ALERT V2 and the ALERT LAB were validated as accurate and precise bacterial measurement systems that enable both in-situ and, respectively, portable analyses of samples to be performed with outstanding simplicity, very limited human intervention, and without having to send samples to an external approved laboratory.

References

A.H. Havelaar, J.M.Melse. (2003). Quantifying Public Health Risk in the WHO Guidelines for Drinking Water Quality. WHO RIVM Report, June 2014, 1–49.

Angelescu, D. E., Hausot, A., Huynh, V., and Wong, J., 2018. An In-Situ Autonomous Bacterial Pathogen Sensor for Water Quality and Environmental Monitoring Applications. Weftec Proceedings, Water Environ. Fed. 3982–4403.

Angelescu, D.., Abi-Saab, D., Hausot, A., Huynh, V., Rocher, V., Azimi, S., Guérin, S., 2020. In-situ monitoring of fecal bacteria in wastewater treatment processes using ALERT rapid microbiology instrumentation. Ed. Int. Water Assoc.

Antonelli, M.; Turolla, A.; Mezzanotte, V.; Nurizzo, C. Peracetic Acid for Secondary Effluent Disinfection: A Comprehensive Performance Assessment. Water Science and Technology 2013, 68, 2638–2644, doi:10.2166/wst.2013.542.

Aquastat, 2020. Water Exploitation Index-Aquastat (https://ec.europa.eu/eurostat/databrowser/view/t2020_rd220/default/table?lang=en) [WWW Document].

Arvaniti, O.S.; Dasenaki, M.E.; Asimakopoulos, A.G.; Maragou, N.C.; Samaras, V.G.; Antoniou, K.; Gatidou, G.; Mamais, D.; Noutsopoulos, C.; Frontistis, Z.; et al. Effectiveness of Tertiary Treatment Processes in Removing Different Classes of Emerging Contaminants from Domestic Wastewater. Front Environ Sci Eng 2022, 16, doi:10.1007/s11783-022-1583-y.

Arzate, S.; Pfister, S.; Oberschelp, C.; Sánchez-Pérez, J.A. Environmental Impacts of an Advanced Oxidation Process as Tertiary Treatment in a Wastewater Treatment Plant. Science of The Total Environment 2019, 694, 133572, doi:10.1016/j.scitotenv.2019.07.378.

Carvalho, P.N.; Finger, D.C.; Masi, F.; Cipolletta, G.; Oral, H.V.; Tóth, A.; Regelsberger, M.; Exposito, A. Nature-Based Solutions Addressing the Water-Energy-Food Nexus: Review of Theoretical Concepts and Urban Case Studies. J Clean Prod 2022, 338.

Collivignarelli, M.C.; Abbà, A.; Benigna, I.; Sorlini, S.; Torretta, V. Overview of the Main Disinfection Processes for Wastewater and Drinking Water Treatment Plants. Sustainability (Switzerland) 2018, 10, doi:10.3390/su10010086.

DEMOWARE, 2017. DEMOWARE Project [WWW Document]. http://www.demoware.eu.

Deviller, G., Lundy, L., Fatta-Kassinos, D., 2020. Recommendations to derive quality standards for chemical pollutants in reclaimed water intended for reuse in agricultural irrigation. Chemosphere 240. https://doi.org/10.1016/j.chemosphere.2019.124911.

Dingemans, M., Bartholomeus, R., Medem, G., 2018. Evaluation of the proposed EU regulation on minimum requirements for water reuse for irrigation.

EPA, 2012. Guidelines for Water Reuse. USA.

European Commission, 2022. COMMISSION NOTICE - Guidelines to support the application of Regulation 2020/741 on minimum requirements for water reuse (2022/C 298/01).

European Parliament, 2020. EU-REGULATION 2020/741 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 May 2020 on minimum requirements for water reuse.

Foglia, A.; Akyol, Ç.; Frison, N.; Katsou, E.; Eusebi, A.L.; Fatone, F. Long-Term Operation of a Pilot-Scale Anaerobic Membrane Bioreactor (AnMBR) Treating High Salinity Low Loaded Municipal Wastewater in Real Environment. Sep Purif Technol 2020, 236, 116279, doi:10.1016/j.seppur.2019.116279.

González-Camejo, J.; Ferrer, J.; Seco, A.; Barat, R. Outdoor Microalgae-Based Urban Wastewater Treatment: Recent Advances, Applications, and Future Perspectives. Wiley Interdisciplinary Reviews: Water 2021, 8.

González-Camejo, J.; Jiménez-Benítez, A.; Ruano, M. v.; Robles, A.; Barat, R.; Ferrer, J. Optimising an Outdoor Membrane Photobioreactor for Tertiary Sewage Treatment. J Environ Manage 2019, 245, 76–85, doi:10.1016/j.jenvman.2019.05.010.

Goodwin, D.; Raffin, M.; Jeffrey, P.; Smith, H.M. Collaboration on Risk Management: The Governance of a Non-Potable Water Reuse Scheme in London. J Hydrol (Amst) 2019, 573, 1087–1095, doi:10.1016/j.jhydrol.2017.07.020.

Grandclément, C.; Seyssiecq, I.; Piram, A.; Wong-Wah-Chung, P.; Vanot, G.; Tiliacos, N.; Roche, N.; Doumenq, P. From the Conventional Biological Wastewater Treatment to Hybrid Processes, the Evaluation of Organic Micropollutant Removal: A Review. Water Res 2017, 111, 297–317, doi:10.1016/j.watres.2017.01.005.

Kobayashi, Y.; Ashbolt, N.J.; Davies, E.G.R.; Liu, Y. Life Cycle Assessment of Decentralized Greywater Treatment Systems with Reuse at Different Scales in Cold Regions. Environ Int 2020, 134, 105215, doi:10.1016/j.envint.2019.105215.

Lavrnić, S., Zapater-Pereyra, M., Mancini, M.L., 2017. Water Scarcity and Wastewater Reuse Standards in Southern Europe: Focus on Agriculture. Water, Air, and Soil Pollution 228. https://doi.org/10.1007/s11270-017-3425-2.

Lavrnić, S.; Zapater-Pereyra, M.; Mancini, M.L. Water Scarcity and Wastewater Reuse Standards in Southern Europe: Focus on Agriculture. Water Air Soil Pollut 2017, 228, doi:10.1007/s11270-017-3425-2.

Maffettone R., Gawlik B.M., Technical Guidance - Water Reuse Risk Management for Agricultural Irrigation Schemes in Europe, Publications Office of the European Union, Luxembourg, 2022, doi:10.2760/590804, JRC129596.

Maffettone, R.; Manoli, K.; Santoro, D.; Passalacqua, K.D.; Wobus, C.E.; Sarathy, S. Performic Acid Disinfection of Municipal Secondary Effluent Wastewater: Inactivation of Murine Norovirus, Fecal Coliforms, and Enterococci. Environ Sci Technol 2020, 54, 12761–12770, doi:10.1021/acs.est.0c05144.

Mara, D. (2006). Guidance note for Programme Managers and Engineers A Numerical Guide to Volume 2 of the Guidelines and practical advice on how to transpose them into National. 2(January 2008), 1–12.

Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, Australian Health Ministers' Conference, 2006. Australian Guidelines for water recycling: managing health and environmental risks.

Ofori, S.; Puškáčová, A.; Růžičková, I.; Wanner, J. Treated Wastewater Reuse for Irrigation: Pros and Cons. Science of the Total Environment 2021, 760.

Radini, S., Marinelli, E., Akyol, Ç., Eusebi, A. L., Vasilaki, V., Mancini, A., Frontoni, E., Bischetti, G. B., Gandolfi, C., Katsou, E., & Fatone, F. (2021). Urban water-energy-food-climate nexus in integrated wastewater and reuse systems: Cyber-physical framework and innovations. *Applied Energy*, *298*. https://doi.org/10.1016/j.apenergy.2021.117268

Rizzo, L., Gernjak, W., Krzeminski, P., Malato, S., McArdell, C.S., Perez, J.A.S., Schaar, H., Fatta-Kassinos, D., 2020. Best available technologies and treatment trains to address current challenges in urban wastewater reuse for irrigation of crops in EU countries. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2019.136312

Russo, N.; Marzo, A.; Randazzo, C.; Caggia, C.; Toscano, A.; Cirelli, G.L. Constructed Wetlands Combined with Disinfection Systems for Removal of Urban Wastewater Contaminants. Science of the Total Environment 2019, 656, 558–566, doi:10.1016/j.scitotenv.2018.11.417.

Salgot, M., Folch, M., 2018. Wastewater treatment and water reuse. Current Opinion in Environmental Science and Health. https://doi.org/10.1016/j.coesh.2018.03.005.

Seco, A.; Jiménez, A.; Ruiz-Martínez, A.; Ferrer, J. AnMBR Technology to Treat Sewage and Reuse It in Agriculture (Tecnología AnMBR Para Tratamiento de Aguas Residuales Urbanas y Reutilización de Agua En Agricultura). RETEMA-Revista Técnica de Medio Ambiente 2018, 207, 8–16.

Seis, W. (2012). Risk assessment of Braunschweig wastewater reuse scheme.

Seis, W. (2022). QMRA (Version 0.1.3) [Computer software] (p. https://doi.org/10.5281/zenodo.6457511).

Seis, W., & Remy, C. (2013). DEMOWARE D3.1 Appropriate and user-friendly methodologies for Risk assessment, Life Cycle Assessment, and Water Footprinting. 1(619040).

Semerjian, L.; Shanableh, A.; Semreen, M.H.; Samarai, M. Human Health Risk Assessment of Pharmaceuticals in Treated Wastewater Reused for Non-Potable Applications in Sharjah, United Arab Emirates. Environ Int 2018, 121, 325–331, doi:10.1016/j.envint.2018.08.048.

Sgroi, M.; Snyder, S.A.; Roccaro, P. Comparison of AOPs at Pilot Scale: Energy Costs for Micro-Pollutants Oxidation, Disinfection by-Products Formation and Pathogens Inactivation. Chemosphere 2021, 273, doi:10.1016/j.chemosphere.2020.128527.

Sgroi, M.; Vagliasindi, F.G.A.; Snyder, S.A.; Roccaro, P. N-Nitrosodimethylamine (NDMA) and Its Precursors in Water and Wastewater: A Review on Formation and Removal. Chemosphere 2018, 191, 685–703.

Tchobanoglous, G.; Stensel, D.H.; Tsuchihashi, R.; Burton, F.L. Wastewater Engineering - Treatment and Resource Recovery. By Metcalf & Eddy, Inc. New York: McGraw-Hill ; 5th ed.; 2014.

TEG EU Technical Expert Group on Sustainable Finance. Taxonomy: Final Technical Report; 2020.

The European House - Ambrosetti, 2022. Libro Bianco 2022. Valore acqua per l'Italia. 3 Edizione.

Thompson, M.; Moussavi, S.; Li, S.; Barutha, P.; Dvorak, B. Environmental Life Cycle Assessment of Small Water Resource Recovery Facilities: Comparison of Mechanical and Lagoon Systems. Water Res 2022, 215, 118234, doi:10.1016/j.watres.2022.118234.

Traini, S. The EU Taxonomy and Water Utilities: How to Align with the New Paradigm to Finance Sustainable Investments. REF Ricerce. In Proceedings of the ECOMONDO. The Green Technology Expo. Rimini, Italy, 2021.

UNEP, 2005. Guidelines for Municipal Water Reuse in the Mediterranean Region.

WHO, 2006. Guidelines for the safe use of wastewater, excreta and greywater.

WHO, 2016. Sanitation Safety Planning - Manual for safe use and disposal of wastewater, greywater and excreta.

WHO, 2016. Quantitative Microbial Risk Assessment: Application for Water Safety Management.

WRE, 2022. Water Reuse Europe (WRE) - (water-reuse-europe.org). [WWW Document].

3. Risk management for bathing water

Water bodies allowed for bathing are increasing in Europe, reaching 21 968 sites in 2020, two thirds of them representing coastal seawater. On the same trend, bathing water quality is also continuously improving, with 83% of sites characterized by excellent water quality status, according to EU regulation, and a decreasing number of poor-quality sites, varying from 2% in 2013 to 1.3% in 2020 (EEA 2021). This aspect is also reflected in urban waters, thanks to an improved management along the water and wastewater cycle, the application of an integrated framework of regulations and the construction of dedicated infrastructures for water treatment and management. Bathing Water Directive (BWD) and Water Framework Directive (WFD) helped to reduce the impacts of point-sources of contamination, such as the discharges of WWTPs. However, potential contamination sources include also diffuse inputs, such as urban and agricultural run-offs, which may occur during precipitation and flooding events. Extreme rainfall events may impact on sewer overflows and thus on surface water quality, since they may increase bacterial loads and concur in environmental contamination, affecting both human health and ecosystem (Penna et al., 2020). Moreover, climate changes and weather events are inducing bigger impacts even on temperate areas. The exposure of waterborne microorganism present in bathing water may conduct, other than to the possibility of diseases, to the distribution of antimicrobial resistant (AMR) organisms (Farrell et al, 2021). Thus, an integrated monitoring appears to be fundamental to control quality parameters and avoid negative health outcomes (Varma et al., 2019).

Nowadays bathing sites can also be found in European Capitals as Berlin, Copenhagen or Amsterdam (EEA, 2020), once considered unsafe and contaminated environments.

The general increase of water quality responds to the increased demand for bathing areas, due not only to a deeper consciousness on health and environmental impacts, but also on its potential for tourism and local economy development (EEA, 2021). Safe bathing areas represents a valuable attractive for recreational activities and tourism industry (Kaas et al., 2011). In a study conducted among bathers in Berlin, 46% of the interviewed showed willingness to pay for the quality improvement of bathing sites (Van L.A. et al., 2022). Public opinion towards bathing quality must be considered for the good management of bathing sites. Different strategies were proposed to inform citizens and costumers about bathing water quality. In a study conducted by Quilliam et al. (2019), two bathing sites in the UK were selected as case studies. From the interviews conducted among the bathers, it emerged that most of the beach-users were not aware of the quality classification signals of the EU regulation and were not informed about the presence of information boards or water quality predictions performed in the sites. On the other hand, the minimum risk level that the interviewed considered as acceptable in contracting a gastrointestinal infection was estimated at 1% in terms of probability of illness, which is actually much stringent than the restrictions required for the classification as “good” quality status. The discrepancy between the awareness on the current quality status and the expected risk levels highlighted the importance of tools for public information and dissemination (Quilliam et al., 2019).

3.1 Current legislation

At European level, the Bathing Water Directive (BWD) 2006/7/EC of the European Parliament and of the Council regulates the quality classification of water bodies, depending on the concentration levels of microbial contaminants *Escherichia coli* and *Intestinal Enterococci*. It also provides indication on monitoring campaigns and data elaboration. The water quality is in fact determined considering the 95th percentiles of the log_{10} measured data, obtained from at least four years of monitoring campaigns, performed during bathing season (H.J. Mälzer et al., 2016). Particular attention is also paid to improved monitoring measures, to ensure health protection and safe recreational uses (Penna et al., 2021).

Bathing site managers are required to monitor water quality regularly and develop the so-called bathing water profiles, to keep the public informed about bathing water quality and beach management. Bathing water profiles include information about water quality and possible sources of health risks, such as WWTP discharges, and thus represent a tool to support managers in risk prioritization.

Even if bathing water profiles should have been ready by March 2011, according to an interview to French local authorities (Agence Régionale de Santé), this process has not been finalized yet by some local authorities.

Minimum monitoring frequency is set to one sampling each month, during the bathing season. However, according to local laws or to the expected more stringent requirements of the regulation currently in revision, sampling frequency will probably increase.

The regulation assumes that bathing sites can be subjected to short-term pollution events, which are caused by well-known sources and are characterised by a duration of less than 72 hours, but in that case monitoring measures and early warning systems (EWSs) must be applied to ensure health protection. With the inclusion of alert systems and prediction tools, the BWD aimed to encourage the use of support tools to protect bathers' health from short term pollution events, establishing that, in case managers adopt EWS, 15% of monitoring samples can be disregarded (Viegas C. et al., 2012). However, no further information is provided on the development and the assessment of EWSs, the parameters or the indicators to be monitored and the thresholds to be considered to set the alarms (W. Seis et al., 2018).

The BWD is in line with the approach carried out for the Water Framework Directive and the Urban Wastewater Treatment Directive, considering a holistic approach for health and environment protection (Water Europe 2022). A review of BWD is currently under development, after a period of feedback collection and public consultation, with an expected increase of requirements stringency from 2023.

3.2 Management improvements and monitoring tools

Bathing water management is performed by local and national public, health and environmental authorities, that should ensure a safe use of recreational waters, both by dedicated monitoring and by applying integrated measures on the upstream flows, which may represent potential sources of pressures for bathing water quality. The integrated approach on the urban water cycle, with controls on wastewater treatment plants (WWTPs) discharges, as well as on combined sewer overflows (CSOs) and urban run-off, helps to reduce pollution and improve bathing water quality.

According to the European standards outlined in the Urban Wastewater Treatment Directive, sewage wastewater should have been treated with at least full biological treatment before being discharged, to protect the environment and bathing water quality. In many European countries, much work has also been done to reduce run-off from farms in the catchment areas into rivers and streams flowing to the bathing waters. Despite these extensive improvements, the risk of short-term pollution events during or following substantial rainfalls still remains. In fact, diffuse sources of pollution and combined sewer overflows, together with punctual malfunctions or limited treatment capacities, may cause environmental and social problems, especially in the surroundings and in downstream locations (Crocetti et al., 2021). Specific actions must be carried out to face those phenomena and minimize the connected risks.

Rain events may affect water quality, introducing short-term pollution and microbial contamination. In recent years, around 4000 of such short-term pollution events were recorded in Europe, meaning that they represent a significant impact on bathing water quality, but also that monitoring is often more implemented to detect those events (EEA, 2021).

Bathing water quality can also affect the local economy, especially as concern touristic sites. Non-compliance with regulatory limits can imply the closure of the bathing site for at least 3 days in most of the cases, with impacts on the local socio-economic activities.

The effectiveness and representativeness of monitoring are also affected by spatial and temporal variability of water quality. Standard grab samples may not be effective in identifying those trends, as well as in detecting unexpected events. Bathing water managers are encouraged to develop tools for monitoring, warning and predicting bathing quality, to ensure health protection from short term pollution (Viegas C. et al., 2012). It must also be noticed that while continuous monitoring and forecasting models aim to detect water quality variability to ensure an optimised control level, measurements campaigns are usually performed on grab instantaneous samples and thus they could not detect the occurrence of single pollution events, nor their duration. On the other hand, prediction models and monitoring tools require calibration in order to consider site-specific conditions and their accuracy must be evaluated against the standard methods.

Standard monitoring campaigns are only describing instantaneous conditions in space and time and their representativeness of the general status of the system is highly affected by their distribution and frequency, as well as the level of steadiness of bacteria concentration (Zhang et al., 2012). A huge amount of efforts, both in terms of money, time and expertise, is required to plan and perform a monitoring sampling campaign (Kaas et al., 2011).

On average, about 8 – 15 measurements are conducted during a bathing season of 100 days, also depending on the site-specific conditions. Costs for the communities are estimated to be over than 100 € for each analysis performed, meaning that the costs for one bathing season monitoring are around 800 – 1500 €.

Moreover, the response time of standard lab analyses is not feasible for real-time warning, since by the time the manager is aware of the results, bathers may have been already exposed to the health hazard (Kaas et al. 2011). In addition to that, due to their discrete nature, grab sampling are not suitable to detect short-term pollution events, which highly affect recreational water quality, especially as concern urbanised areas (WHOCC, 2011). In fact, discharges affected by rainy events were observed to have major impacts on water contamination (Viegas et al., 2009; Nevers and

Whitman, 2005; Wyer et al., 1994), and steady condition rarely occurs in coastal beach water (Boehm et al., 2005; Heberger et al., 2008; Hou et al., 2006).

Analytical analysis performed with standard methods alone, even if carried out following the regulation requirements of at least 4 measurements during the bathing season, are not able to detect pollutant sources. Mathematical modelling are thus useful tools to assess water quality and identify the sources of potential pressures and impacts (Bruschi et al., 2021). Digital tools, using innovative sensors or predictive models, could play a crucial role in monitoring, forecasting and decision support. Using those tools, early warning systems (EWSs) can be implemented to evaluate water quality and support decision making, providing relevant information to the competent authorities or to the public (Water Europe 2022), minimising health related risks and optimising bathing site management.

3.3 Early warning systems for bathing water

Early warning systems (EWSs) are tools developed to support monitoring, control and decision making, since they can detect anomalies or faults in real time and can make predictions to allow rapid intervention.

EWSs are generally based on models to create simulations and perform predictive scenarios, which are calibrated according to the real data provided. Different modelling approaches can be followed, depending also on the characteristics and the complexity of the system, the available data, and the required level of detail.

Deterministic models aim to represent the real system by describing its physical and chemical mechanisms with mathematical functions. They could include different sub-models to describe each specific aspect, such as hydraulics, weather conditions, diffusion, bacteria regrowth or decay rate. Deterministic models allow a detailed description of the system, as well as the performance of different scenarios, but they require a deep knowledge of the site-specific conditions, the mechanics and the correlations between all the parameters involved. On the other hand, statistic models perform probabilistic analysis on the observed data to define system behaviour. They require less information and do not include the understanding of the physical system, but their results are highly affected by the input data provided, both in terms of quantity and variability, and cannot respond to system changes.

Deterministic and statistic approaches have also been compared in other environmental studies, such as in model development to predict air pollution (G. Hoshyaripour et al., 2016). As concern bathing sites, different models to simulate and predict water quality have been developed, especially for coastal areas (Heberger et al., 2008; Jung et al, 2011; Kay et al., 1994; Lin et al., 2008; Zhang et al., 2012). In the Portuguese Estoril coast, a deterministic Lagrangian model has been developed for 48 hours predictions at three bathing sites, combining real-time data from hydrometric stations monitoring, historical data on coastal water and streams together with the modelling tool MOHIDWater. (Viegas et al., 2012). In Denmark and Sweden the Bathing Water Forecast System (BWF system) has been implemented by DHI (DHI Water Forecast, 2011). The system has been applied in Copenhagen since 2002 (Erichsen et al. 2003) and it was extended to 11 Danish and 3 Swedish municipalities. The BWF system includes hydrodynamic-ecological models (MIKE by DHI model tools: 1D, 2D and/or 3D) for the simulation of the physical conditions of the

sites and of bacteria behaviour. The BWF system allows real-time quantification and predictions for 3 or 4 days for bacteria concentration (Kaas et al., 2011).

Under the CADEAU project (assimilation of national water quality data in Coastal Areas for a marine Directives oriEnted downstreAm prodUct), a downstream coastal application of the Copernicus Marine Environment Monitoring Service (CMEMS, https://marine.copernicus.eu) was developed. The tool is based on a coupled hydrodynamic-biogeochemical modelling system integrated with observational data. The service was tested in the Italian Adriatic coastal area of Chioggia to provide information on bathing water quality, through the definition of indexes for water quality assessment (Bruschi et al., 2021).

In the framework of the EU Interreg Italy-Croatia Project WATERCARE, an integrated water quality platform was developed to support decision making about bathing water management. The tool uses hydro-meteorological models, coupled with automatic sampler that activates after rainfall events to validate the results of a forecasting system which predicts water quality (Penna et al., 2021).

In Scotland, the Scottish Environment Protection Agency (SEPA) manages the environmental monitoring of bathing sites. During the bathing season, the online service "BeachLine" provides and posts online daily water quality predictions in 30 Scottish bathing sites. The forecasting model is based on the previous 24-48h precipitations, the measured riverflows and levels monitored by gauging stations. The predictions allow managers to inform the public of predicted bathing water quality, either via the message 'Water quality is forecast to be acceptable today' or to make population aware of potentially poorer water quality due to a short-term pollution event (https://www2.sepa.org.uk/bathingwaters/Predictions.aspx).

3.4 Material and methods

3.4.1 Paris case study

The Seine is characterised by straight sections with low turbulence, that make it navigable for about two-thirds of its length. Its hydrographic regime shows maximum discharge in January and a minimum flow of about 70-100 m3/s in August. The city of Paris plays a significant impact on its basin, due to highly urbanised areas, such as Île de France, which with its 16 million inhabitants constitute about 28% of the French population. Sewage management includes 7 WWTPs, of which 2 are located in the city of Paris, and a series of combined sewer overflows, which, together with diffuse urban run-off, constitute the predominant source of contamination in wet periods (Van L.A. et al., 2022), (Servais et al., 2011).

Moreover, in 2024 Paris will held the next Olympic and Paralympic Games and for that occasion 23 bathing sites were identified on the Seine and Marne Rivers, of which 5 are located in the city centre (Van L.A. et al., 2022). Paris area is strongly committed to provide permanent and safe opportunities for bathing in the urban river as a legacy of the Olympics and Paralympic games 2024. Many efforts have already been done aiming at reducing drainage system impact on rivers. A more efficient and risk oriented digital approach has been carried out, with the active participation to European projects such as Digital Water City (DWC), which supported the development of an EWS for safe bathing water.

Models that could address microbial quality and quantify the impacts of point discharges of CSOs and WWTPs grew thus high interest, since they could evaluate bacteria contamination in surface water, analysing the impacts of point discharges from CSOs and WWTPs and the influence of rain events (Servais et al., 2011) (Van L.A. et al., 2022).

3.4.2 ProSe model

The deterministic model ProSe was developed in the context of the PIREN-Seine programme (Interdisciplinary Research Programme on water and the Environment of the Seine basin) for the study of Seine and Marne rivers in Paris. It includes hydraulic modules, as well as modules for the simulation of Faecal Indicator Bacteria (FIB) (Servais et al., 2011). More in detail, since it was observed that bacteria behaviour was deeply affected by the fraction attached to solid particles, the sub-model that describe bacteria behaviour include the definition of the settling fraction, the first order kinetic decay rate, the sedimentation rate, and the resuspension. Discharges from CSOs and WWTPs were considered as the main sources of contamination.

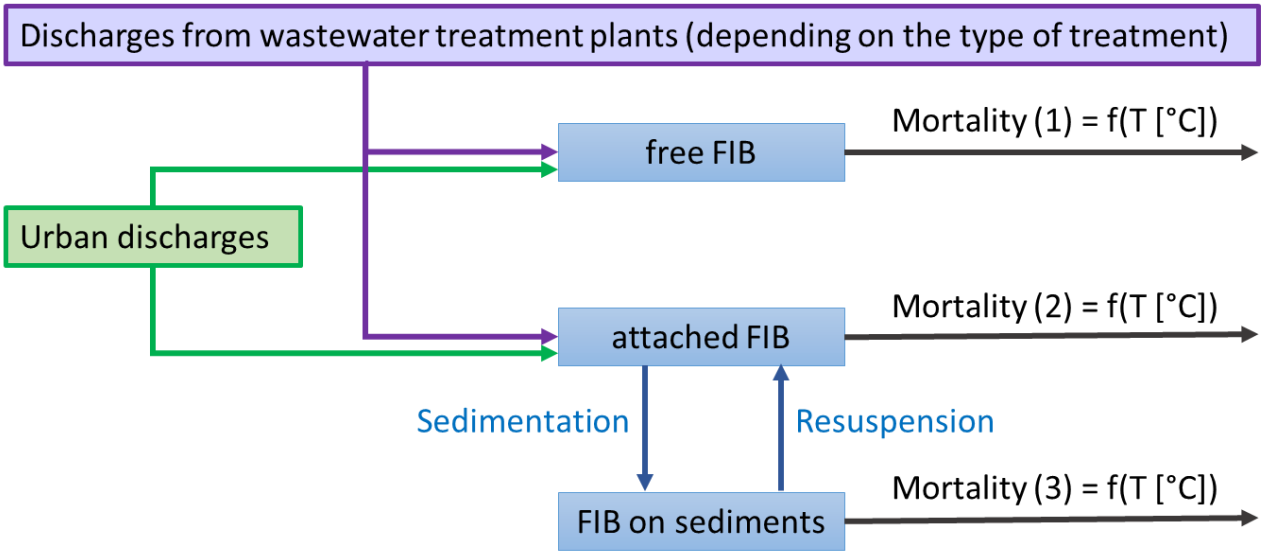


Figure 47: Schematic of ProSe model.

Through the use of sub-models and measured data, which provide information about flowrates, rainfall, chemical characteristics and FIB concentrations, ProSe model simulates river water quality and bacteria contamination. Simulated *E.coli* values are compared with measured data for model calibration. Additional sampling campaigns were performed for model calibration, coupling lab analysis with innovative sensors for bacteria determination (D. Angelescu, 2018), as well as their correlation with physical-chemical characteristics, such as Ammonia, Dissolved Organic Carbon (DCO) or Chemical Oxygen Demand (COD). Sub-models include dependencies amongst multiple variables, such as temperature, rainfall, punctual discharges, decay rate and wastewater characteristics. Real data were used, as well as experimental correlations between *E.coli* and DCO and literature assumptions.

Results on the first developed version showed in general good agreement between simulated and measured FIB values, even if significant deviations were detected in correspondence of rainy events (from Poulin et al., 2013).

The deterministic model can be adapted to simulate infrastructure changes in the sewer network or future scenarios for CSOs and WWTPs discharges. In fact, since it is connected to different sub-models, each of them specialised to investigate a definite aspect from hydrology to bacteria contamination, ProSe includes the representation of the physical system and thus is suitable to evaluate different scenarios, including future designs and infrastructure changes.

On the other hand, the accurate description of the real field conditions requires a high level of complexity, with long calculation time. The process workflow implies first to run simulations from each sub-model, then the simulated intermediate outputs must be fed manually as inputs to the ProSe. Moreover, since the interest was focused on the urban city of Paris, efforts were required to reduce the extension of the simulated area. ProSe provides hourly values of bacteria concentration, but results do not include information about accuracy or sensitivity analysis yet, even if new upgrades are going to be implemented to take into account uncertainties. Among technical and operative issues, one of the main obstacles to the exclusive use of ProSe as EWS is that, due to its structure and the complex mechanism for feeding input data, sensors cannot be directly connected to the model to provide predictions, making ProSe not suitable for real-time simulations. Nevertheless, its strong connection with the physical system and the possibility to perform different scenarios on future configurations, make ProSe a highly valuable tool to obtain a robust dataset of bacteria concentration trends, that could take into account different environmental conditions and system configurations.

3.4.3 AI: SWIM model

The statistic model AI:SWIM is a data-driven models for real-time prediction of bathing water quality, especially at rivers impacted from urban areas. AI:SWIM uses data of river flows, rainfall, CSOs and WWTPs discharges, which are rapidly available in most cases, to predict *E.coli* concentrations, expressed as time trends of geometric mean with corresponding uncertainty ranges, based on 95^{th} percentiles. Data can be temporally aggregated into daily or hourly values, according to the local availabilities and the most appropriate time-step required for the predictions.

Random Forest (RF) regression techniques are applied to estimates a probability of exceeding a defined threshold, using the provided $\log_{10}$ transformed FIB concentrations to forecast a trend of FIB concentrations along the time, characterised by a predicted mean and uncertainty range, defined by the related predicted quantiles.

In that way, the trend of the predicted concentrations is coupled with the related uncertainty range and users or bathing water managers obtain information not only on the expected value of microbial contamination, but also on its variability or affordability.

The model can be used through a friendly web-app interface, which allows the user to upload his own data and see the results (https://dwc-ds2.xyz/accounts/login/?next=/).

The application is open-source and uses only open-source technologies like Django (web framework), Leaflet (mapping library), Celery (Task manager), Redis (message broker), and the Orion Context Broker. Using the latter component makes the application Powered by FIWARE, which is a European initiative for developing standardized data models and IoT solutions.

The application includes:

- A graphical user interface (GUI) and content management system (CMS), which allows users to manage multiple variables and prediction models.
- The ability to create spatial variables (e.g., grouping all rain gauges in a specific area) by via an interactive map.
- Calibration and interactive analysis of state-of-the art machine learning and probabilistic forecasting models for predicting bathing water quality.
- An interface for using the FIWARE Orion Context Broker for real-time data transfer
- A scheduling functionality for defining the time and frequency when of predictions should be done.

From a practical use point of view, the model requires first of all the definition of a Bathing Spot, which represents the site where water quality has to be predicted and management measures are to be applied in order to ensure a safe recreational use. The variable related to each bathing spot is the Faecal Indicator Bacteria (FIB), that is the dependent variable to be predicted by the model. Then, the other variables that are fed to the model and used to perform the predictions are defined as Predictors. The categories included for the predictors can be of type rainfall, river flow, WWTP and Network (CSOs) discharges. However, other data can be uploaded, such as Temperatures or environmental conditions. Predictors are then grouped into the so-called Features groups, which allow to aggregate the data over classes of variables of the same type (e.g., rainfall, CSO outlets, WWTP discharges) into spatial averages. Depending on their availability, input data can be uploaded at different time-steps and aggregated into hourly or daily values. All the input data can be uploaded with the localisation of the monitoring point where they are measured. Then, area of interests can be selected directly from the visualisation map, in order to choose the most appropriate variables for each desired prediction model, also depending on the localisation of the bathing site and its distance from the available monitoring stations.

Once the bathing spot and the features groups are defined, the model performs its predictions to obtain FIB concentrations at the desired location in the defined conditions. Random Forest regression techniques are applied to predict the dependent variable (FIB) values. Random Forest is an ensemble learning method, which uses bootstrapping aggregation technique to combine the result of different algorithms and obtain the final prediction improving its accuracy (F. Bagherzadeh et al., 2021). Through this process, a forest of decision trees is defined by randomly sub-sample the given dataset and the results of the different trees are aggregated to obtain the output prediction (F. Bagherzadeh et al., 2021). In particular, the function used in Python environment is *RangerForestRegressor* from *skranger* package, since it also supports quantile regression, using the *predict_quantiles* method, other than methods for data splitting, model tuning through a grid search of the best hyperparameters values with the *GridSearchCV* function, and the evaluation of the importance of each variable.

3.5 Development of an EWS for Paris

In order to develop an EWS for the city of Paris, which was able both to realistically represent the physical conditions of the system and that could perform rapid and affordable predictions, it was decided to integrate the Prose and the AI:SWIM models.

However, models have not been integrated on the basis of the current configuration of Paris sewage system, since new interventions on the sewer network have been already planned to improve CSOs management and reduce overflows, as well as to optimise the WWTPs disinfection treatments. For that reason, the ability of ProSe to simulate scenarios was used to obtain a dataset that could represent the future conditions of the sewage management, providing a strong dataset to the statistic model, that on the other side was not flexible to adapt himself to system variations. It was decided to carry on the simulations on the basis of an historical observed period, for which hydrologic, meteorological and quality data had already been collected and analysed. Starting from the available data, some preliminary elaborations were conducted to adapt them to the scenario that wanted to be simulated. In detail, a four years time period, from 2016 to 2020, was selected. Data from CSOs discharges were adapted to simulate the new interventions, as well as the effluent concentrations of WWTPs flows, in order to obtain simulated FIB concentrations on the "Pont d'Iena" bathing site. The FIB data generated by ProSe, together with the rain gauges, environmental temperatures, CSOs and WWTPs discharges were then provided to the statistical model. The statistic model was then used and validated against the ProSe generated data, considered as "real measurements". The predictions on microbial water quality obtained from AI:SWIM were tested against the ones already generated by ProSe to verify the level of agreement between the two models and evaluate if by the integration of the two approaches was possible to perform accurate forecasting of water quality and support bathing water managers in decision making.

3.5.1 Paris data generation with ProSe

To test the possibility of integrating the representativeness of the physical system and the possibility to simulate design scenarios of deterministic modelling with the ability to perform rapid predictions of a statistic model, ProSe was used to generate FIB data, which were provided as input to a statistic model.

Three different scenarios were progressively simulated with ProSe model, considering subsequential changes and interventions on the sewer network and on the WWTPs, planned by the sewer managers and the water utility. In particular, CSO discharges are going to be regulated, reducing their maximum outflows, while WWTPs are going to be implemented with tertiary UV disinfection treatments. Progressive interventions were simulated as follows:

- Scenario SC4: Reduction of dry weather discharges and reduction of WWTPs effluent FIB concentrations by 3 log units.
- Scenario SC6: Implementation of Scenario SC4 and further reduction of CSOs discharges during rainy weather events.
- Scenario SC7: Implementation of Scenario SC6 with further reduction of rainy weather discharges and 5% reduction of the impermeable area in the catchment.

The output from ProSe model were used to feed the statistical model, to develop an EWS for the prediction of *E.coli* on the future configuration.

Since the Scenario SC7 was the most completed, according to the interventions planned in the sewer network and in the WWTPs, its results were used to feed the AI:SWIM model. In particular, FIB concentrations at the bathing site "Pont d'Iena" were considered to train and test the model, in

order to verify the level of agreement between them and thus evaluate the possibility of their integration for the EWS development.

3.5.2 Paris data elaboration with AI:SWIM

ProSe model reflects the physical system of Paris infrastructures and is flexible to simulate alternative scenarios of water management. However, it is not designed to detect short-term variations as for rainfall events. On the other hand, statistical models require in general less efforts to provide predictions, even if they are not able to simulate system changes or alternative scenarios. For this reason, the Early Warning System was developed integrating the deterministic model with a statistical model, based on machine learning and Random Forest regression. The input data were provided from ProSe simulations as well as local data, such as information on the localisation of the bathing site, rain stations, temperatures, river flow meters, CSOs and WWTP discharges.

The selected point for the definition of the bathing spots where to perform the predictions was Pont d'Iena, in Paris city centre (Figure 48). Values of *E.coli* concentrations obtained by ProSe modelling were aggregated into daily means and fed to train and test the model. Then, the daily averages of CSOs and WWTPs discharges, river flow meter, temperatures and rain gauges data were defined as predictor variables (Table 26).

Table 26: Input data to statistic model.

name	name abbreviation	description	unit	unit abbreviation
Escherichia Coli	*E.coli*	Faecal indicator bacteria	Most probable number	MPN/100mL
Rainfall	P	wet precipitation within 24h	Millimetres per day	mm/d
Flow	Q_avg	average river flow per day	Cubic meter per second	m^3/s
Discharge	Q_cum	cumulative discharge volume per day	Cubic meter per day	m^3/d
Temperature	T	Daily temperatures	Centigrade degrees	°C

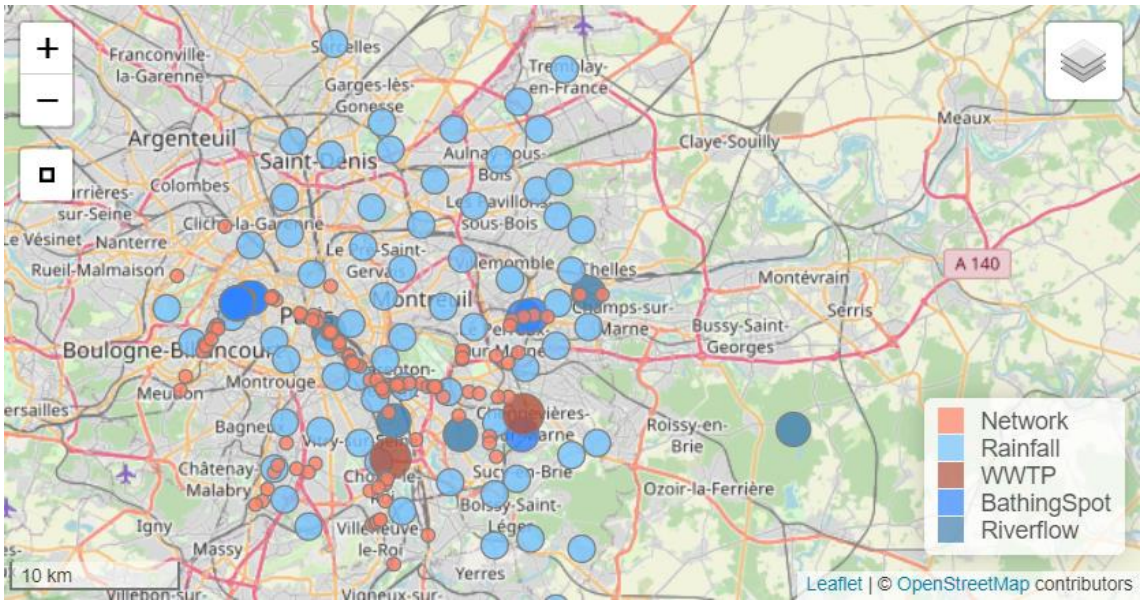


Figure 48: Map with the localisation of predictors.

Once uploaded all the data into the model, features groups were created for each variable type, in order to obtain aggregated data to perform the predictions. Data from the different rain stations were grouped, considering the catchment area that could most affect the water quality of Pont d'Iena bathing site. For the river flow, data from the nearby Austerlitz meter were selected, as well as the two nearest WWTPs, respectively of Seine Valenton and Marne Avalon, which were considered separately into two different feature groups. For CSOs, a first test was conducted considering a unique feature group that included all the discharges. Since that huge quantity of data conducted to an overfeeding of the model, a new model was performed considering only the CSOs that resulted of biggest importance on model predictions. Features groups were thus created singularly for each element, since punctual discharges may have had different and significative impacts.

Once uploaded all the data required, Random Forest regression was applied to predict FIB concentrations.

FIB dataset was split, using *TimeSeriesSplit* function of *sklearn* package, into train-data and test-data. The *RangerForestRegressor* function was used to obtain the predictions, together with the related quantiles to represent the uncertainty range around the predicted values.

A grid search was conducted to optimise the arguments of the function used, in order to obtain the best combination of hyperparameters that allowed the maximisation of the agreement between the two models predictions, evaluated through the R^2 index.

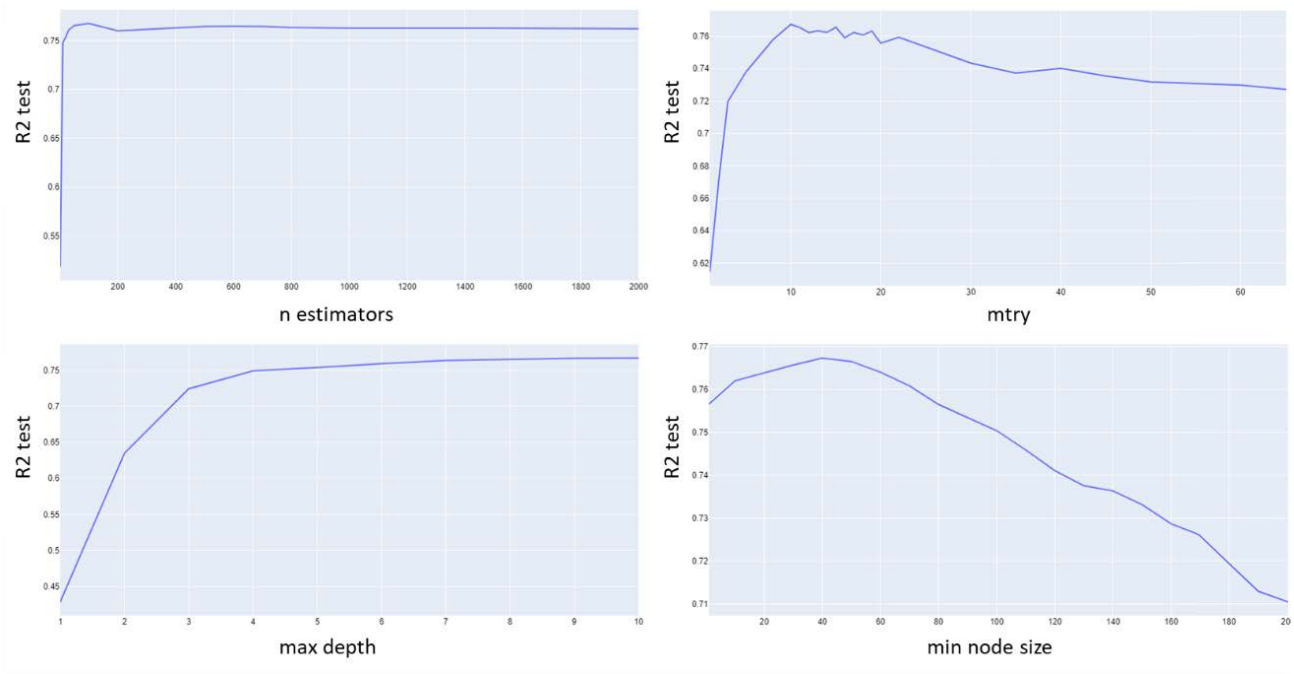


Figure 49: R^2 values depending on single hyperparameter.

From the analysis (Figure 49) it was obtained a number of trees (*n_estimators*) equal to 100, a number of features to split on each node (*mtry*) of 10, minimal node size (*min_node_size*) of 40, while importance and maximal tree depth were left as default values, equal to *importance* and to no limitations, respectively (Figure 50). The intervals for choosing the most appropriate hyperparameters were defined considering also the physical properties and meanings of each of them. In fact, high values for the number of trees allow less uncertainties due to the random nature of the function. The features consider the number of variables and the number of splits performed for each of them. In the developed model, each variable was aggregated into daily means, considering up to 5 days before the selected data. Finally, the depth and the minimum size of each node are linked, since they impact on how many ramifications would be performed for each tree.

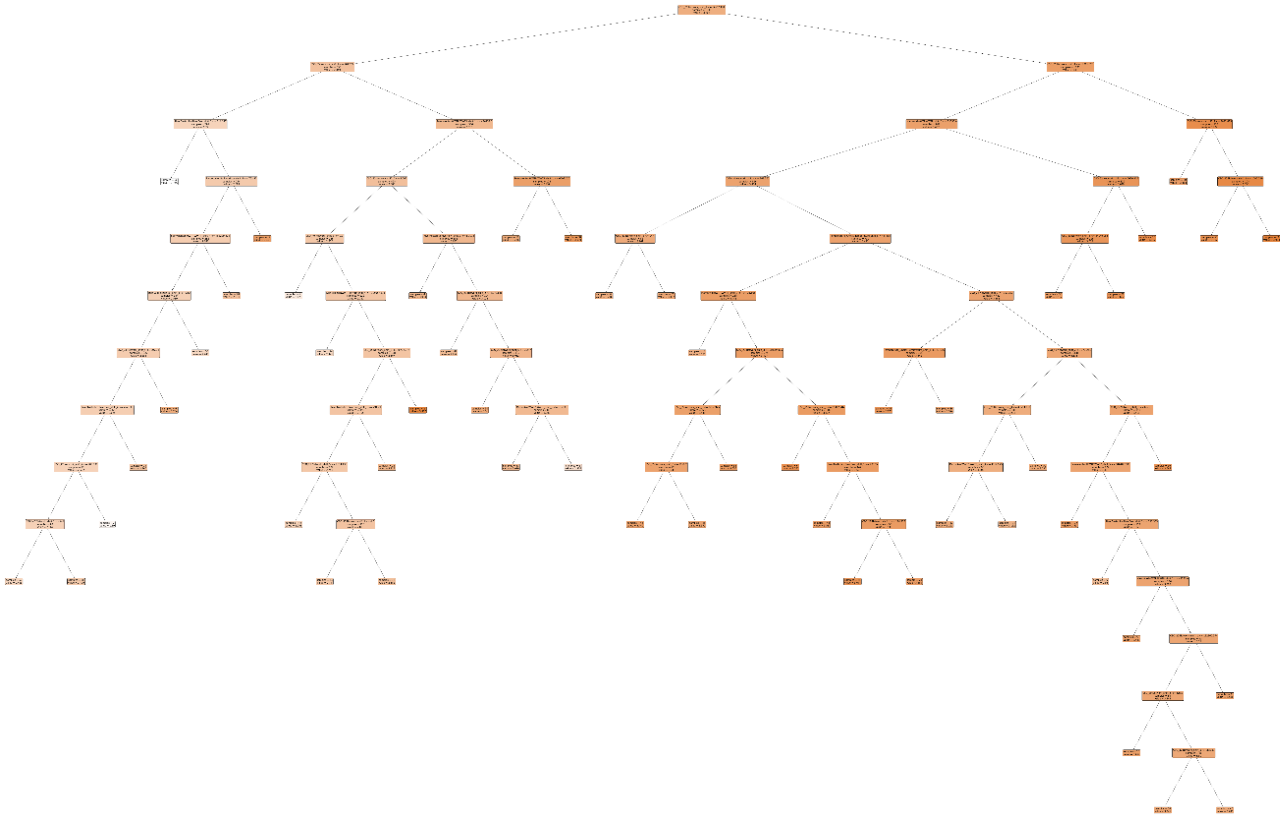


Figure 50: Example of a decision tree structure.

The model was fitted using the training-data and validated with the test-data. The importance of each independent variable was evaluated and sorted to estimate which features groups were the most impacting.

Finally, statistic indicators were calculated to evaluate the performances of the model using training and test data, as summarised in Table 27.

Table 27: Statistic metrics description.

Metric	Meaning	Explanation
R^2	Variation for the dependent variable predictable from the independent variables	High values of R^2 indicate good model accuracy
Mean Square Error (MSE)	Standard deviation of the prediction errors	Low values of RMSE indicate good model accuracy
Number of detected contaminations	measurements larger than 1000 MPN/100mL	Refers to the number of measurements above a specific threshold (i.e., 900 *E.coli*/100mL)
% below 95th percentile		Refers to the number of measurements below 95th percentile
% below 90th percentile		Refers to the number of measurements below 90th percentile
% in 95% prediction interval		Refers to the number of measurements in 95% prediction interval

% Predicted contaminations (true positives)	percentage of predicted contaminations, which were confirmed with measurements	If a predicted contamination is confirmed with a measurement, the model predicted correctly. This indicator is equivalent with a true-positive prediction
% Unpredicted contaminations (false negatives)	days without warning (i.e., predicted 90th percentile < 900 MPN/100mL) with high measurements (e.g., *E. coli* > 900)	If a measured contamination is not predicted, the model predicted incorrectly. This indicator is equivalent with a false-negative prediction

In particular, the R^2 and the Mean Square Error (MSE) parameters give information on prediction accuracy, meaning that a good fitting is achieved when high values of R^2 and low values of SME are obtained (Y. Sun et al., 2021). However, other than the most common indicators, the percentages of correctly predicted contaminations (true positives rates) and unpredicted contaminations (false negative rate) were calculated, since they were considered most suitable to describe the performance of the model in supporting decision making from the bathing site managers.

From a support to decision point of view, in fact, the most important attribute that the statistic model should satisfy, rather than estimating precisely the exact value of FIB concentration, is the ability to distinguish between days characterised by good water quality and when bathing can be permitted, from days where health risks are above the acceptable level and the bathing site should be closed.

Moreover, indexes were also expressed considering not only the mean values, but also the different percentiles, according to the BWD prescriptions.

3.6 Results

Generally, good performances of statistic indicators can be observed, with R^2 values around 0.85 and 0.77 for the training and the testing dataset, respectively (Figure 51 and Table 28). Accordingly, observed values of MSE are equal to 0.07 for the training data and 0.09 for the test data.

Observing the distribution of predicted values, it can be noticed that about 92% of the test data are below the 90th percentile, while 97% was reached as concern data inside the 95th prediction interval.

The best performances are expressed in terms of percentage of correctly predicted contaminations. It can be observed that the two models agree in all the cases in the detection of non-compliant days and there are no events of false negatives outcomes.

Table 28: Statistic metrics evaluation for Paris model.

Metric / Score	Training	Test
R^2	0.85	0.77
Mean squared error	0.04	0.09
Sample size	965	482
% below 95th percentile	-	96.0
% below 90th percentile	-	92.0
% in 95% prediction interval	-	97.0
Correctly predicted contaminations (True-positive rate)	-	1.0 (272/272)
Unpredicted contaminations (False-negative rate)	-	(/272)

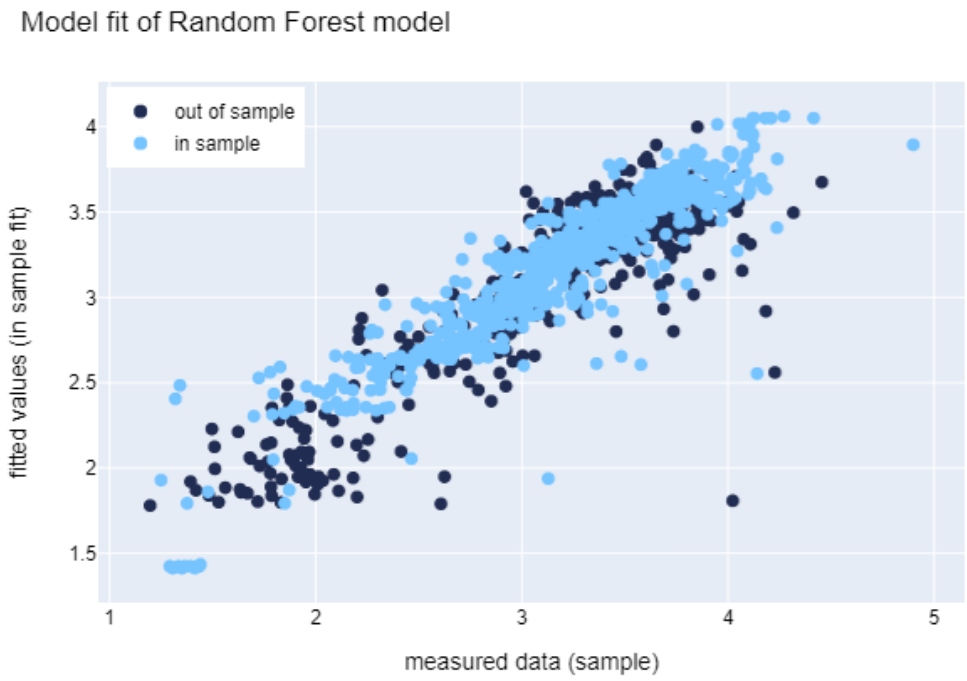


Figure 51: Model fitting.

Analysing the relative importance of the predictors (Figure 52), it can be observed that CSOs and WWTP discharges have a major impact on the model outputs, rather than rain gauges or temperatures. In particular, it was observed that the most impacting CSOs were also the ones characterised by the presence of extreme peaks in their trends, which may have affected water quality.

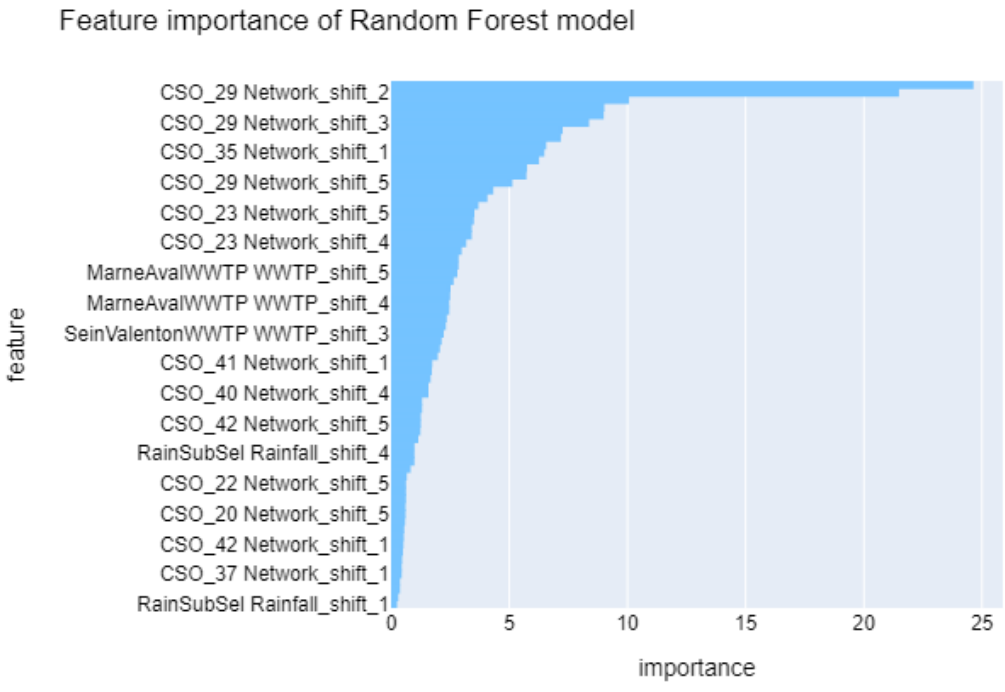


Figure 52: Importance of the variables.

Generally, a good level of agreement can be observed between the two models (Figure 53). Some discrepancies were observed in the winter periods of 2018, perhaps due to interventions on the sewer network, which accounted for some anomalous CSOs discharges.

The predicted trend of the mean values is in line with the concentrations provided by the ProSe model. Nonetheless, the ranges calculated through the *quantile* function show high uncertainties, meaning that the predicted daily values could be subjected to high variations. However, it must also be noticed that the data provided by ProSe model did not take into account errors or variabilities, so a proper comparison including the respective model uncertainties could not be performed.

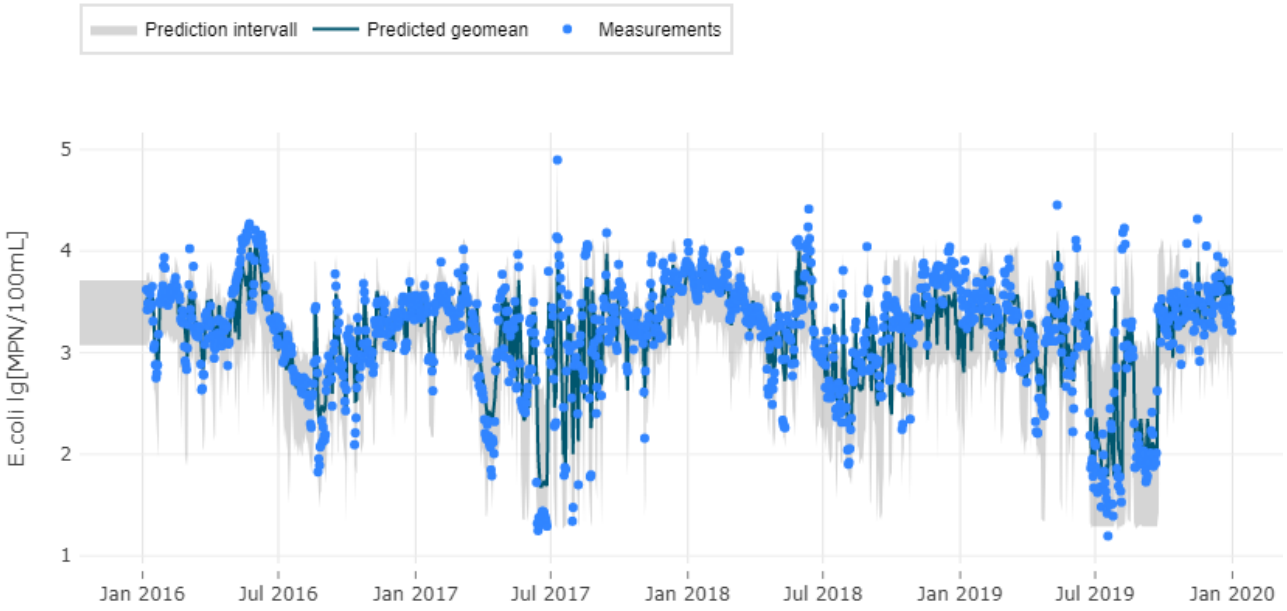


Figure 53: Predicted trends.

3.7 Conclusions

Bathing water management can be supported by the application of digital tools to guarantee a continuous monitoring and allow rapid reactions in case of extreme event occurrence. Early warning systems should be applied to predict water quality and ensure a safe use of recreational waters, providing alarms in case the risk of being exposed to microbial contamination is considered not acceptable. However, there is still lack of models that could be easily implemented by the managers and used to inform public. Deterministic models were commonly used to simulate scenarios, but require the description of the physical system through defined mathematical functions. On the other hand, recently statistic models are diffusing since they can be more easily applied and are more flexible to use the available data. In Paris case-study, a deterministic model has already been developed to support urban water management. On the other hand, it was not designed to perform short-term predictions and its results were susceptible to extreme events like intense rainfalls. The statistic model AI:SWIM, if used alone, can be fed with available input parameters and provide fast predictions, but it requires a huge amount of data to be calibrated and do not consider physical characteristics and eventual changes on the real system. For these reasons, in Paris, in order to develop an Early Warning System to predict bathing water quality, also considering the interventions planned the sewage management, the deterministic model was used to provide an affordable dataset of the future scenario, while the statistical model was then applied to make the predictions on microbial water quality. Tests on 4 years' time series were performed and results

showed a good agreement between the two models, with R^2 values of 0.77, all true positive contaminations detected correctly and no false negatives detected, meaning that they can be integrated each other to compensate the lacks and valorise the strength of each one.

References

Bagherzadeh, F., Nouri, A. S., Mehrani, M. J., & Thennadil, S. (2021). Prediction of energy consumption and evaluation of affecting factors in a full-scale WWTP using a machine learning approach. Process Safety and Environmental Protection, 154, 458–466. https://doi.org/10.1016/j.psep.2021.08.040

Bruschi, A., Lisi, I., de Angelis, R., Querin, S., Cossarini, G., di Biagio, V., Salon, S., Solidoro, C., Fassina, D., Ancona, S., & Silvestri, C. (2021). Indexes for the assessment of bacterial pollution in bathing waters from point sources: The northern Adriatic Sea CADEAU service. Journal of Environmental Management, 293. https://doi.org/10.1016/j.jenvman.2021.112878

Crocetti, P., Eusebi, A. L., Bruni, C., Marinelli, E., Darvini, G., Carini, C. B., Bollettini, C., Recanati, V., Akyol, Ç., & Fatone, F. (2021). Catchment-wide validated assessment of combined sewer overflows (CSOs) in a mediterranean coastal area and possible disinfection methods to mitigate microbial contamination. Environmental Research, 196, 110367. https://doi.org/10.1016/j.envres.2020.110367

Farrell, M. L., Joyce, A., Duane, S., Fitzhenry, K., Hooban, B., Burke, L. P., & Morris, D. (2021). Evaluating the potential for exposure to organisms of public health concern in naturally occurring bathing waters in Europe: A scoping review. In Water Research (Vol. 206). Elsevier Ltd. https://doi.org/10.1016/j.watres.2021.117711

Globevnik, L., Snoj, L., & Šubelj, G. (2021). European Bathing Water Quality in 2020.

Hoshyaripour, G., Brasseur, G., Andrade, M. F., Gavidia-Calderón, M., Bouarar, I., & Ynoue, R. Y. (2016). Prediction of ground-level ozone concentration in São Paulo, Brazil: Deterministic versus statistic models. Atmospheric Environment, 145, 365–375. https://doi.org/10.1016/j.atmosenv.2016.09.061

Kaas, H., Erichsen, A. C., & Roberts, C. (2011). Early warning of bathing water quality-an operational water forecast service.

Mälzer, H. J., aus der Beek, T., Müller, S., & Gebhardt, J. (2016). Comparison of different model approaches for a hygiene early warning system at the lower Ruhr River, Germany. International Journal of Hygiene and Environmental Health, 219(7), 671–680. https://doi.org/10.1016/j.ijheh.2015.06.005

Neves, R. J. J., Fernandes, R., Mateus, M., Viegas, C., & Neves, R. (2012). Modelling tools to support an early alert system for bathing water quality. Environmental Engineering and Management Journal, 11(5), 907–918. http://omicron.ch.tuiasi.ro/EEMJ/

Penna, P., Baldrighi, E., Betti, M., Bolognini, L., Campanelli, A., Capellacci, S., Casabianca, S., Ferrarin, C., Giuliani, G., Grilli, F., Intoccia, M., Manini, E., Moro, F., Penna, A., Ricci, F., & Marini, M. (2021). Water quality integrated system: A strategic approach to improve bathing water management. Journal of Environmental Management, 295. https://doi.org/10.1016/j.jenvman.2021.113099

Poulin, M., Servais, P., Mouchel, J.-M., Therial, C., Thérial, C., Lesage, L., Rocher, V., Goncalves, A., Masnada, S., Lucas, F., & Flipo, N. (2013). Modélisation de la contamination fécale en Seine : impact des rejets de temps de pluie. https://www.researchgate.net/publication/305816033

Quilliam, R. S., Taylor, J., & Oliver, D. M. (2019). The disparity between regulatory measurements of E. coli in public bathing waters and the public expectation of bathing water quality. Journal of Environmental Management, 232, 868–874. https://doi.org/10.1016/j.jenvman.2018.11.138

Radini, S., Marinelli, E., Akyol, Ç., Eusebi, A. L., Vasilaki, V., Mancini, A., Frontoni, E., Bischetti, G. B., Gandolfi, C., Katsou, E., & Fatone, F. (2021). Urban water-energy-food-climate nexus in integrated wastewater and reuse systems: Cyber-physical framework and innovations. *Applied Energy*, *298*. https://doi.org/10.1016/j.apenergy.2021.117268

Seis, W., Zamzow, M., Caradot, N., & Rouault, P. (2018). On the implementation of reliable early warning systems at European bathing waters using multivariate Bayesian regression modelling. Water Research, 143, 301–312. https://doi.org/10.1016/j.watres.2018.06.057

Servais, P., Poulin, M., Flipo, N., Lesage, L., Rocher, V., Goncalves, A., Masnada, S., Lucas, F., & Mouchel, J. M. (2007). Vers un modèle de la contamination fécale en Seine par temps de pluie.

Sun, Y., Clarke, B., Clarke, J., & Li, X. (2021). Predicting antibiotic resistance gene abundance in activated sludge using shotgun metagenomics and machine learning. Water Research, 202. https://doi.org/10.1016/j.watres.2021.117384

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION. (2006). DIRECTIVE 2006/7/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union.

Van, L. A., Nguyen, K. D., le Marrec, F., & Jairy, A. (2022). Development of a Tool for Modeling the Fecal Contamination in Rivers with Turbulent Flows—Application to the Seine et Marne Rivers (Parisian Region, France). Water (Switzerland), 14(8). https://doi.org/10.3390/w14081191

Varma, K., Tripathi, P., Upadhyaya, S., Srivastava, A., Ravi, N. K., Singhal, A., & Jha, P. K. (2022). Assessment of mass bathing event (Kumbh- 2019) impact on the river water quality by using multivariate analysis and water quality index (WQI) techniques at Sangam (Prayagraj), India. Groundwater for Sustainable Development, 17. https://doi.org/10.1016/j.gsd.2022.100750

Water Europe. (2022). Bathing Water Directive. Public health starts with a water-smart management of bathing waters.

WHO CC for Health Promoting Water Management & Risk Communication, & University of Bonn - IHPH Institute for Hygiene and Public Health. (2011, December). Dynamic Modelling of Bathing Water Quality for Early Warning. www.oresund.badevand.

4. Sustainability assessment in the integrated urban water and reuse system

Currently, comprehensive models and tools are missing that consider the interconnections amongst catchment, sewer network, wastewater treatment plant (WWTP), river and climatic system in a holistic approach and define relevant monitoring requirements and trustable information provision. Cyber-physical systems provide a technological ground for an efficient management of such integrated systems. Digital solutions and serious games addressing the nexus in urban and peri-urban water management are also presented to facilitate innovative practice aspects and to foster public involvement. Adaptable digital solutions can help to understand stakeholders' perception of water quality and its governance and to improve levels of awareness and collaboration between utilities, authorities, farmers and citizens.

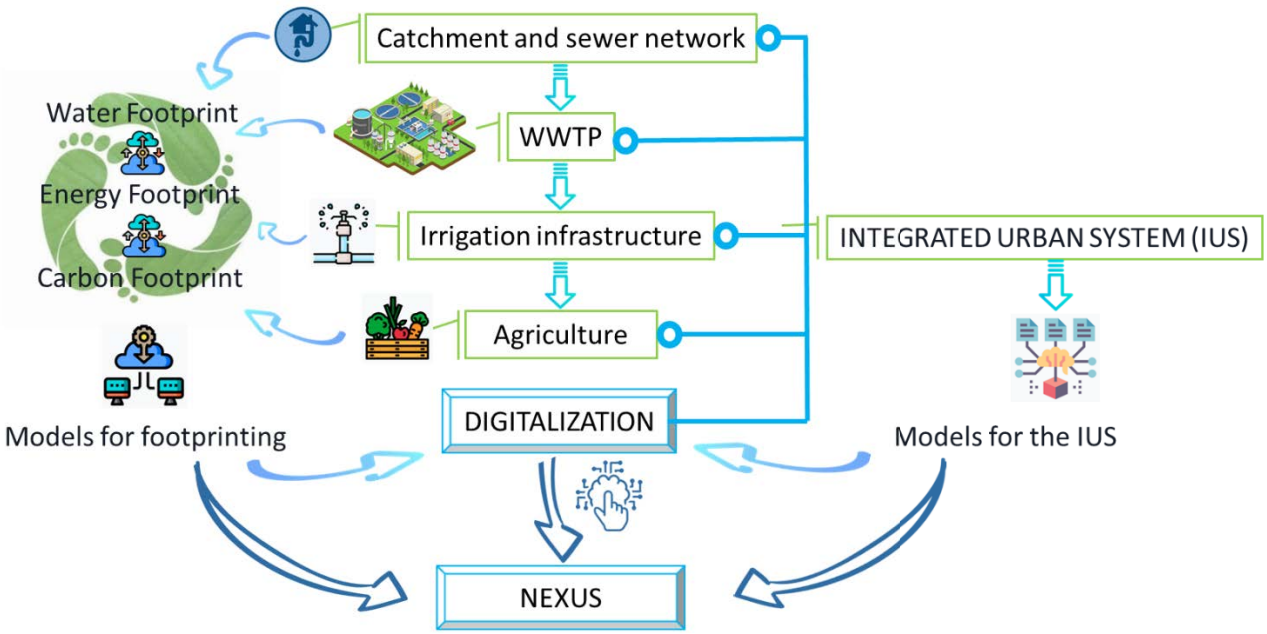


Figure 54: Interconnections in integrated urban systems (Radini et al., 2021)

WWTPs generate significant amounts of operational data sourced from heterogenous sources such as sensors and laboratory analyses. The optimisation of the performance of WWTPs can be possible by using models and tools to simulate processes. WWTPs management implies a series of impacts on different sectors, connected into the water-energy-food-climate (WEFC) nexus. Catchment, sewer network, WWTP and water bodies receptors are highly interconnected. Characteristics, efficiencies or variations in each stage of the urban water cycle can affect the other stages. In order to quantify the WEFC nexus, all the steps that involve wastewater production, transportation, treatment and reuse should be considered. Integrated Urban Wastewater Systems (IUWS) have been developed to assess those linkages. Integrated models can help providing a wider panorama of all the interconnections that implies the urban wastewater cycle, from its catchment to the final destination. Urban Wastewater System (UWS) modelling was developed to consider the interconnections amongst catchment, sewer network, WWTP and river system following a holistic approach. Benchmark Simulation Model BSM-UWS is an open-source system-wide model used to simulate, predict and evaluate different strategies in UWS. BSM-UWS is applied to provide an

integrated system assessment. The toolbox uses different sub-models for each UWS stage, connected through interfaces in which mass-balances are verified. The BSM2 dynamic influent pollutant disturbance scenario generator is used to simulate the catchment model is simulated using, in order to obtain flow and concentration of pollutants in different wastewater sources: domestic, industrial, stormwater and infiltration to sewer. Sewer network sub-model considers the transport of wastewater and first flush loads from the catchment to WWTP, considering the presence of storage tanks and pumping stations. WWTP is modelled through ASM2d, while river water system is simulated using the IWA River Water Quality Model. Performances of each sector are assessed using evaluation indexes. For the sewer network overflow duration, frequency, volume and overflow quality index (kg pollutant units/day) are considered. WWTP efficiency is evaluated considering influent quality index (kg pollutant units/day) and effluent quality index (kg pollutant units/day). River water system is evaluated considering dissolved oxygen (DO) and unionized ammonia (NH_3) concentrations. Three main control strategies were used by Saagi and others (2017). Sewer network system efficiency is evaluated considering the flowrate at WWTP inlet, manipulated by varying the flows from the storage tanks. Concerning the WWTP, the DO concentration in the aeration tanks is measured, modulating the oxygen transfer coefficients k_{La}. River water system is evaluated through the concentration of ammonia, controlled by the thresholds set up for the by-pass flows in the WWTP. BSM-UWS is applied on different tools. WESTforIUWS, developed by Dansk Hydraulisk Institute in 2016, developed water quality-based indicators, including uncertainty and sensitivity analysis. SIMBA# water, developed by the German Institute für Automation und Kommunikation in 2016, uses hydrological and hydrodynamic models for the sewer network, biochemical and physical processes to simulate the WWTP and modules for biochemical processes in the river. CITY DRAIN (Achleitner et al., 2007) is an open-source MatLab based toolbox that uses hydrological models for sewers and river systems as well as a simplified model for WWTP (Achleitner et al., 2007). It allows the users to create new blocks in addition to the existing model library (Ramesh. Saagi, 2017). The models and tools that are commonly used to measure carbon, energy and footprints in WWTPs are summarized in Table 29.

Table 29: A summary of some of the models and tools used to measure carbon, energy and water footprints in WWTPs and to quantify the nexus.

Footprint	Model/method	Tool	Reference
Carbon footprint	EF, IPCC Guidelines	Carbon Footprint Calculation Tool (CFCT, 2014)	(Gustavsson & Tumlin, 2013)
	Mass balances, ASM1, sub-models for secondary settler and AD	CF TOOL CTRL	(Baeza et al., 2017)
	Diffusive Emissions Estimation Model DEEM		(Mannina et al., 2016; Rodriguez-Garcia et al., 2012)
	Activated Sludge Models for Nitrogen ASMN		(Mannina et al., 2016; Porro et al., 2012)

	BSM2G		(Flores-Alsina et al., 2012; Mannina et al., 2016)
	BSM-e		(Mannina et al., 2016; Sweetapple et al., 2013)
Energy footprint	ENERWATER	On-line tool	(S. Longo et al., 2019b)
Water footprint	Water Footprint Network (WFN)		(A.Y. Hoekstra et al., 2011)
Integrated footprint	BSM-UWS	WESTforIUWS	(R. Saagi et al., 2017; Ramesh. Saagi, 2017)
		SIMBA# water	(R. Saagi et al., 2017; Ramesh. Saagi, 2017)
		CITY DRAIN	(Achleitner et al., 2007; R. Saagi et al., 2017; Ramesh. Saagi, 2017)

In recent years modelling development gave a great contribution for the estimation of WWTP footprints, providing support tools for decision-making processes. WEFC nexus models can be used to evaluate designed solutions or scenarios, and support decision making. Even if more specific models are being constructed to fit WWTP conditions, still not all the footprint assessments can rely on specific tools for WWTP modelling. Different models are based on different hypothesis for process simulation or are focused on different parameters. A comprehensive tool that considers all the sectors of the WEFC nexus is still missing.

Nexus quantification depends on accurate, open access information. Decision-makers are often hindered to compare management options, make informed decisions balancing economic, social and environmental interests, and subsequently evaluate and prioritize potential sustainability solutions. To overcome this bottleneck, a mechanism is needed for data and monitoring requirements and trustable information provision.

4.1 Nexus assessment of complex territorial systems

An urban water management considers water supply, wastewater treatment and disposal as an integrated system (Radini et al., 2021). The modern thinking about urban water systems is moving to ensure that adaptive measures are applied for climate change mitigation by reducing energy use and greenhouse gas emissions. Water treatment is one of the major carriers of the water-energy-food-climate (WEFC) nexus, and although the relationship between water and energy is well recognized, there is still a lack of adequate analysis of the cyber-physical framework to address and assess urban and peri-urban WEFC nexus in an integrated approach. Regional and local characteristics are also highly effective on the urban WEFC nexus. The nexus that interlinks water and energy consumption, carbon emissions, environmental impacts and food production in agriculture (water-energy-food-climate, WEFC) can be assessed through a systematic approach of design, scientific investigation and policy instruments focused on synergies and trade-offs at physical, digital, socio-economic, and governance level (Radini et al., 2021; Zhang and Liu, 2022). WEFC nexus means a shift of paradigm in wastewater management, from simply considering the environmental impacts related to wastewater discharge to assessing all the impacts (positive or negative) related to the processes with the ultimate goals of carbon neutrality. Nexus is thus a novel tool that can be combined with the risk and nutrient management plans since water reuse not only has to be safe but sustainable, with the aim to assure water and food production in the following

decades. Important to note is that the nexus way-of-thinking should be applied not only to technical and environmental factors related to water reuse, but also to socio-political issues, considering the local dimension as well (Marinelli et al., 2021).

In recent yars, wastewater sector started to recognise wastewater as a resource and makes effort to recover materials and energy (Puchongkawarin et al., 2015). There is an increasing number of studies on WWTPs that develops innovative technologies for efficient recovery of valuable resources, such as chemicals, nutrients, bioplastics, enzymes and water (Akyol et al., 2020; Batstone et al., 2015; Frison et al., 2015). It is estimated that wastewater contains 1.3 MJ/person/day (6.5 MJ/L) in terms of chemical energy, 20% of the phosphorous consumed and 10%-30% of nitrogen needed in agriculture. In this regard, economic, environmental and industrial incentives take place in the circular economy concept of the water sector (Puyol et al., 2017). In recent years, great efforts have been made to promote the sustainability of WWTPs via two main approaches: improving energy efficiency and resource recovery (Mo & Zhang, 2012). Wastewater treatment processes are highly energy demanding, with high operational cost, and significant sources of GHG emissions, whose reduction has been recently mandated by the EU and other countries' policies (Capodaglio & Olsson, 2020) and raised as global concern (Ashrafi et al., 2014; Bani Shahabadi et al., 2010). CH_4, N_2O and CO_2 are the main GHGs emitted in WWTPs. Emissions are categorized as direct, if they are released during treatment processes, such as biologic treatment, sludge processing or biogas combustion. Emissions due to energy and chemical consumption, transport, waste disposal and treated water discharge are referred as indirect, because even if they are not emitted inside the plant boundaries, they are dependent on WWTPs activities or management decisions. In recent years, particular attention has given to N_2O emissions, as its Global Warming Potential (GWP) is 273 times higher than CO2 (IPCC, 2021). The US Environmental Protection Agency (US EPA) estimated that wastewater sector was responsible for 5% of global non-CO_2 GHG emissions in 2005, with a predicted increase by 27% by 2030 (Caniani et al., 2019). Up to date, many models and tools have been developed and integrated to determine the footprints of the WWTPs. These methodologies can help to take an action towards a more sustainable and energy-efficient urban wastewater management.

Water and carbon footprints of WWTPs are connected via energy footprints. The study of Gu et al. (2016) showed that on average 13.38 L of freshwater is required to produce 0.4 kWh of electric energy, used as input for treating 1 m^3 domestic wastewater, with a consequent emission of 0.23 kg CO_2 during the process. However, the reduction of Grey Water Footprint due to wastewater treatment was 6.78 m^3/m^3 of sewage treated in Chinese WWTPs. Over the past few decades, energy recovery from wastewater has increased (Hao et al., 2019; Stillwell et al., 2010), and also been developed and applied in WWTPs. In fact, carbon neutrality and serious reduction in operation costs, and energy consumption can be achieved in energy self-sufficient WWTPs (Gu et al., 2017; Hao et al., 2019; Jenicek et al., 2013).

4.1.1 Nexus evaluation in Peschiera Borromeo water reuse system

The evaluation of the water reuse-carbon-energy-food-climatic (WEFC) nexus in the integrated peri-urban wastewater treatment and reuse system connected to Peschiera Borromeo WWTP was performed to quantify the benefits of water reuse.

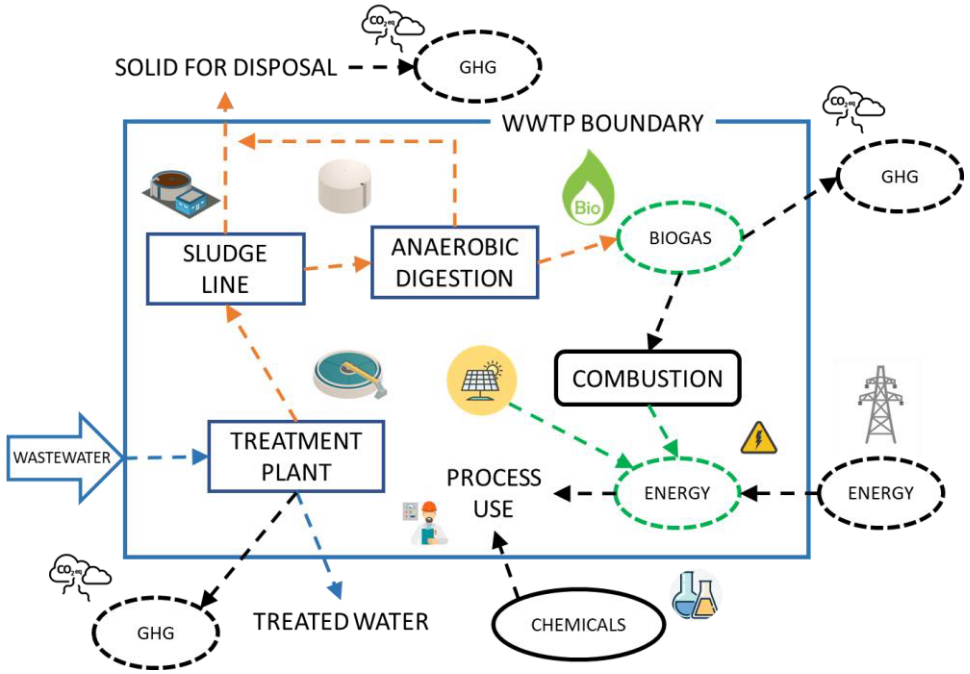


Figure 55: Schematisation of Peschiera Borromeo WWTP system

To analyse the differences in respect to the non-reuse scenario, where treated wastewater is supposed to be discharged, three different reuse scenarios were set up, considering the three water quality classes that according to the EU Regulation 741/2020 are feasible for water reuse in agriculture irrigation (class A, B or C).

Energy footprint was performed according to the recently standardised ENERWATER methodology (Longo et al., 2018), while carbon footprint assessment was evaluated according to the methodology reported in Marinelli et al., 2021. Nutrient content in treated wastewater was taken into account, in order to evaluate the possible savings in terms of synthetic fertilisers.

Generally, it was found that the reuse scenarios allowed an overall reduction of carbon and energy footprints. Considering the scenario where the plant configuration is structured to deliver reclaimed water of quality class C, energy consumptions were estimated to be lowered up to 7.1% and carbon emissions were minimised up to 2.7%, in respect to the condition where treated wastewater is simply discharged. However, when comparing the reuse scenarios, upgrading water quality from class C to class A resulted in increments in energy and carbon footprint of 5.7% and 1.7%, respectively. This aspect is balanced when considering that higher quality crops could be cultivated with reclaimed water in class A, with bigger economic revenues and high recovery of nutrients (e.g., recovery of 154,450 kg N/y for tomato cultivation).

Table 30: Nexus evaluation for reuse and non-reuse scenarios.

Category	Unit	Non-Reuse	Reuse		
			Class C	Class B	Class A
Energy footprint	MWh/y	16'374	15'196	15'427	16'121
Carbon footprint	tonCO_2eq/y	24'505	23'849	23'952	24'260
Treated wastewater	millions of m^3/y	25.9			
Nutrients in the wastewater effluent	mgN/L	7.8			
	mgP/L	0.6			

Results of this work showed that transferring the WEFC nexus from theory to practice can realize sustainable resource management in the operating environment by providing a reduction in greenhouse gas (GHG) emissions, overall energy savings, reduction in water stress and optimization of agricultural practices.

4.2 LCA for water reuse in Peschiera Borromeo WWTP

Life cycle assessment, as standardised tool to quantify the environmental impacts of processes through the entire production chain, can be useful to evaluate scenarios where irrigation or fertigation using reclaimed water are contemplated and to quantitatively demonstrate their benefits in comparison to conventional treatment schemes (Radini et al., 2021; Sgroi et al., 2018). Life Clycle Assessment (LCA) can contribute to raise awareness of decision makers, stakeholders and the general public, so they can complement health risk and nutrient management plans.

4.2.1 Materials and methods

LCA was performed to assess the environmental impacts of Peschiera Borromeo UWWTP, in the current configuration and in the design one, characterized by reclaimed water reuse in the surrounding agricultural area. The final aim was to identify and quantify the benefit or burdens of plant upgrading. The comparison was mainly carried out through a Life Cycle Thinking approach, but it required preliminary static evaluation such as mass and energy balance.

As described in the previous chapters, Peschiera Borromeo WWTP consists of two water lines (L1 an L2) and a sludge line with biogas recovery and final sludge disposal as soil improver. The overall treatment capacity is 516000 PE. In order to enable the reuse of the final effluent for agricultural purposes, the following proposed scenarios focused only on the Line 2 of Peschiera Borromeo WWTP were analysed. The environmental impacts of the current configuration were compared to the ones of alternative reclamation solutions addressing water reuse.

The baseline scenario refers to the current treatment chain of Line 2 where the final effluent is discharged on surface water and the irrigation and nutrient demand are supplied by freshwater and spreading of mineral fertilizers, respectively.

To comply with the water reuse regulation, the proposed reuse scenarios (Figure 56) involve upgrading or process implementation of Line 2 as follows:

- UV disinfection at higher UV dose (Scenario 1),

- Chemical disinfection using peracetic acid PAA (Scenario 2)
- Biological treatment with UASB followed by AnMBR (Scenario 3).

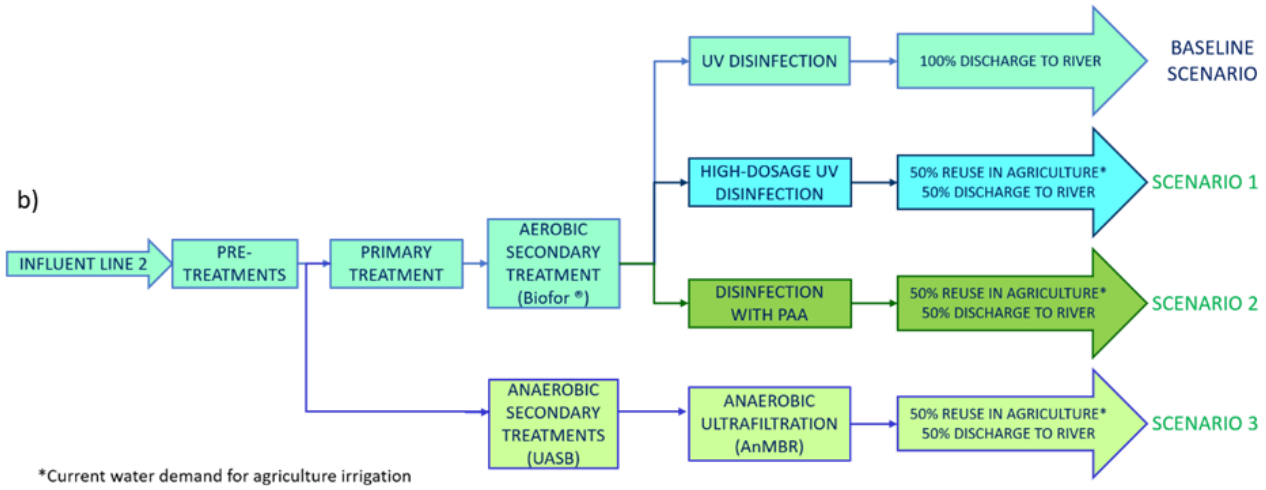


Figure 56: Scenarios of Peschiera WWTP

In Scenario 1, the existing UV disinfection operates at a dose of 80 mWs/cm^2 to ensure a 3.5 log reduction (DEMOWARE, 2016) required to achieve a quality effluent of Class A. In Scenario 2, the UV disinfection is substituted by chemical disinfection unit of 2200 m^3, with a contact time of 49 min and a dosage of 5 mgPAA/L to guarantee the same log reduction (Antonelli et al., 2013) of Scenario 1. Finally, in Scenario 3, an UASB reactor is installed replacing the aerobic secondary treatment. The UASB reactor works at ambient temperature and has a volume of 24106 m^3, with a hydraulic retention time (HRT) of 9 hours. Then, the UASB is coupled with an anaerobic hollow-fiber ultrafiltration membrane (AnMBR) as tertiary treatment. The membrane (267842 m^2) with a nominal pore size of 0.03 µm operates with a specific flux of 10 L/m^2/h. The ultrafiltration technology in Scenario 3 provides pathogen-free effluent. Therefore, all the alternative scenarios are modeled to reach reclaimed water of class A quality (*E. coli* < 10 CFU/100 ml). The physical system boundaries of LCA were defined according to the goal and scope of the study, i.e. the comparison of different tertiary treatment schemes. It included not only the water line processes (L2) but also the water and nutrient demand of the surrounding irrigation area (1500 ha). In fact, the described scenarios are assumed to treat the entire flowrate actually treated by Line 2 (64282 m^3/d), while the effective request of water for irrigating the surrounding area is almost half of the WWTP flow. Therefore, 32959 m^3/d are reused in agriculture and 31323 m^3/d are discharged in the Lambro River. At the same time, the nutrient demand of crops is first covered by the N and P contents of the reclaimed water and then by a supplementary amount of mineral fertilizer, if needed. To model foreground and background processes, the following data were considered: the volume and the quality of all water streams, direct GHG emissions from processes, energy consumption, production and transportation of chemicals, wastes disposal (incineration or landfill), surface water withdrawal and production and spreading of fertilizers. To compare the environmental performance of the different scenarios, 1 m3 of treated wastewater was selected as the functional unit.

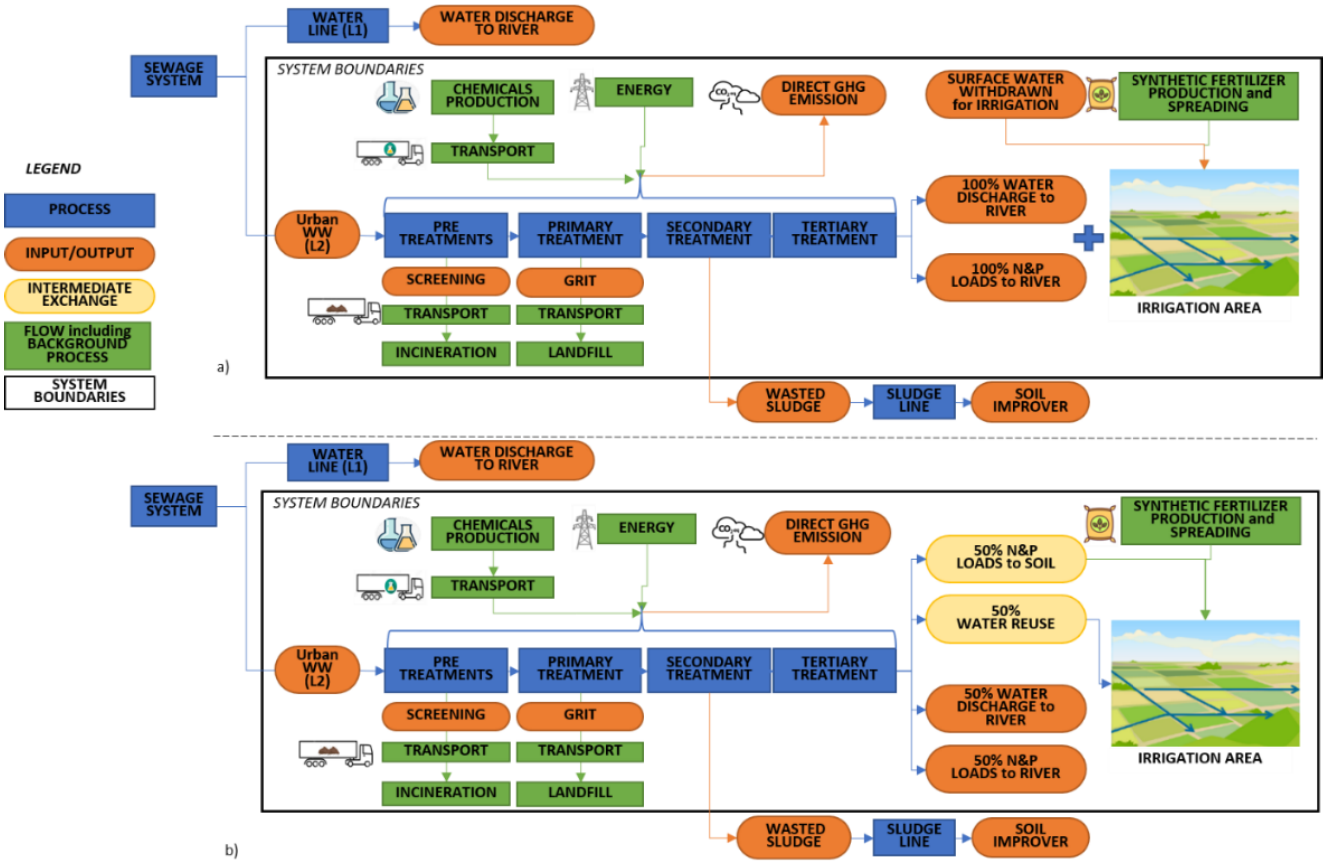


Figure 57: System boundaries for the life cycle assessment: a) baseline configuration; b) alternative scenarios.

A detailed summary of the Life cycle inventory (LCI) of all the scenarios is reported in Table 31.

Table 31: Life cycle inventory of the operation stage for the four scenarios.

		Baseline scenario	Scenario 1	Scenario 2	Scenario 3
		No reuse	Reuse of class A reclaimed water		
Parameters	**Units**	**UV**	**High dosage UV**	**PAA**	**AnMBR**
Q treated (L2)	m^3/d	64,282	64,282	64,282	64,282
Q discharged to river	m^3/d	64,282	31,323	31,323	31,323
Q required by crop	m^3/d	32,959	32,959	32,959	32,959
Q surface water withdrawn	m^3/d	32,959	0	0	0
Q water reused for irrigation	m^3/d	0	32,959	32,959	32,959
TN effluent concentration	g/m^3	8	8	8	24
TN required by crop	kg/d	658	658	658	658
TN added by water	kg/d	0	275	275	791
TN added by mineral fertilizers	kg/d	658	383	383	-
Excess TN to soil	kg/d	-	-	-	133
TN discharged to surface water	kg/d	536.35	261.35	261.35	751.73
TP effluent concentration	g/m^3	1.04	1.04	1.04	1.94
TP required by crop	kg/d	82	82	82	82

TP added by water	kg/d	-	34	34	64
TP added by mineral fertilizers	kg/d	82	48	48	18
Excess TP to soil	kg/d	0	0	0	0
TP discharged to surface water	kg/d	67.12	32.71	32.71	60.88
Consumed electricity (secondary treatments)	kWh/d	9792	9792	9792	900
Consumed electricity (tertiary treatments)	kWh/d	2517	5472	43	12,381
Consumed electricity (whole plant)	kWh/d	20,318	23,273	17,844	21,290
Produced electricity	kWh/d	0	0	0	1350
Self-produced heat	MJ/d	0	0	0	4236
PAA at 16% w/w	kg/d	0	0	2009	0
Citric acid at 100% w/w (membrane cleaning)	kg/d	0	0	0	93
NaOCl at 15% w/w (membrane maintenance)	kg/d	0	0	0	618

The life cycle impact assessment was carried out by applying the "ReCiPe 2008 Midpoint (H) V1.13 no LT" (results without long-term emissions) method for the following impact categories: climate change (CC), fossil fuel depletion (FD), freshwater eutrophication (FE), mineral resource depletion (MRD), water depletion (WD), freshwater ecotoxicity (T-FET), human toxicity (T-HT) and terrestrial ecotoxicity (T-TET).

The considered background processes were modelled through the Ecoinvent v3 database. Nevertheless, some assumptions were made according to previous literature experiences:

- The peracetic acid production (PAA), since no available data are present in the Ecoinvent database, is modelled through the production processes of acetic acid (CH3COOH) and hydrogen peroxide (H2O2) assuming that the production of 1 kg of PAA requires 0.45 kg of CH3COOH, 0.79 kg of H2O2 and 0.28 kg of water (Buonocore, Mellino, De Angelis, Liu, & Ulgiati, 2018)
- The polyelectrolyte is implemented as polyacrylamide, which is a kind of polymer used for sludge coagulation (Zhang et al., 2020).
- Trucks 16–32t EURO 4 were selected as transport vehicles for sludge and wastes disposal. In the case of chemicals, vans less than 3.5t were the preferred option due to the small amount transported.
- The processes "disposal, plastics, mixture, 15,3% water, to municipal incineration" and "disposal, paper, 11,2% water, to municipal incineration" are used to estimate the environmental burdens caused by the disposal of screening waste, assuming that such waste is only composed by paper and plastic (Buonocore et al., 2018).
- The process "disposal, inert waste, 5% water, to inert material landfill" can be considered for the grit disposal (Buonocore et al., 2018; Lorenzo-Toja et al., 2016)

- The mineral fertilizers considered are TSP (triple superphosphate) and CAN (calcium ammonium nitrate), in line with other LCA including fertilizer offset (Flores-Alsina et al., 2010; Pasqualino et al., 2011; Pintilie et al., 2016; Yoshida et al., 2018).
- The amount of avoided fertilizers were estimated according to plant availability of 30% for N, while 70% of P is considered to be available for the crops (Bengtsson et al., 1997).
- The emissions associated with the physical application of sludge-based or mineral fertiliser are mainly from the combustion of diesel used by machines.
- Electricity production was based on the consumption of Italian specific electricity mix.

4.2.2 Results

The baseline scenario shows the largest environmental impact in all categories, except for freshwater eutrophication, human toxicity, and terrestrial ecotoxicity. As expected, the highest benefit is observed in water depletion category since the withdrawal of freshwater is replaced with reclaimed water reuse. Scenario 1 and 2 show a significant reduction in freshwater eutrophication due to the lower amount of P directly discharged to the river. On the other hand, Scenario 3 rises the impact on freshwater eutrophication since the UASB + AnMBR effluent is highly rich in nutrients that leads to higher rate of PO4-release. However, due to the savings of producing and spreading mineral fertilizer, this scenario has a lower impact on fossil fuel depletion (68%). A slight reduction of 3% and 6% in fossil fuel depletion impact is observed also in Scenarios 1 and 2, respectively, compared to baseline scenario, since they are strongly related to fossil fuel combustion required in energy production and in the transport of the disinfection agents. Looking at the toxicity-related categories, the toxicity in the water environment is higher in the Baseline since traces of heavy metals present in the effluent are fully discharged into the river. However, increased toxicity levels for terrestrial and human categories are observed in the alternative scenarios where the toxic compounds are partially sent to the soil, due to irrigational water reuse.

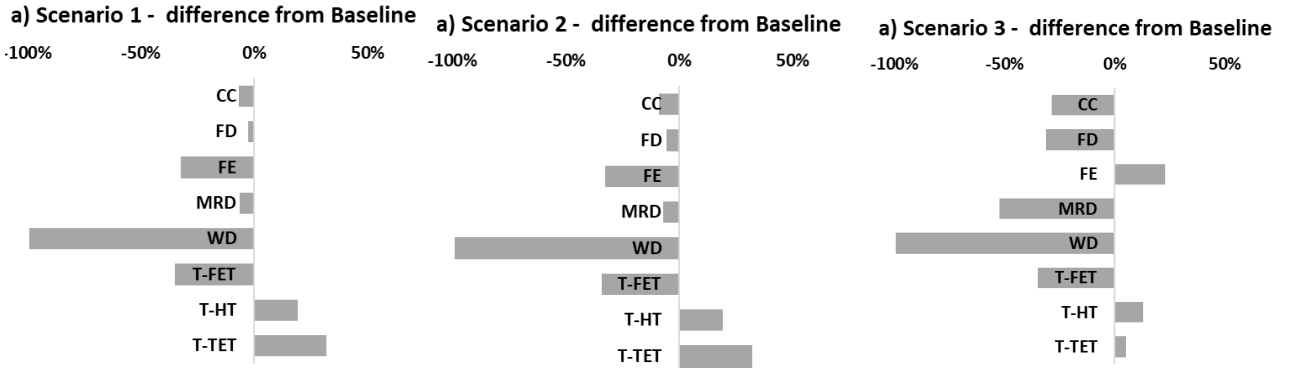


Figure 58: LCA results of the different scenarios

In conclusion, lower impacts were obtained in terms of climate change (-28%), fossil fuel depletion (-31%), mineral resource depletion (-52%) and freshwater ecotoxicity (-35%) in the treatment scenario based on anaerobic treatment coupled to ultrafiltration in comparison to the baseline scenario based on conventional activated sludge system coupled to ultraviolet disinfection.

4.3 Parametric local and territorial carbon footprint

Carbon footprint is an evaluation of GHG emissions due to WWTPs activities. General guidelines on Carbon footprint inventory are provided by UNI EN ISO 14064-1:2019-1. However, we still miss a unified methodology to assess carbon footprint from WWTPs (IPCC, 2013; Mannina et al., 2019). One of the simplest ways to evaluate carbon footprint is using Emission factors (EFs) that normalize the emissions with respect to a process parameter. Depending on the category, there are many databases that collect and update EFs. IPCC Guidelines provide EFs specific to different processes or activities and developed an online database, the EFDB*2, that can be freely consulted. On the other hand, more specific EFs can be evaluated at national level. For instance, ISPRA and SINANET developed national databases for the main activities in Italy (ISPRA, 2020), while the Swiss national GHG inventory has been updated under the UNFCCC in 2020, covering the years 1990-2018 (Federal Office for the Environment FOEN, 2020). According to IPCC an EF of 1.6% for of the total N-load is suggested (IPCC, 2019); to increase reliability of the EF estimates in large WWTPs, a country-specific EF is also proposed. Reliable EFs estimates require long-term sampling campaigns to capture the seasonal variability of N2O emissions, and thus this is rarely applied in practice due to the associated costs and labour demands. Many tools have been proposed, such as the Carbon Footprint Calculation Tool (Gustavsson & Tumlin, 2013) developed in the project entitled “Calculation of the CF from Swedish WWTPs” (SVU 12-120). More sophisticated tools use models to simulate treatment processes. First, steady-state process models were developed to assess GHG emissions. In recent years, dynamic models are also applied to evaluate strategies for GHG emissions mitigation (Mannina et al., 2016).

The need to develop a methodology for carbon footprint assessment dedicated to wastewater utilities arises from the requirements of the National authority ARERA, stated in the document 917/2017/R/idr about the *Regulation of The Technical Quality of The Integrated Water Service* (RQTI). Specifically, with the G5.3 indicator ARERA has introduced the calculation of the carbon footprint of the wastewater treatment service, which should be evaluated according to the UNI EN ISO 14064-1 standard. UNI EN ISO 14064-1 standard details the methodology for quantifying greenhouse gas emissions at the organization level, considering both direct and indirect activities. The standard is not specifically declined for the Integrated Water Service and therefore requires detailed assumptions for its contextualization and application to the water sector.

The developed methodology for parametric calculation is based on the product between the “Data activity”, which quantifies the specific flow of each emission source, and the corresponding “Emission factor”. The quantified emission data were then converted into CO2 equivalent on the basis of the Global Warming Potential (GWP) factors, as reported in the 2019 IPCC guidelines and summarized in Equation 1.

$$Emission\ contribution\ \left(\frac{tonCO_2e}{y}\right) = Activity\ data\ \left(\frac{quantity}{y}\right) * EF\ \left(\frac{tonGHGs}{quantity}\right) * GWPs\ \left(\frac{tonCO2e}{tonGHG}\right)$$

Where is it:

- GHG emission: quantification of GHG emitted by the activity, expressed in terms of tons of CO_2 equivalent (tons CO_2e), in the reference period.
- Activity data: quantity, generated or used, which describes the activity, and which can be expressed in terms of energy (e.g., J, MWh, ...), mass (e.g., ton, kg, ...) or volume (e.g., m^3, l, …).
- EF: emission factor, which express in a specific way the greenhouse gas emissions generated in relation to the reference quantities.
- GWP "Global Warming Potential", represents the climate-altering power of each greenhouse gas and express the emissions of different GHGs into CO_2 equivalent.

WWTP are considered responsible for greenhouse gas emissions, as CO_2, CH_4 and N_2O are mainly generated in the organic matter degradation and nutrient removal processes (IWA Publishing, 2018). As concern direct emissions, CO_2 is generally produced by the oxidation processes of organic matter, methane derives to a greater extent from anaerobic processes that can occur in the plant or already in the sewer network, while N_2O emissions occur during the nitrification and denitrification phases of biological processes. The gases are transferred into the atmosphere mainly through diffusion or stripping phenomena.

The production of direct N_2O emissions is mainly caused by the biological processes of nitrification and denitrification. However, the main emission phase essentially takes place during the aerated phase, in which the gaseous molecules produced are transferred into the atmosphere, also based on the characteristics of the oxygen transfer systems present in the biological compartments. From the analysis of the technical-scientific literature it appears that the direct emissions of N_2O for the wastewater purification processes present a considerable variability, in an indicative range from 0.5 to 20% of the incoming nitrogen load (Campos et al., 2016). During the nitrification process, the main parameters influencing N_2O production are the concentration of NH^{4+}, NO^{2-} and dissolved oxygen, the presence of metals and the pH conditions. In particular, limiting dissolved oxygen concentrations could lead to an increase in N_2O emissions. In addition, high influential nitrogen loads and high concentrations of NH^{4+} lead to higher N_2O emissions, as they lead to high oxygen consumption. Retention times also affect N_2O production. in particular, it is observed that low SRTs lead to NO^{2-} accumulations due to incomplete nitrification, causing an increase in N_2O emissions. Changes in the operating conditions in the reactor, such as in the case of processes with intermittent aeration, lead to higher emissions of N_2O. it was also observed that, in conditions of pH equal to 8, the emissions of N_2O are greater than those observed at neutral pH. During the denitrification process, the parameters that most determine the production of N_2O are the load of organic carbon, the concentrations of NO^{2-} and NO^{3-} and the environmental conditions of SRT and pH. Low C: N ratios generally cause a substantial increase in N_2O emissions. The type of carbon source also influences N_2O emissions, noting that rapidly biodegradable sources lead to lower emissions. Again, low SRT conditions, which involve incomplete denitrification reactions, cause an increase in N_2O emissions. The accumulation of NO^{2-}, NO and NH_3 lead to an increase in the production of N_2O. The partial nitrification processes, therefore, in which there are high concentrations of NO^{2-}, involve greater quantities of N_2O. Furthermore, as explained for the nitrification processes, intermittent oxygen conditions cause high N_2O emissions. In nitrification conditions with low levels of dissolved oxygen, simultaneous denitrification could reduce the accumulation of NO^{2-} linked to the

denitrification phase alone and therefore the related emissions. As for the environmental conditions, pH values below 6.5 cause an increase in the quantities emitted.

Table 32 summarizes the main measures for minimizing N_2O emissions from wastewater treatment processes and, where quantifiable, the relative mitigations that can be obtained, it is specified that those most applicable in the most common water utilities.

Table 32: Interventions for the minimization of N2O emissions from wastewater treatment processes

Intervention	Impacts	Reference
Monitoring of precursors parameters NO_2^-, NO e NH_2OH	Increase of N_2O production of 44% in aerobic systems with nitrates	(Desloover et al., 2012)
Keep dissolved oxygen > 2mg/l in aerobic phase and ensure adequate aeration phases with intermittent aeration		(Desloover et al., 2012)
Ensure adequate substrate levels, through gradual feeding, sufficient mixing and volumes		(Desloover et al., 2012)
Ensure low concentrations of free ammonia and free nitrous acid		(Desloover et al., 2012)
Avoid transient conditions between anoxic and aerobic conditions	EF in aerobic conditions: 0.17-0.24% Nin vs EF intermittent aerobic phase: 0-0.39% N influent	IWA 2018
Avoid load peaks of ammonia nitrogen	Critical load of 1.6 mgN-NH_3/gTSS	(Desloover et al., 2012)
Ensure sufficient SRT	Increase of EF up to 16% with low SRT (3d)	IWA 2018
Ensure sufficient Cu availability	Reduction of 54.7-73.2% with 10-100 µgCu/l	IWA 2018
Avoid pH in nitrification phase > 8		(Desloover et al., 2012)
Avoid pH in denitrification phase < 5-6		(Chen et al., 2018)
Stability of microorganisms AOB		(Desloover et al., 2012)
Bio-augmentation with AOB		(Desloover et al., 2012)
Bio-augmentation with species such as Pseudomonas stuzeri		(Desloover et al., 2012)
Limit aeration speed and turbulences		(Desloover et al., 2012)
Optimise aeration efficiency, without over-aeration, optimisation of bubble dimension		(Desloover et al., 2012)
Reduce COD removal in the upstream units or provide external carbon sources	Reduction of N_2O production up to 95%	(Chen et al., 2018)
Selection of external carbon sources, preferring methanol to ethanol		(Desloover et al., 2012)
Ensure sufficient HRT to denitrification process		(Desloover et al., 2012)
Odour treatment (biofiltration) of covered biologic units		(Desloover et al., 2012)
Microalgae process for nitrogen removal	Emissions 0.005% kgN-N_2O/kgN influent; consumption of CO_2 in photosynthesis and production of 17 kgO_2/kgN removed	(Campos et al., 2016)

Passive aeration technologies (biologic with rotative contact) or aeration systems without bubbles (MABR)		(Desloover et al., 2012)
Bioelectrochemist process for the removal of N_2O without oxygen		(Desloover et al., 2012)
Biologic gas treatment gas with nitrification and denitrification bacteria or microalgae in biofilters or scrubber	Removal N_2O efficiencies of 75-99%	(Campos et al., 2016)
Collection of N_2O from filtration/nitrification units and use as oxidant for the combustion of the CH_4 produced in anaerobic digestion		(Campos et al., 2016)
Increase of freeboard temperature in thermos-valorisation plants	Emission reduction from 560 to 110 mgN_2O/m^3 increasing T from 844°C to 876 °C	(SaÈnger et al., 2007)
Adding of auxiliary fuels, through methane, ethane or propane injection in thermos-valorisation plants	Emission reduction of 70% with ethane and prophane, reduction of 30% with methane	(Liu and Gibbs, 2000)

Where possible, the average, maximum and minimum emission factors have been plotted in Figure 59, distinguished on the basis of the type of biological process.

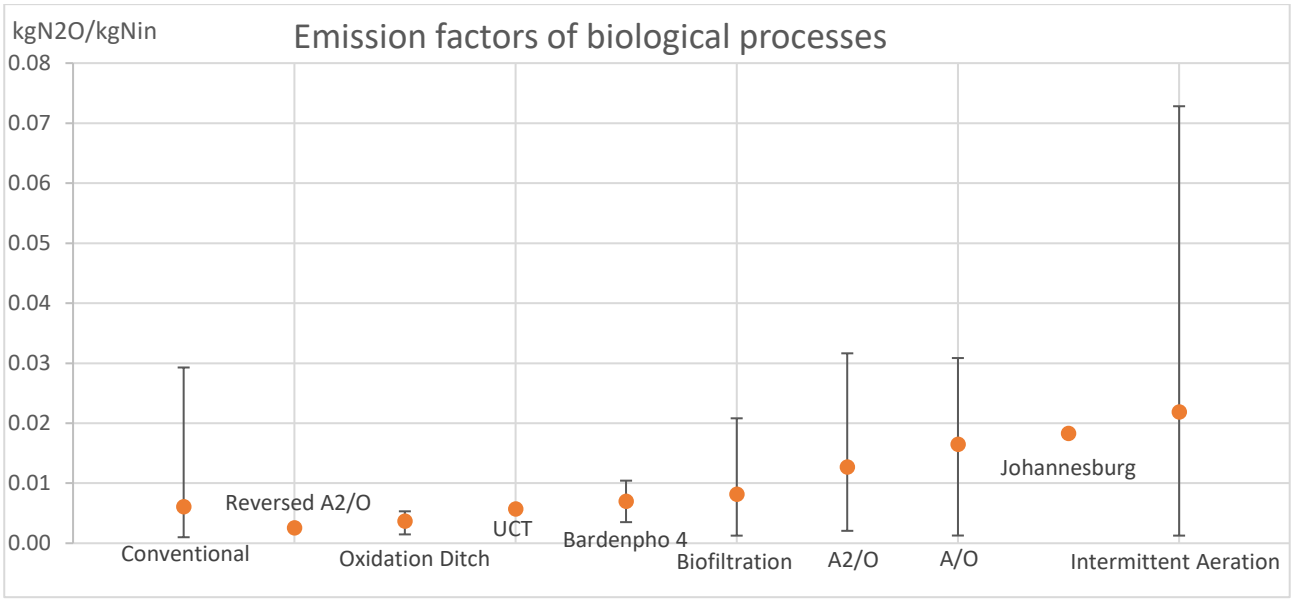


Figure 59: Emission factors of the main biological processes

From the analysis of the results, it can be seen that all the emission factors show very high standard deviations, linked to the site-specific conditions of the plants and the characteristics of the influent wastewater. The emission factors vary in ranges that tend to be comparable for conventional configurations (DN) and biologic nutrient removal (BNR) but increase in an evident way for processes with intermittent aeration. As an indication only, without specific details on the characteristics of the processes, the average emission factor of a conventional treatment is more than three times lower than any configurations that apply processes with aerobic / anoxic phases.

CH_4 emissions are generally generated along the sewer system and during anoxic and anaerobic conditions, while they are released during aerated processes. Methane emissions can vary

considerably, depending on the characteristics of the influent, the environmental conditions and the process parameters. As the temperature increases, an increase in emissions is observed, so in the summer seasons the highest emissions are observed. The relationship between dissolved oxygen and CH_4 is less evident, as higher concentrations inhibit the generation of methane, but at the same time the aerated conditions facilitate the stripping of dissolved CH_4. The characteristics of the influent, such as the C:N ratio and, in particular, the incoming concentration of COD, affect methane emissions. Emission mitigation operations may include the coverage of aerated units, the oxidation of CH_4 in activated sludge processes by methanotrophic bacteria or the reduction of their generation. In addition, the methane produced during the anaerobic digestion processes can be used as a renewable energy source, thus reducing the impacts due to the use of fossil sources. To reduce the generation of CH_4, actions can be carried out on the wastewater entering the sewer network, trying to avoid sedimentation conditions along the pipelines, reduce the run-off times, limit the length of the networks and increase the frequency of cleaning operations. Generally, the formation of anoxic conditions and the generation of fermentation processes must be avoided, both during treatment and upstream along the sewer system.

Table 33 summarizes the main interventions for the minimization of CH_4 emissions from wastewater treatment processes and, where quantifiable, the relative mitigations that can be obtained, it is specified that those most applicable in most of the water utilities.

Table 33: Interventions for the minimization of CH4 emissions from wastewater treatment processes

Intervention	Impacts	Reference
Cover treatment units and storage of sludge, with biofiltration air treatment		(Campos et al., 2016)
Recovery through biogas production	35-45% of energy in wastewater can be converted into CH_4	(Campos et al., 2016)
Recovery of fugitive CH_4 emissions from anaerobic digestion	Energy recovery of 10-30%	(Chen et al., 2018)
Avoid low temperatures in anaerobic processes in the water line (AnMBR)	Loss of 10-90% of CH_4 produced	(Chen et al., 2018)
Oxidation of CH_4 in anaerobic condition with sulphates, nitrates, nitrites, Mn^{+4} o Fe^{+3} as electron acceptors		(Campos et al., 2016)
In situ biogas sparging	Action on gas-liquid balances with reduction of dissolved CH_4 up to 50%	(Chen et al., 2018)
Membranes in sweep-gas contact	Reduction of dissolved CH_4 up to 92.6%	(Chen et al., 2018)
Membranes with vacuum aspiration	Desorption of 77%-86% of dissolved CH_4, CH_4 recovery	(Chen et al., 2018)
Downflow hanging sponge DHS	Removal up to 97%-99% of dissolved CH_4, recovery of gas with high concentration of CH_4 (30%)	(Chen et al., 2018)
Oxidation anaerobic denitrification DAMO		(Chen et al., 2018)
Microbial Fuel Cells MFC		(Chen et al., 2018)

WWTPs are also responsible for indirect emissions related, for example, to energy consumption and the use of reagents, as these resources are characterized by an intrinsic carbon footprint, linked to their production, processing and transport activities. It is specified that a careful analysis of the

technical-scientific literature of the sector has been carried out for the categories relating to direct emissions related to the processes and for those indirect on the receiving water body, which represent the contributions most connected to the site-specific characteristics of the individual plant configurations, specific purification yields and construction details of the sewer networks serving the treatment plants. In addition to the analysis of the international guidelines (IPCC - Intergovernmental Panel on Climate Change, 2006 version and 2019 update), an in-depth analysis of the technical-scientific literature of the sector was conducted, and is continuously updated also with targeted measurement campaigns, to identify specific and appropriate emission factors for the wastewater treatment sector and to select the most suitable ones for describing the particular conditions characteristic of the individual activities or plants of the organization. The operational boundaries of the wastewater treatment service should reasonably be associated with the individual installations under the control of the organisation. It should be noted that the main direct emissions of GHGs are caused by the degradation processes of the organic substance and the removal of nutrients, by the biogas combustion systems, by fugitive losses in the transfer and temporary storage systems. The main sources of direct emission and / or removal are therefore found in the aerated compartments of the water line, in any odour treatment units, in the biogas conveying and combustion sections or in the storage sites of the final sludge produced. The indirect emission categories, on the other hand, include a wider range of activities (e.g., energy consumption or use of reagents), however external with respect to the operational boundaries of the organization. It is specified that indirect emissions must be accounted for, to the extent that they can be attributed to operational, managerial or operating choices directly linked to the organization's activity.

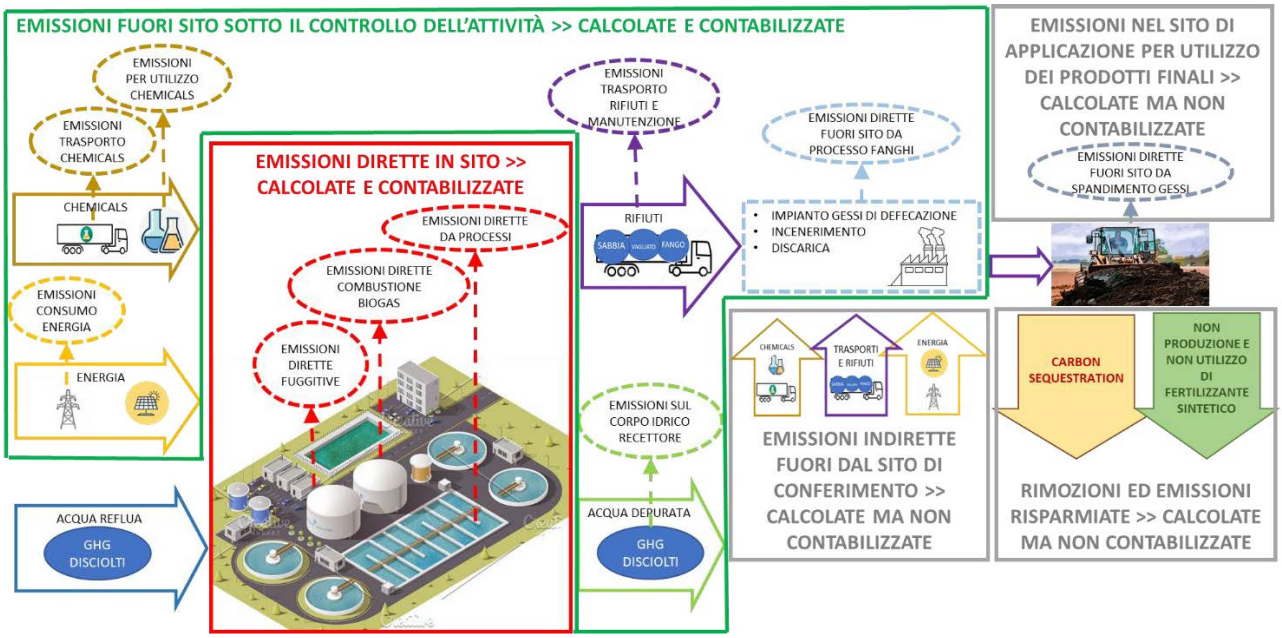


Figure 60: schematic representation of system boundaries and emissions

The approach is schematized in Figure 60, distinguished between:

– direct emissions on site calculated and accounted for in the final report of the CF (border highlighted in red in Figure 60) as they are directly emitted within the WWTPs.

- indirect off-site emissions calculated and accounted for in the final report of the CF (border highlighted in green in Figure 60) as a direct consequence of management decisions for which the organization is responsible.
- indirect off-site emissions calculated but not accounted for in the final report of the CF (border highlighted in grey in Figure 60) as it is only partially a direct consequence of management decisions for which the organization is responsible.

More specifically, the direct emissions on site (in red in Figure 60) are mainly caused by the generation of greenhouse gases during the treatment processes, by the combustion of the biogas produced in the plant and by fugitive emissions caused by losses in the treatment and transport of biogas or due to the temporary storage of sludge produced in the plant. Off-site emissions, on the other hand, under the control of the activity (in green in Figure 60) include indirect emissions that fall under the responsibility of the organization. The use of reagents and the choice of the type of these in the purification processes, for example, represents an operational decision of the operator, who has identified a specific chemical treatment method, compared to other alternative processes, or a particular type of reagent or a particular supplier. Therefore, the indirect emissions related to the production and supply of these reagents, although outside the operational confines of the company, fall indirectly under the responsibility of the organization, which has chosen to use them and determine, in this sense, their value. final of the CF. Similarly, the emissions for energy consumption are attributable to the control of the company, which can act on the choice of processes with higher or lower energy demand, on the type and efficiency of electro-mechanical equipment, as well as on the purchase of energy from fossil or renewable sources. Indirect emissions on the receiving water body also fall within this definition, since they are partially dependent on the removal efficiencies of the treatment processes. In addition, indirect emissions related to the transport of waste to the respective delivery sites, established by the organization, and to the handling due to internal maintenance activities are considered. Similarly, with regard to the destinations of the final sludge, the direct emissions generated in the post-processing and final delivery sites were quantified and accounted for in the indirect category. This aspect is essential to include and distinguish in a clear and evident way the managerial choice of the final delivery destination (e.g., composting, direct spreading, thermal processes, gypsum and carbonate production, etc.) which cannot fail to disregard operational and exercise of the organization. On the other hand, the indirect off-site emissions outside the control of the company (grey in Figure 60) were calculated but not included in the final report of the carbon footprint of the plants, as they are not directly attributable to the control of the organization. These include indirect emissions for the energy consumption of the delivery site, direct emissions at the site of use of the final products (e.g., spreading in agriculture) and the related removals or mitigations of emissions (e.g., carbon sequestration, non-use of synthetic fertilizers, etc.).

Table 34: Emission categories declined for the wastewater service.

Declined category	Activity data	Emission factor (EF)	Ref.
Direct emission	Biogas produced (Nm^3/y),	g CO_2/kg burned CH_4 g CH_4/kg burned CH_4 g N2O/kg burned CH4	International databases (IPCC, 2019b)

from combustion	methane content (%CH_4)		
Direct emissions from WWTP processes in mainstream	Influent and effluent COD and TN loads (ton/y)	kg N_2O/kgTNrem kg CH_4/kg CODin kg CO_2/kg CODrem	Measured, Literature, International databases (Aboobakar et al., 2013; Ahn et al., 2010; Caniani et al., 2019; Foley, de Haas, Hartley, et al., 2010; Joss et al., 2009; Masuda et al., 2018; Ribera-Guardia et al., 2019; Wang et al., 2016);
Direct fugitive emissions	Biogas produced (Nm^3/y) and methane content (%CH_4); sludge produced (ton/y) and characterisation (%TS, %N, %P, %K, %C)	kgN_2O/tonTS kgCH_4/tonTS kgCO_2/tonTS	Literature (Kirkeby et al., 2005; Majumder et al., 2014; Scheutz & Fredenslund, 2019; Willén et al., 2016)
Indirect emissions for energy consumption	Electricity consumption (MWh/y), other fuel combustion also renewable (Nm^3/y or l/y)	tonCO_2eq/GWh	National and International databases (IPCC, 2019a; ISPRA, 2018)
Indirect emissions for waste transport	km travelled	gN_2O/km gCH_4/km gCO_2/km	National databases http://www.sinanet.isprambiente.it/it/sia-ispra/fetransp
Indirect emissions for chemical transport	km travelled	gN_2O/km gCH_4/km gCO_2/km	National databases http://www.sinanet.isprambiente.it/it/sia-ispra/fetransp
Indirect emissions for chemical consumption	Quantity used (kg/y)	kgCO_2eq/kg reagent	Literature, International databases (Gustavsson & Tumlin, 2013)
Indirect emissions on the water body	Effluent COD and TN loads (ton/y)	g CO_2/kgCODeff g CH_4/kgCODeff g N_2O/kgTNeff	Literature and Measured
Indirect emissions and mitigation for sludge disposal	Dried sludge	kg N_2O/tonTS kg CH_4/tonTS kg CO_2/tonTS	Literature, International databases (Boldrin et al., 2009; Chai et al., 2015; Chen & Kuo, 2016; Han et al., 2018; IPCC, 2019c; Kirkeby et al., 2005; Piippo et al., 2018; Yuan et al., 2018; https://www.climfoot-project.eu/)
Indirect emissions from sludge reuse	Dried sludge	kg N_2O/tonTS kg CH_4/tonTS kg CO_2/tonTS	(Boldrin et al., 2009; Bruun et al., 2006; IPCC, 2019c; Kirkeby et al., 2005)

Mitigations: carbon sequestration and synthetic fertiliser substitution	Dried sludge	kg N_2O/tonTS kg CH_4/tonTS kg CO_2/tonTS	(Foley, de Haas, Yuan, et al., 2010; Kirkeby et al., 2005)

TS=total solids, CODin= influent chemical oxygen demand; CODeff= effluent chemical oxygen demand; CODrem= removed chemical oxygen demand; TNrem= removed total nitrogen; TNeff= effluent total nitrogen; K=potassium; C=carbon.

When water utilities manage a huge number of WWTPs, parametric calculation is performed only for the most significative plants, in terms of size and representativity. At the end of the parametric calculation phase of the selected plants, the determination of the territorial Carbon Footprint was carried out, with the use of the specific carbon footprint coefficients, represented by the ratio between the carbon footprint emitted by the plant and the effective potentiality.

$$\text{Specific coefficient of carbon footprint } \left[\frac{\text{tonCO2e}}{\text{PE} * \text{y}}\right] = \frac{\text{Carbon Footprint } \left[\frac{\text{tonCO2e}}{\text{y}}\right]}{PE}$$

The specific coefficients were graphed in respect to the effective potentiality (Figure 61), to identify different classes depending on the size of the plants. In fact, usually, WWTPs of greater size are also characterized by better treatment efficiencies and optimization of energy consumption, returning lower specific CF data (tonCO_2eq/year/AE).

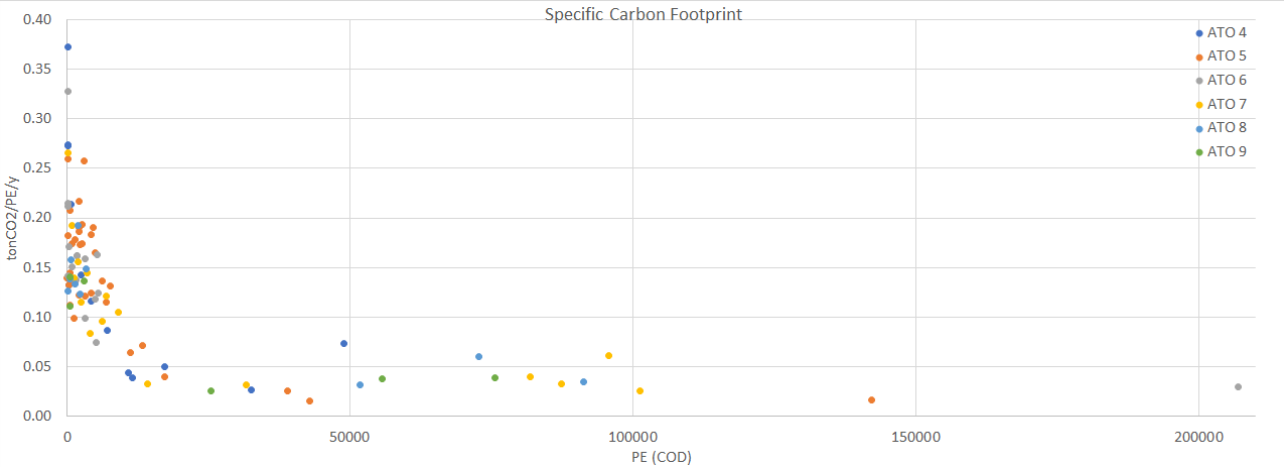


Figure 61: Specific carbon footprint of a water utility, managing different territorial districts (ATO)

The data obtained for each class were averaged and applied to the WWTPs in the same size-class for which the parametric CF was not calculated. Finally, the emissions due to the home-work transport of employees and the consumption of general goods and services of the company were added.

4.3.1 Application in Italian water utilities

The developed methodology was applied to four Italian water utilities, who manage wastewater treatment in North and Central Italy:

- Hera Group manages the Integrated Water Service of 228 municipalities in North-Centre of Italy, for a total of 3.6 million users, and controls 871 WWTPs, for a total nominal served population of 5607369 PE.
- Acegas manages 11 WWTPs in the North-East of Italy, for a total nominal served population of 587800 PE.
- Ireti manages the integrated water service in North-Centre of Italy, for a total nominal population of 585640 PE, with 49 WWTPs.
- Alto Trevigiano Servizi operates in 53 municipalities in the North-East of Italy, with a total served population of 411484 PE. The basin is characterised by 5 WWTPs with a design capacity of more than 40000 PE, by 3 WWTPs with the capacities range of 15000–40000 PE, 27 WWTPs with less than 15000 PE capacity, and 28 septic tanks.

The criteria used to identify the plants to be subjected to the parametric calculation were: a) the treatment capacity of each plant and b) the representativeness of the plants in respect to the size classes of the WWTPs and the site-specific conditions of the territory.

In particular, it was decided to select WWTPs in order to cover an overall representativeness of more than 90% of the total population served.

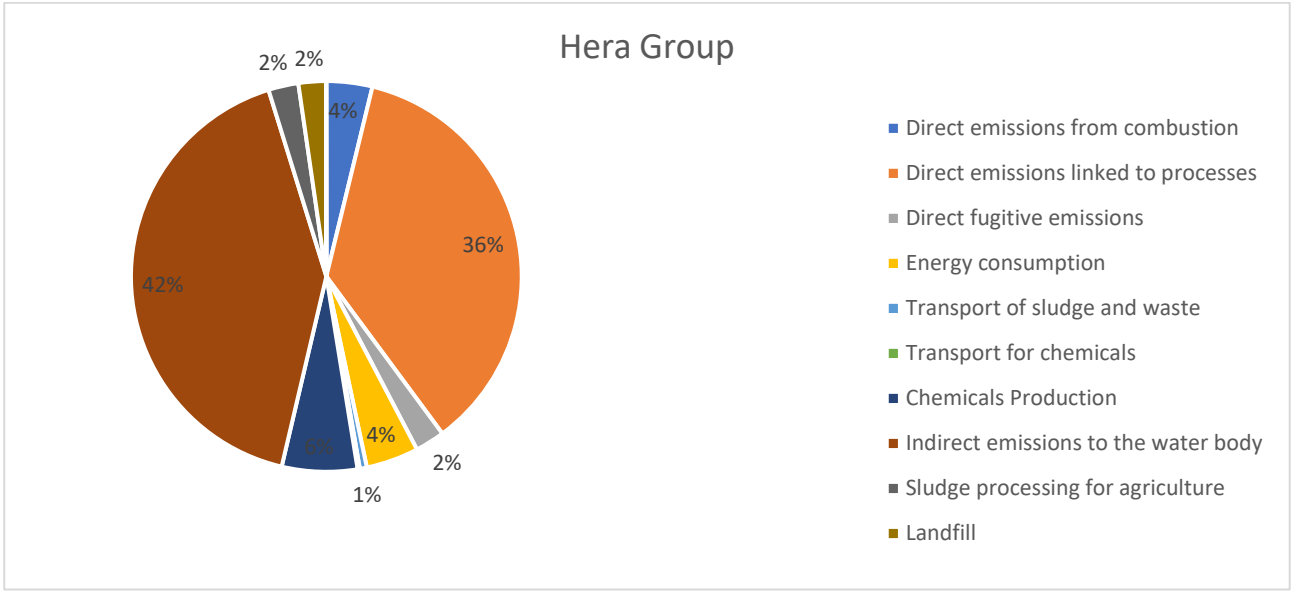


Figure 62: Carbon Emissions of Hera Group divided by category

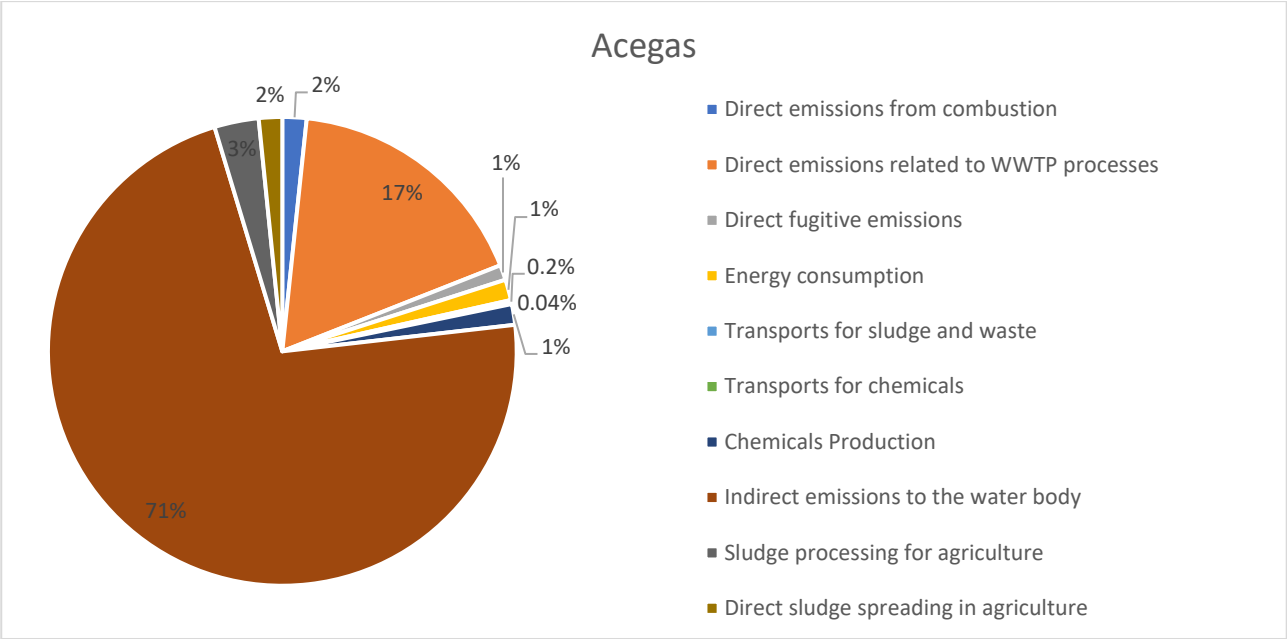


Figure 63: Carbon Emissions of Acegas divided by category

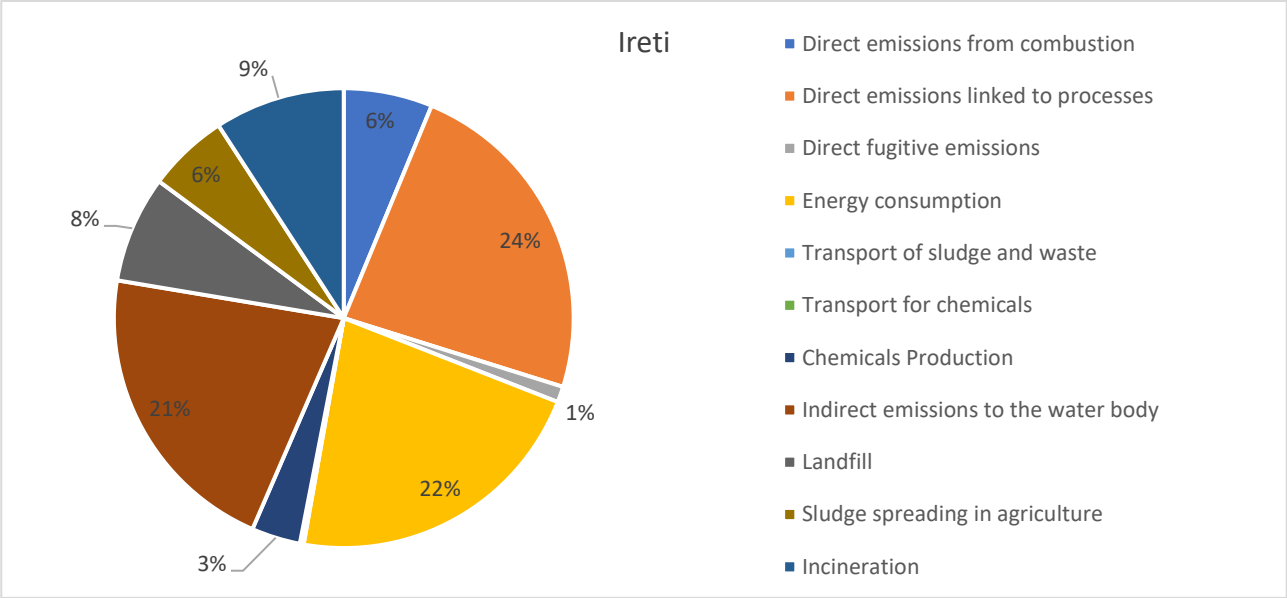


Figure 64: Carbon Emissions of Ireti divided by category

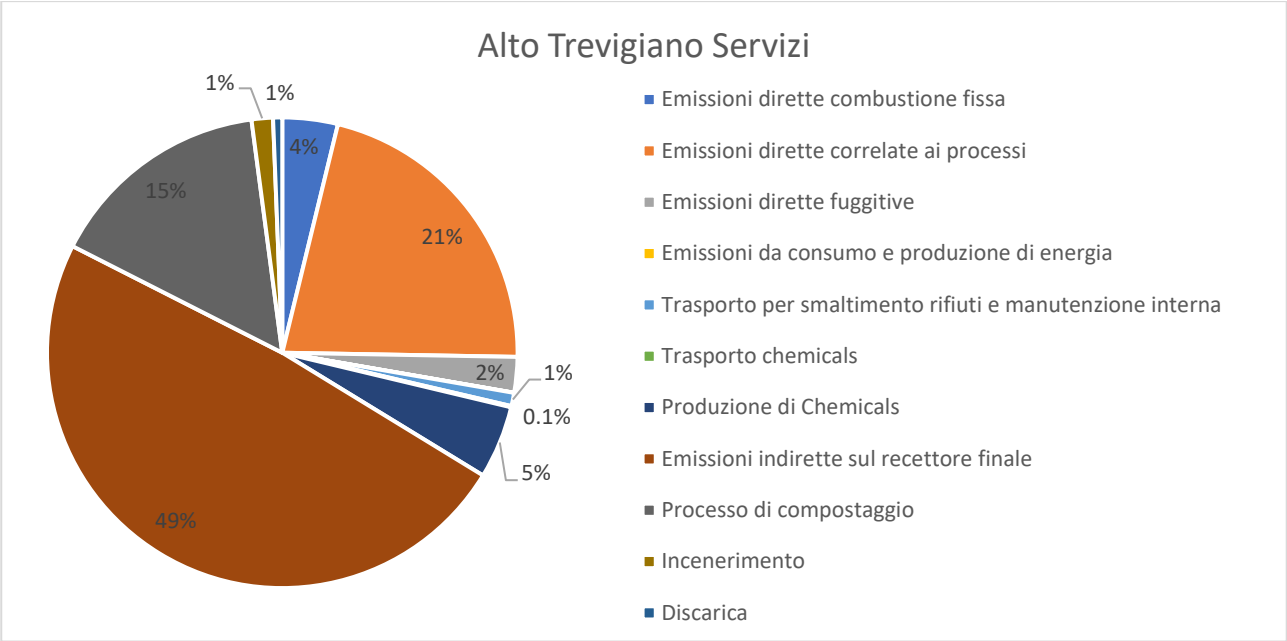


Figure 65: Carbon Emissions of Alto Trevigiano Servizi divided by category

Since the water utilities used their electricity from certified renewable sources, the CO_2 equivalent emissions related to its consumption is considered to be zero. In fact, the use of renewable sources of energy is considered as a strategy to mitigate carbon footprint.

Comparing the graphs in Figure 62 - Figure 65, it can be noted that the most impacting emissions categories are the direct emissions related to WWTP processes and indirect emissions to water body for all the organisations considered.

The quantification of the emissions attributed to each category can support defining mitigation activities.

Given that the direct emissions from processes and the indirect emissions on the receiving water body are more variable according to the specific plant conditions, the process parameters and the obtainable removal efficiencies, 3 different scenarios have been hypothesized and analysed, assuming to act on the optimization of removal efficiencies and minimization of emissive factors. In particular, the analysis was carried out with reference to N_2O emissions, as they are more dependent on the processes that occur in biological treatment.

First, it was assumed to act on the removal efficiencies, in order to obtain a better quality of the effluent in terms of total nitrogen concentrations and consequently to obtain lower emissions on the receiving water body due to the dissolved gases. The plants were grouped into two bands, depending on their size, respectively greater and less than 20000 PE, assuming two levels of mitigation, with minimum removal thresholds for total nitrogen divided by size:

- Level E1: E% Ntot equal to 60% in plants less than 20000 PE and equal to 80% in larger plants.
- Level E2: E% Ntot equal to 60% in plants less than 20000 PE and equal to 90% in larger plants.

A hypothesis of mitigation of direct emissions from processes was also evaluated, applying efficiency measures in the transformation yields of biological processes such as to obtain a reduction of between 20% and 40% of the emission factor. A further level was therefore defined:

• Level D1: 20% reduction of the emission factor of direct emissions of N2O from processes.

• Level D2: 40% reduction of the emission factor of direct emissions of N2O from processes.

As an example, mitigation scenarios performed for the Organisation 1 were schematised in Table 35. Scenarios have been compared with the state of the art, highlighting the possible reductions.

Table 35: Proposed mitigation scenarios

Mitigation scenario	**Intervention**	**Reduction % in respect to the state of the art**
		tonCO_2e/y
STATE OF THE ART	STATE OF THE ART	-
A	Use of alternative chemicals	> 1.3%
B	Delivery of sludge for processing in agriculture instead of in landfill	2%
C	Improvement of N removal efficiencies at 60% in WWTPs < 20000 PE and between 80%-90% in WWTPs > 20000 PE	Up to 6%
D	Improvement of N removal efficiencies at 60% in WWTPs < 20000 PE and between 80%-90% in WWTPs > 20000 PE + reduction of direct emissions from processes between 20%-40%	Up to 11%
A + B	SCENARIO 1	> 3%
A + B + C	SCENARIO 2	> 9%
A + B+ D	SCENARIO 3	> 14%

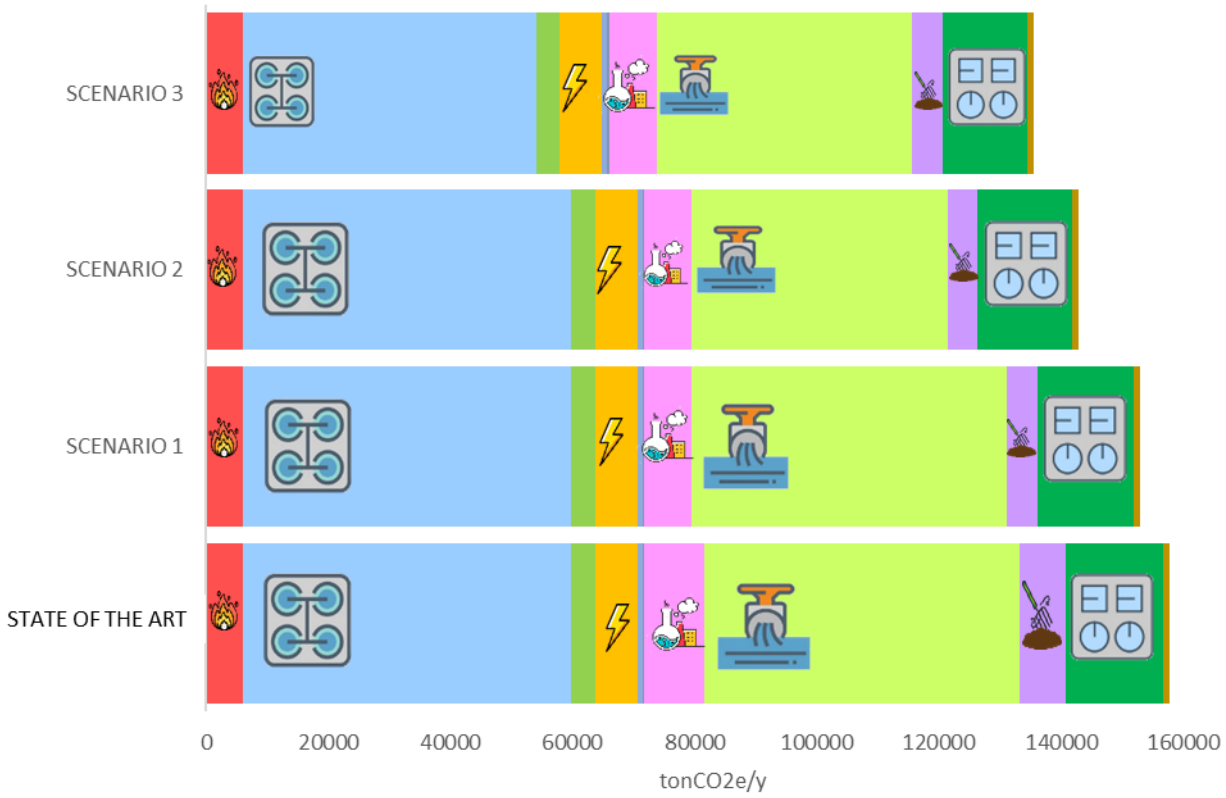


Figure 66: Carbon footprint reduction for the different scenarios analysed

4.3.2 Results and discussions

In summary, from the results of the analysis of the proposed mitigation scenarios, it appears that:

- Delivering the sewage sludge, currently destined for landfills, to processing plants for reuse in agriculture can reduce the expected impacts by an average of 2% on the territorial carbon footprint.
- By optimizing removal efficiencies, reaching between 80% and 90% for WWTPs bigger than 20000 PE and to 60% for smaller plants, it would be possible to mitigate the emissions to the water body, reducing further the impacts of at least 6% on the territorial carbon footprint. In this case it is crucial to select the processes that, with the same nitrogen removal, are less emissive than climate-altering gases.
- Applying processes that can reduce the direct N_2O emissions of 20% - 40% would lead to further mitigations, up to at least an 11% reduction on the territorial carbon footprint.
- It should be noted that the proposed scenarios do not include extremely site-specific mitigations, difficult to quantify a priori, associated with the direct or indirect emission of methane, that could further improve the total CF of the service.
- It should be noted that targeted mitigation actions of direct emissions from processes and indirect emissions on the water body can be identified for the single WWTPs, integrating the analysis with on-site monitoring and measurement activities, also accompanied by multi-parametric analysis and data analytics techniques, to correlate treatment processes and emissions, to define and highlight any critical issues and / or targeted interventions for reducing the carbon footprint.

4.4 Sustainability of phosphorus recovery

Rock phosphate was included in the list of EU critical raw materials due to its importance to the EU agri-food sector. Europe has no significant phosphate mines and the concentration of rock P in geopolitically sensitive regions was identified as a threat to European food security; ninety percent of the phosphate rock reserves are located in just five countries: Morocco, China, South Africa, Jordan and the United States. The fertilizer industry is the largest user of P therefore alternative sources to rock phosphate are urgently needed. As highlighted in the Circular Economy Package (2016), the EU has prioritized the recovery and safe reuse of plant bioavailable P from food and municipal waste streams, including agro-food industry sectors. Large resource streams, typically considered as waste and rich of P (wastewater, sludge and manure), are produced by food industry and can reach the environment, where P is lost. These 'waste' streams constitute secondary sources of P, which could be recovered and reused to shift to a circular economy.

The increasing demand and price, and the scarce quantity of primary P make the closing of P loop an urgent need. Moreover, the recent EU Fertilizer Directive (27/03/2019) recognizes the market demand of struvite and considers it as a secondary raw material for fertilizers. In this context, it is possible to rethink struvite as a valuable resource for a regenerative economy. Within the Revised EC Fertilizer Regulations that aim to harmonise the requirements for fertilisers produced from phosphate minerals and from organic or secondary raw materials in the EU, new possibilities for their production and marketing on a large scale are stated. The final STRUBIAS report (September 2019) nominated materials containing STRUvite (recovered phosphate salts), BIochar (including hydrochar), or incineration AShes as suitable candidate materials and the report provides technical guidelines for their quality. Nowadays, a lot of P-recovery technologies exist to recover P-salts, P-ashes, P-acids and most of them are applied to urban wastewater, sewage sludge and manure as reported in the ESPP–DPP–NNP Phosphorus Recovery Technology Catalogue, February 2020 (info@phosphorusplatform.eu).

The EIT Climate-KIC project PROSUMER aimed to perform a techno-economic and environmental feasibility study of Phosphorus recovery and reuse in fertilizers applied to Italian Prosumers (producers and consumers of P). Prosumer aims to explore the potential of P loop closing in Italian food industry. The techno-economic and environmental feasibility about the implementation of technologies to recover P (e.g., in form of struvite) and reuse it in fertilizers were evaluated with the engagement in Italian food sector involving also fertilizer producers. The main expected impact is the awareness that new valuable opportunities exist rethinking what now is considered waste, generating an effective circular economy.

As highlighted in the Circular Economy Package (2016), the EU has prioritized the recovery and safe reuse of plant bioavailable P from food and municipal waste streams. Phosphorus is present in wastewater in organic and inorganic form, soluble or particulate in variable percentages depending on the origin and the point of the plant where the analysis is carried out. In general, the partition of phosphorus in water depends on several factors. Generally, the presence of soluble inorganic phosphorus is due to the solubilization of the minerals crossed by the spring waters, while

polyphosphates derive from the use of detergents. Organic phosphorus can be hydrolysed and generate soluble inorganic orthophosphates or particulate phosphorus (Metcalf&Eddy, 2014).

From a theoretical point of view, phosphorus recovery is possible from three origins: liquid phase, sludge and sludge ashes. 90-95% of the incoming phosphorus in WWTPs is entrapped in the sludge, and phosphorus recovery from the liquid phase at the current stage of development will not exceed 20% to 40%, while P-recovery technologies from the sewage sludge and sludge ash have a higher potential for P-recovery (Shaddel et al.,2019).

4.4.1 Material and methods: P-recovery technologies and products

Figure 67 presents an overview of the main commercial technologies for phosphorus recovery and products. The most applied approach is struvite precipitation with high P concentration from the sludge in side-stream configuration or from industrial wastewater, mainly food processing, followed by calcium phosphate precipitation and phosphoric acid production. P-recovery, especially in wastewater treatment plant (WWTPs) with enhanced biological phosphorus removal (EBPR) and anaerobic digestion may help to improve plant economy via improved sludge treatment and dewaterability and to create an additional income via product sales. Other technologies are still either in research (laboratory) or development (pilot) phase, improving maturity, lowering costs, and gathering data for full-scale design (Shaddel et al.,2019).

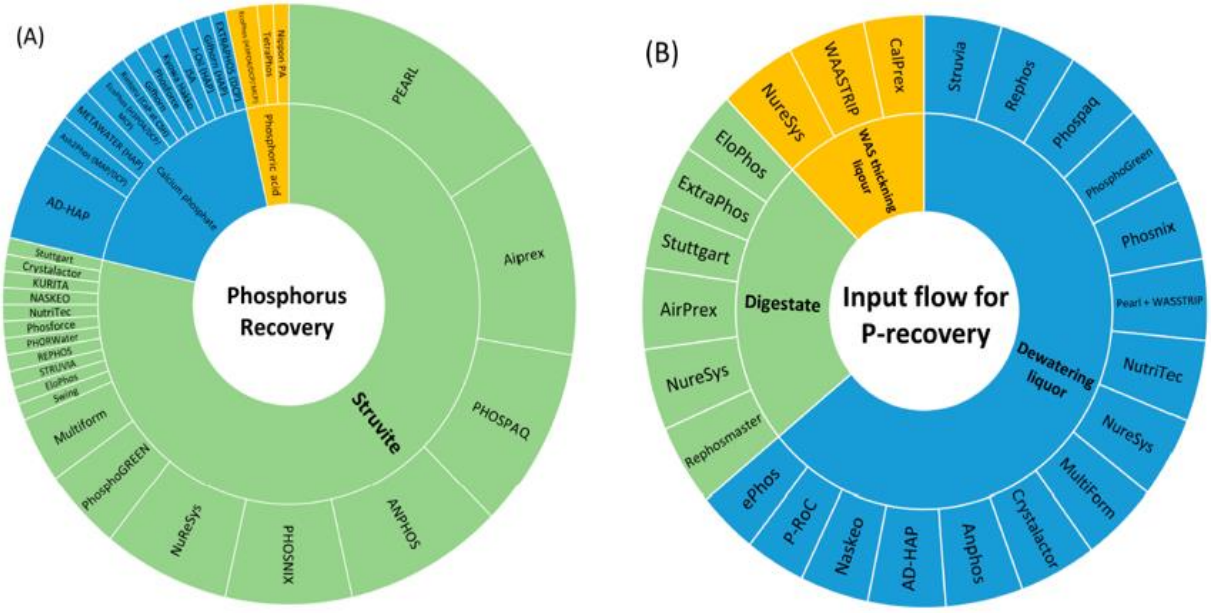


Figure 67: (A) Overview of the main products and the commercial technologies for phosphorus recovery, where area dedicated to each technology corresponds to the number of facilities in operation. (B) The input flow for P-recovery in different P-recovery technologies (Shaddel et al.,2019).

Although the panorama of technologies is very large and diversified, in the following sections only some technologies for P-recovery are described in detail, focusing on the those developed at full scale level, with high number of case studies in the world and/or with successful case studies in the sector of agro-food industries. The results of STRUBIAS report demonstrated that the precipitated phosphate salts and derivates achieve similar results to mineral fertilizers. Food processing industries are potential sources of recovered phosphorus, especially dairy and potato processing

industries. The recovered P is completely bio-available for plant nutrition and fertilisers produced since the recovered P is characterized by a Relative Agronomic Efficiency (RAE) of 105%, compared to traditional synthetic fertilizers. Moreover, unlike most of mineral fertilizers, precipitated phosphate salts are insoluble in water, while soluble in acid solutions, enhances P uptake from plant roots and lowers leachate from diluting waters.

It should be noted that the recovery rates (in terms of percentage of recovered P) are not always comparable between the different technologies, since they are generally calculated considering the phosphorus contents in different flows.

Therefore, three different **P-recovery rates** are introduced:

1. Recovery of P with respect to the influent P of the treatment plant: it is the percentage of recovery relating to P that enters the WWTP;

*Recovery % = (recovered P / WWTP influent P) * 100*

2. Recovery of P with respect to P that enters the recovery process: it is the percentage of recovery of the phosphorus present in the matrix directly sent to the recovery process;

*Recovery % = (P recovered / P influent to the recovery process) * 100*

3. Recovery of P with respect to P content in sludge or ashes destined for recovery: it is the recovery percentage of the phosphorus content in liquid sludge, fed as it is or after treatment, to the recovery process (point 3.1) or relating to the phosphorus content in the ashes, fed as it is or after pre-treatment, to the recovery process (point 3.2).

*3.1 Recovery % = (P recovered / P content in liquid sludge) * 100*

*3.2 Recovery %= (P recovered / P content in the ashes) * 100*

In Table 36 some of the most advanced technologies are provided for phosphorus recovery from waste and wastewater streams.

Table 36: Comparison of P recovery in different technologies

TECH	TRL	MATRIX	P-INFLUENT	RECOVERED PRODUCTS	P- RECOVERY
-	-	type	mgP/L	type	%
AIRPREX	9	liquid	150-250	struvite	10-25
PEARL OSTARA	full scale	liquid	100-900	struvite	10-30
Crystallactor	full scale	liquid	>25	CaP, MgPO4, struvite	10-40
GIFHORN	full scale	liquid after leaching		struvite	49
STUTTGART	full scale	liquid after leaching		struvite	45
STRUVIA	full scale	liquid	>50	struvite,CaP	11
NuReSys	full scale	liquid	60-175	struvite	80-90
PHOSPAQ	9	liquid	50-65	Struvite	70-95
ANPHOS	full scale	liquid	>50	Struvite	80-90
ASHDEC	7	Ash		calcined ash with CaNaPO phase	98
MEPHREC	pilot scale	Sludge and/or Ash		10-25%P rich slag	80
LEACHPHOS	pilot scale	Ash		CaP o Wet Struvite	70-80

LEGEND:

RED %P recovery in respect to the P-influent to the WWTP

BLUE %P recovery in respect to the P-influent to the recovery process

GREEN %P recovery in respect to the P-content in sludge or ash influent to the recovery treatment process

Since 90-95% of the incoming phosphorus in WWTPs is embedded in the sludge, and phosphorus recovery from the liquid phase (anaerobic rejected liquor) at the current stage of development will not exceed 20% to 40%, while P-recovery technologies from the sewage sludge and sludge ash have a higher potential for P-recovery up to 70-80% (Shaddel et al.,2019 and P-Rex, 2015).

The economic market for recovered P salts is focused mainly on fertilizer production industry (Table 37). According to P-REX report (2013), 5 productive sites are already using or are interested on using recovered P and other 13 companies are showing interest on the future development of P recovery technologies. Nowadays, some of the most diffused technologies of P recovery in form of struvite are developing market solutions in order to make phosphorus recovery a sustainable practice also from an economic point of view. In Table 37 the commercial characteristics of the recovered products are presented with the application of different technologies.

Table 37: Products Market Characteristic.

PRODUCT	DIMENSIONS	COMPOSITION	MARKET/COMMERCIALIZATION
AIRPREX	Box of 0.5 kg, 2 kg, 5 kg, 16 kg	21% P_2O_5 of DM, 7% N, 8% MgO	Available on the market as **Berlin Pflanze** or sold to fertilizer industries (ICL fertilizers)
STRUVIA	-	29% P_2O_5 of DM	-
CRYSTALACTOR	Granules 1 mm	-	-
PEARL OSTARA	Granules < 5 mm; available in SGN 90, 150, 300, 450	28 % P, 5% N, 10% Mg	Struvite produced as **Crystal Green** or sold to fertilizer industries (ICL fertilizers)
GIFHORN	-	28 % P_2O_5 of DM	
STUTTGART	-	27 % P_2O_5 of DM	
NURESYS	-	N: 5.6 w/w %, P: 12.6 w/w %, Mg: 10 w/w %	Struvite produced as **BioStru** or sold to fertilizer industries (Timac Agro)
PHOSPAQ	Granules 2 – 4.5 mm	20% P_2O_5, 4% N, 11% MgO	Struvite produced as **Vitalphos**

4.4.1.1. Airprex

The AirPrex® reactor is a technology that recovers P from the digestate. The crystallization of the mineral struvite is set under optimized conditions (pH =8 and the presence of sufficient magnesium after addition of magnesium chloride ($MgCl_2$)). The internal reactor design includes a cylindrical wall, dividing the reactor into two sectors and promoting a streaming loop. This looping moving bed allows good contact between chemical reagents and enables the struvite crystals to grow and to sediment in the conic bottom of the reactor, whereas the smaller particles are kept in the loop until they become large enough to settle down where the struvite can be extracted. The reactor is designed as airlift reactor, where air is pressed in through the bottom and released on top. Air compression for aeration requires considerable amounts of electric energy. On the other hand, consumption of caustic soda for pH adjustment and energy consumption for dewatering are saved. The airlift has two functions: the increase of pH by CO_2 stripping and the maintenance of fluidized bed to enable crystallization and sufficient hydraulic retention time for crystal growth. Since the struvite product is crystallized within the wet sludge, it incorporates some sludge particles and gets a brown colour. However, after the separation from sludge, a sand washer removes the most co-precipitated and co-settled sludge particles from the struvite and improves the quality and purity of the crystals. The final fertilizer product is collected and dried in containers. Figure 68 illustrates the mass flows and the most important characteristics of Airprex process, while Table 38 summaries the economic valorisation of the produce.

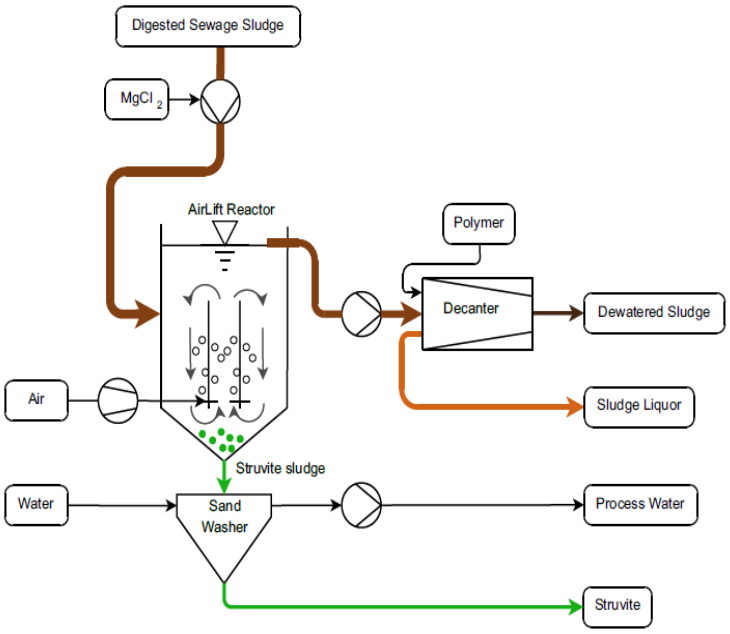


Figure 68: Flow scheme – AIRPREX.

Struvite recovered with AirPrex® technology in the WWTP of Waßmannsdorfis is available on the market under the name Berliner Pflanze since 2008 and distributed in Berlin and Brandenburg region. It's a slow-release fertilizer available in different household size package, recommended for flowering plants. In 2015 Berliner Pflanze won the GreenTec Award.

Table 38: Berliner Pflanze formats and prices.

SIZE	PRICE

0.5 kg	€ 2.50
2 kg	€ 4.00
5 kg	€ 10.00
16 kg (8x2kg)	€ 28.00

In the WWTP of Amsterdam West, AirPrex technology is installed to treat the digestate and recovery 95% of orthophosphate as struvite. The struvite produced is characterized by a dry matter content > 90%, with 50 g/kg of N and 126 g/kg of P. The water company Waternet sells the struvite to ICL fertilizer, who uses it for the production of tailor-made fertilizers.

4.4.1.2. Pearl Ostara

Ostara's Pearl technology can be applied to the sludge after anaerobic digestion or to the rejected liquor after dewatering unit (Figure 69). The removal of the phosphorus takes place by crystallization, thanks to the addition of magnesium at controlled pH in a two-phase fluid bed reactor in up flow configuration. Pearl technology can be implemented with WASSTRIP (Waste Activated Sludge Stripping to Remove Internal Phosphorus), which manages the release of phosphorus upstream of the digester, increasing the efficiency of the removal of nutrients, protecting the units from clogging and improving dewatering of the sludge. The chemicals used for the precipitation and the maintenance of pH are MgCl2 and NaOH, respectively. The electricity consumption required for drying the recovered granules varies according to the availability of residual heat. The final product is marketed under the name Crystal Green, with a formula N-P of 5-28, containing also 10% of Mg. For instance, typical dimensional characteristics of the reactor to highlight a larger diameter of the reactor in the upper part are: The lower part has a standard diameter of 0.8 m and the sedimentation area has a standard diameter of 4.2 m. However, different types of reactor configurations are available depending on the amount of phosphorus fed.

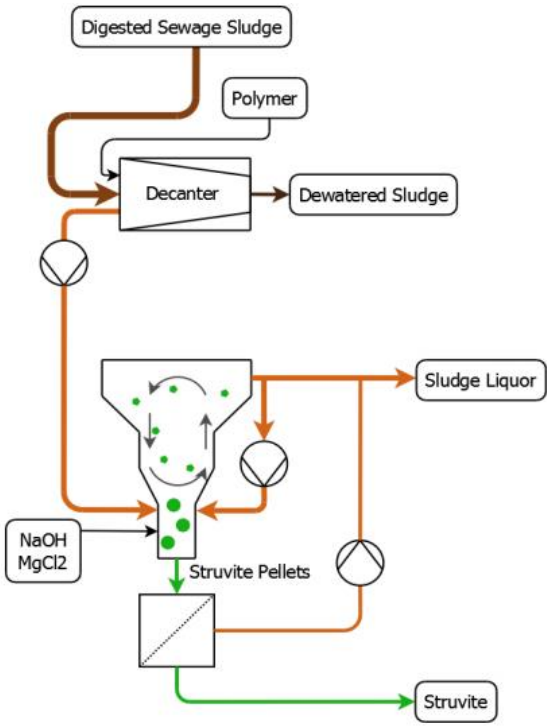


Figure 69: Flow scheme – PEARL OSTARA.

Crystal Green is the commercial product of Pearl technology. It is a struvite fertilizer characterized by a nutrients content of 28 % P, 5% N, 10% Mg. Crystal Green is 4% water soluble and 96% citrate soluble, allowing the phosphorus release in response to plant's organic acid production, improving uptakes and yields and reducing nutrient losses. Crystal Green has got a low salt index, compared to monoammonium phosphates or di-ammonium phosphates, being 7.7 against values of 29 and 27, respectively, according to the Jackson Method. Low values of salt index reduce salt injury that may happen when dosing fertilizer granules near the seed. Ostara has entered into a partnership agreement with ICL Specialty Fertilizers, that will market and sell Ostara's Crystal Green® phosphate fertilizer into the European Union, providing turf and lawncare professionals an enhanced efficiency phosphorus nutrition solution high-performing, sustainable and environmentally responsible. The struvite produced by Pearl® is a Root Activated™ continuous-release phosphorus and will be used in the production of the commercial fertilizer Sierrablen Plus.

4.4.1.3. NuReSys

The Nutrient Recycle System technology recovers phosphorus through the struvite crystallization. In general, the process is applied to the effluent flow from the anaerobic digestion as it is or to the separated liquid phase downstream of the dewatering unit. Nuresys is divided into two phases which take place in different reactors. The first involves CO_2 stripping with the consequent increase of pH. In the second phase, $MgCl_2$ is dosed to the wastewater in the crystallization reactor, where the struvite is precipitated. In addition, a solution of NaOH is added in the reactor for pH control in between 8 and 8.5, to maintain the suitable conditions for crystallization. The process takes place in continuous mode and the reactor is equipped with a single blade rotor for the collection of crystals on the bottom, from which they are removed discontinuously. The struvite obtained was identified as 100% pure by XRD analysis. The collection is quite simple because grains have a specific density of 1.7 t/m^3 and they can be easily separated from the effluent. The product called BIOSTRU can be used as a fertilizer or as a soil conditioner (Desmidt, 2015). Figure 70 illustrates the flow scheme and Table 39 summaries the most important characteristics of the Nuresys process.

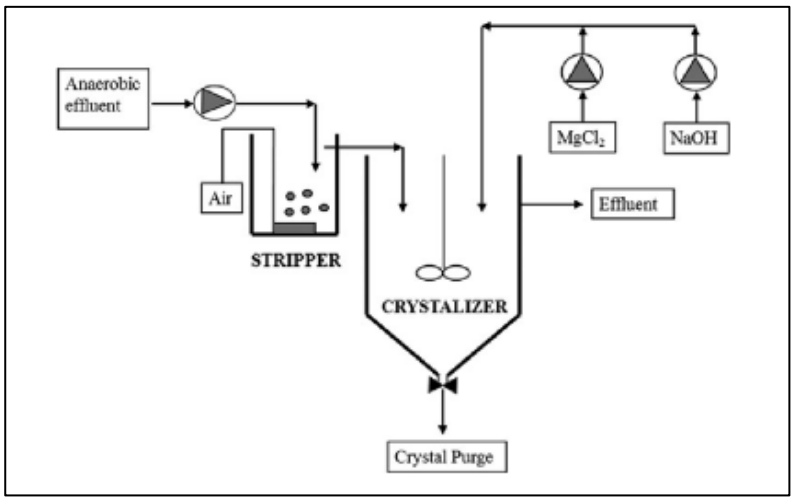


Figure 70: Flow scheme – NURESYS.

NuResys technology is applied at full scale for P recovery from wastewaters of dairy and potato processing industries, mainly in Belgium and in the Netherlands, treating mainly UASB effluents.

Table 39: NuReSys case studies.

PLANT	YEAR	TREATED MATRIX	Q in [m^3/h]	P-PO_4 influent [mgP/l]	P-PO_4 effluent [mgP/l]	Struvite Production kg/d
Humana Milchunion, Everswinkel (Germany)	2006	UASB effluent	125	60-65	20-15	600
Agristo, Harelbeke-Hulste (Belgium)	2008	UASB effluent	60	100	20-15	600-800
Clarebout Potatoes, Nieuwkerke (Belgium)	2011	UASB effluent	120	150	20-15	1800
Clarebout Potatoes, Nieuwkerke (Belgium)	2012	UASB effluent	120	130-175	20-15	1700-2000

The struvite produced by NuReSys process is commercialised as Biostru©. It is characterized by a nutrient content of 5.6% N, 12.6% P, 0 K and 10% Mg (% w/w). The struvite is not water soluble, while it is soluble in acidic soil conditions. All the nutrients are completely available for plant uptake. The product is crystalline, with a dry matter content of 98-100 %, a particle density of 1.7 g/cm3 and a low content of heavy metals. Biostru© can be applied directly or blended with other fertilizer to optimise nutrient ratios. It is available for domestic use in 1-10 kg package. It so can be used in conventional farming especially in horticulture. The current price of the Biostru© varies from 80 to 120 €/ton. It gained the end of waste status on Flanders and can be used as a fertilizer in Flanders and Wallonia. In France it has the permission of NF U 42-001-1.

As reported in Table 40, NuReSys sells the struvite produced on its plants to fertilizer industries, such as the company Timac Agro, that produces the granular fertilizer ''Physiostart P Plus'' using the recovered struvite. Physiostart P Plus may be used in the livestock sector, in agriculture and horticulture. A study campaign on agronomic efficiencies of Physiostart P Plus has been carried on 2014-2016 with experiments on pot, greenhouses and field with different crops (corn, potatoes and lettuce) in collaboration with Delphy, WUR, Aeres and University of Milan. Results showed a DMY comparable with mineral fertilizers. (W. van Geel, 2015).

Table 40: Experimental tests for Physiostart P Plus.

Object	Fresh Yield (t/ha)	Dry Matter %	Dry Matter Yield (t/ha)	Net Energy Value (g/kgDM)	Starch (g/kgDM)	N-tot (g/kgDM)	P_2O_5 (g/kgDM)
Untreated	53.3	32.9	17.2	1002	364	11.7	4.1
Physiostart NP	54.1	34.1	18.1	1009	376	11.6	4.3
Physiostart P-Plus	54.6	33.9	18.2	998	359	11.7	4.1

4.4.1.4. PHOPSPAQ

PHOSPAQ ™ was developed by Paques as a commercial solution to recover phosphate and prevent uncontrolled precipitation of struvite (Figure 71). With the air insufflation made by large bubbles, COD is biologically converted into new biomass and CO_2, while with the addition of magnesium

oxide (MgO) the phosphate and ammonium precipitate as struvite (MgNH4PO4*6H20). The dosage of MgO as an alternative to $MgCl_2$ and it eliminates the need of dosage of caustic soda (NaOH) to keep the pH between 8.2 and 8.3. The technology is applicable for a Phosphorus load greater than 100 kg P/ d, 50 mg PO4-P / L and 200 mg NH4-N / L. The struvite granules are removed from the aqueous phase by means of a hydro-cyclone, followed by a pressure and a screw and transferred to a container. The crystals have an average size of about 0.7 mm and comply with the requirements of the European legislation on fertilizers. The advantages of PHOSPAQ ™ are therefore the combined removal of phosphate and COD in a single reactor and aeration, which provides optimal conditions for struvite formation and carbon dioxide stripping, that increases the pH and stimulates the precipitation of struvite and, finally, a good quality of the crystals are produced. The removal efficiency of P is around 70-95%.

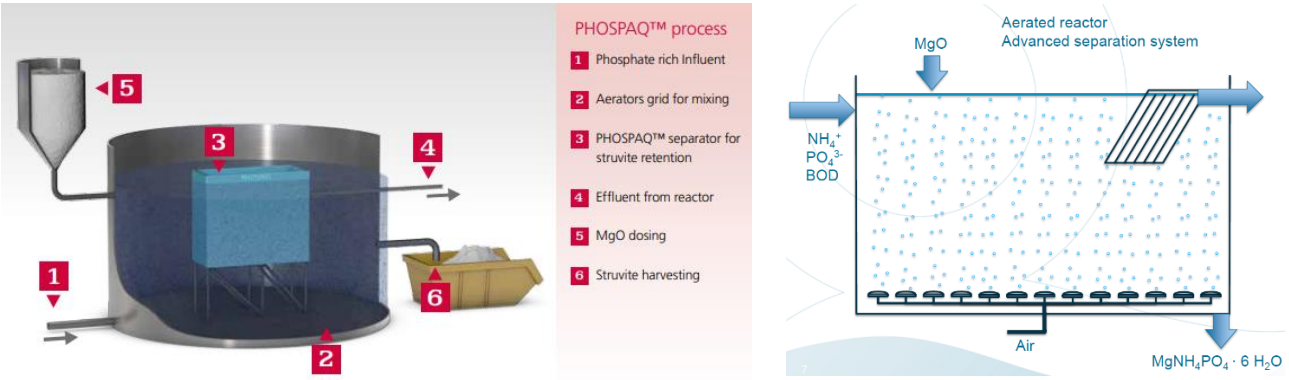


Figure 71: PHOSPAQ reactor.

Phospaq™ technology is applied in a full-scale plant (Table 41), treating the agro-industrial process water, often combined also with municipal wastewater. At LOMM and OLBURGEN WWTPs, in Netherlands, industrial wastewaters from potato processing industry are treated with upflow anaerobic sludge blanket (UASB) reactor and its effluent is sent to Phospaq™ process for further P recovery by struvite crystallization.

Table 41: Phospaq™ industrial case studies.

CASE STUDY	SCALE	SECTOR	FLOW	INFLUENT P	RECOVERY	QUANTITY
LOMM, NL	Full scale	Agro-industry potato processing	UASB effluent	125 kg/d	75% P; 19% N	720 kg struvite/d
OLBURGEN, NL	Full scale	Agro-industry potato processing	UASB effluent	200 kg/d	82% P; 92% N	1200 kg struvite /d

Lomm WWTP

The treatment plant of Lomm, the Netherlands, receives wastewater from a potato processing factory. Wastewater is first sent to an anaerobic reactor and is subsequently treated in a Phospaq™ reactor. According to Remy et al., 2013, influent concentrations are about 62 mgP/l, while the effluent contain about 15 mgP/l. The Phospaq™ process about 120 kg P/d, of which approximately 75% is recovered, with a struvite production of 720 kg/day. A more enhanced removal is possible, but considered not economically feasible, due to the MgO consumption.

Olburgen WWTP

In the WWTP of Waterschap Rijn en Ijssel, in Olburgen (NL), process waters of Aviko–Royal Cosun potato industry are treated with Phospaq™ technology since 2006, recovering struvite for fertilizer industries. A study conducted in 2013 by Remy et al. showed the performance of Phospaq™ reactor, with a daily P recovery of about 150 kg/d and a struvite production of 1200 kg/d. The Phospaq™ reactor is equipped with an aeration system, providing efficient mixing and enhancing stripping of CO_2, which is advantageous for struvite precipitation. MgO is dosed to the reactor to further increase the pH. Influent phosphate concentrations ranged from 50 to 140 mg/L, while in the effluent reduced to 14 mg/L, resulting in an average removal of 82%.

In 2014 the DBFO company Waterstromen BV, in collaboration with Melspring-Olmix, implemented in the WWTP for the direct production of Vitalphos, a long release NP fertilizer. Industrial waters are treated in a UASB reactor, producing more than 3,000,000 m^3 of biogas every year. The effluent from the process is mixed with reject water and sent to Phospaq™ reactor, followed by a one stage granular sludge nitritation/anammox process. Combining Phospaq™ technology with Anammox® technology allows to remove phosphorus and ammonium from nutrient (N & P) rich liquors. The struvite produced is then dewatered and dried at low temperature, using the heat produced in the CHP system. The WWTP produces 1,000 ton/year of fertilizer, that is sold in the Netherlands and abroad for horticultural and sports applications.

VitalPhos is the commercial product of PHOPSPAQ technology (Figure 72). It is a struvite-based fertilizer with 20% P_2O_5, 4% N and 11% MgO. It's a grey-brown granulated product, with an average dimension of 2 – 4.5 mm, patented for organic farming.

Marathon VitalPhos (analyse en specs)	
Nitrogen	4 %
ammoniacal nitrogen	4 %
phosphorus pentoxide total P205	20 %
soluble in water and neutral ammonium citrate	19 %
potassium oxide soluble in water K_2O	1 %
magnesium oxide total MgO	11 %
organic matter from potato water	10 %
Physical properties	
Grain size	**2–4,5mm 90 %**

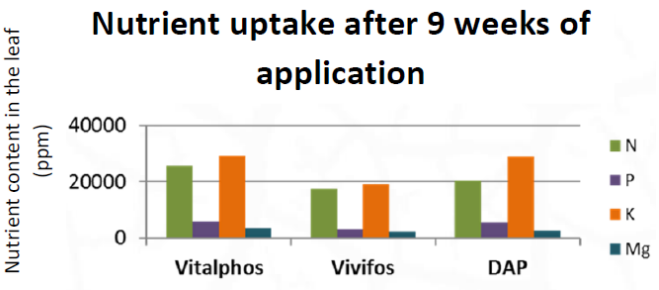


Figure 72: Characteristics of the commercial product VitalPhos

4.4.2 Results and market value analysis

The economic value of precipitated phosphate salts and derivates is directly related to the purity of the product, the bioavailability of P for the plants and the low content in metals, especially Cadmium, that is present in phosphate rocks at concentration of about 200 mg/kgP_2O_5 and whose removal from contaminated soils are estimated as 27-28 €/ton P. Recovered fertilizers, as struvite, contain also other value nutrients, such as magnesium, that is valued at 130€/ton Mg, and nitrogen, with a value around 320€/ton N. The STRUBIAS report dated in 2019 estimated the potential economic value of struvite about 158 €/ton, based on its chemical and agronomic characteristics, considering a struvite composition of 12.6% P, 9.9% Mg and 5.7% N, against the price of 988 €/ton indicated for the DAP fertilizer ($(NH_4)_2HPO_4$) in 2017. In techno-scientific literature, different

economic values of P-recovered products are reported, as indicated in Table 42. Struvite economic market value is about 435 €/ton.

Table 42: Market prices of P salts reported in literature.

PRODUCT	€/ton	REFERENCE
Struvite ($MgNH_4PO_4 \cdot 6H_2O$)	250	Ueno and Fujii, 2001
	300	Product PhosCare, SUEZ company
	188-314	Münch and Barr, 2001
	464	Shu et al., 2006
	763	Dockhorn et al., 2009
	390	Nieminem et al., 2010
	489	Desmidt et al., 2014
Idrossiapatite/Calcium Phosphate		
$Ca_3(PO_4)_2$	729	Gaanstra et al. 1998
$CaHPO_4 \cdot 2(H_2O)$ (Brushite)	290	Montag and Pinnekamp 2009
$Mg_3(PO_4)_2$	Mainly used in pharmaceutical/food industry. Price of the reused product not provided. Price of the pure product of about 190-360 €/ton	
$Fe_3(PO_4)_2 * 8H_2O$	10	Yang Wu et al.,2019

4.4.3 Discussion

Nowadays, phosphorus recovery from waste streams is not considered economically feasible yet, because of the still high operative and capital costs for P recovery technologies and the uncertainties related to the recovered products market. However, the value of P recovery could be enhanced, if a wider systemic analysis is applied, considering also related recoveries, such as energy production, or services, as environmental protection from eutrophication, that are linked with phosphorus recovery. Other nutrient resources, as N, K and Mg, could be recovered and valorised together with phosphorus for agricultural application. Technologies of phosphorus recovery reduce the quantity and the related costs of sludge disposal, respect to conventional chemical removal processes. Moreover, struvite precipitation decreases the potential of scaling in pipes and pumps (that represents a cost issue of approximately ≥ $100,000/y for a 25 MGD treatment plant), extending the life of the facilities and decreasing the operating and maintenance costs (Mayer et al., 2016). The increasing stresses on water availability boost water reuse practices and encourage nutrient recovery, both in agricultural applications, valorising the nutrients content in waste streams, and in potable or industrial use, combining water reuse with phosphorus recovery technologies in order to obtain cleaned water and recovered resources.

Food processing industries are a potential source of recovered phosphorus, especially for dairy and potato processing industries. Potential recovery is estimated to be around 2000 tonP/y, mainly in form of precipitated phosphate salts and derivates. The phosphorus recovered is completely bio-available for plant nutrition and fertilisers produced with recovered P are characterized by a relative agronomic efficiency of 105%, compared to traditional synthetic fertilizers. Market opportunities are not yet fully developed, because of legislative uncertainties and lack of confidence due to the

origin of the recovered source. However, the new Fertilizer Regulation 2019/1009 opened to the use of recovered material for fertilizer production. Moreover, many technology providers have developed robust market strategies in order to make the practice of phosphorus recovery economically sustainable, boosting on pure and safe products with demonstrated agronomic efficiencies. One of the most promising commercial applications might be organic farming, where recovered phosphorus could be valorised, according to the principles of circular economy.

References

Achleitner, S., Möderl, M., & Rauch, W. (2007). CITY DRAIN © - An open source approach for simulation of integrated urban drainage systems. Environmental Modelling and Software, 22(8), 1184–1195. https://doi.org/10.1016/j.envsoft.2006.06.013

Akyol Ç, Foglia A, Ozbayram EG, Frison N, Katsou E, Eusebi AL, et al. Validated innovative approaches for energy-efficient resource recovery and re-use from municipal wastewater: From anaerobic treatment systems to a biorefinery concept. Crit Rev Environ Sci Technol 2020;50:869–902. https://doi.org/ 10.1080/10643389.2019.1634456.

Aquaminerals website. https://aquaminerals.com/struvite-in-fertiliser-for-maize/

ARERA (2017). 562/2017/R/IDR, URL: https://www.arera.it/it/docs/17/562-17.htm

Ashrafi O, Yerushalmi L, Haghighat F. Greenhouse gas emission and energy consumption in wastewater treatment plants: Impact of operating parameters. Clean - Soil, Air, Water 2014;42:207–20. https://doi.org/10.1002/ clen.201200158.

Bani Shahabadi M, Yerushalmi L, Haghighat F. Estimation of greenhouse gas generation in wastewater treatment plants - model development and application. Chemosphere 2010;78:1085–92. https://doi.org/10.1016/j. chemosphere.2009.12.044.

Batstone DJ, Hülsen T, Mehta CM, Keller J. Platforms for energy and nutrient recovery from domestic wastewater: a review. Chemosphere 2015;140:2–11. https://doi.org/10.1016/j.chemosphere.2014.10.021.

Caniani D, Caivano M, Pascale R, Bianco G, Mancini IM, Masi S, et al. CO2 and N2O from water resource recovery facilities: evaluation of emissions from biological treatment, settling, disinfection, and receiving water body. Sci Total Environ 2019;648:1130–40. https://doi.org/10.1016/j.scitotenv.2018.08.150.

Capodaglio AG, Olsson G. Energy issues in sustainable urban wastewater management: use, demand reduction and recovery in the urban water cycle. Sustain 2020;12. https://doi.org/10.3390/su12010266.

Dairy Foods. https://www.dairyfoods.com/gdpr-policy?url=https%3A%2F%2Fwww.dairyfoods.com%2Farticles%2F83569-alto-dairy-installs-phosphorus-removal-system.

European Sustainable Phosphorus Platform Newsletter, 2020. http://www.phosphorusplatform.eu/images/download/ESPP-eNews-005-November-2016_2.pdf

Foglia, A., Andreola, C., Cipolletta, G., **Radini, S.**, Akyol, Ç., Eusebi, A. L., Stanchev, P., Katsou, E., & Fatone, F. (2021). Comparative life cycle environmental and economic assessment of anaerobic membrane bioreactor and disinfection for reclaimed water reuse in agricultural irrigation: A case study in Italy. *Journal of Cleaner Production*, *293*. https://doi.org/10.1016/j.jclepro.2021.126201

Frison N, Katsou E, Malamis S, Oehmen A, Fatone F. Nutrient removal via nitrite from reject water and polyhydroxyalkanoate (PHA) storage during nitrifying conditions. J Chem Technol Biotechnol 2015;90:1802–10. https://doi.org/ 10.1002/jctb.4487.

Gu Y, Dong YN, Wang H, Keller A, Xu J, Chiramba T, et al. Quantification of the water, energy and carbon footprints of wastewater treatment plants in China considering a water-energy nexus perspective. Ecol Indic 2016;60:402–9. https:// doi.org/10.1016/j.ecolind.2015.07.012.

Gustavsson, D. J. I., & Tumlin, S. (2013). Carbon footprints of Scandinavian wastewater treatment plants. Water Science and Technology, 68(4), 887–893. https://doi.org/10.2166/wst.2013.318

Hao X, Li J, van Loosdrecht MCM, Jiang H, Liu R. Energy recovery from wastewater: Heat over organics. Water Res 2019;161:74–7. https://doi.org/ 10.1016/j.watres.2019.05.106.

https://www.colsen.nl/en/projects?werkgebied%5B2%5D=2

https://www.olmix.com/

https://taurus.ag/product/crystal-green/

IPCC (2019). URL: https://www.ipcc.ch/report/ar5/syr/

IPCC. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia,. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press; 2013.

ISO 14064-1 (2019). Greenhouse gases - Part 1: Specification for the quantification, monitoring and reporting of organization emissions and removals.

ISPRA (2018). Fattori di emissione atmosferica di gas a effetto serra e altri gas nel settore elettrico.

Jenicek P, Kutil J, Benes O, Todt V, Zabranska J, Dohanyos M. Energy self-sufficient sewage wastewater treatment plants: Is optimized anaerobic sludge digestion the key? Water Sci Technol 2013;68:1739–43. https://doi.org/ 10.2166/wst.2013.423.

Marinelli, E., **Radini, S.**, Akyol, Ç., Sgroi, M., Eusebi, A. L., Bischetti, G. B., Mancini, A., & Fatone, F. (2021). Water–energy–food–climate nexus in an integrated peri-urban wastewater treatment and reuse system: From theory to practice. *Sustainability (Switzerland)*, *13*(19). https://doi.org/10.3390/su131910952

Marinelli, E., **Radini, S.**, Foglia, A., Lancioni, N., Piasentin, A., Eusebi, A. L., & Fatone, F. (2021). Validation of an evidence-based methodology to support regional carbon footprint assessment and

decarbonisation of wastewater treatment service in Italy. *Water Research*, *207*. https://doi.org/10.1016/j.watres.2021.117831

Metcalf & Eddy, 2014. Wastewater Engineering Treatment and Resource Recovery. Fifth edition, AECOM

Nutri2Cycle project website. https://www.nutri2cycle.eu/

NUTRIent MANagement and Nutrient Recovery Thematic Network, 2020. https://nutriman.net/farmer-platform/product/id_293.

Nutrient Platform NL. https://www.nutrientplatform.org/en/success-stories/

P-Rex, 2015 deliverables https://zenodo.org/record/242550

Puchongkawarin C, Gomez-Mont C, Stuckey DC, Chachuat B. Optimization-based methodology for the development of wastewater facilities for energy and nutrient recovery. Chemosphere 2015;140:150–8. https://doi.org/10.1016/j. chemosphere.2014.08.061.

Puyol D, Batstone DJ, Hülsen T, Astals S, Peces M, Kr¨omer JO. Resource recovery from wastewater by biological technologies: opportunities, challenges, and prospects. Front Microbiol 2017;7:1–23. https://doi.org/10.3389/ fmicb.2016.02106.

Radini, S., Marinelli, E., Akyol, Ç., Eusebi, A. L., Vasilaki, V., Mancini, A., Frontoni, E., Bischetti, G. B., Gandolfi, C., Katsou, E., & Fatone, F. (2021). Urban water-energy-food-climate nexus in integrated wastewater and reuse systems: Cyber-physical framework and innovations. *Applied Energy*, *298*. https://doi.org/10.1016/j.apenergy.2021.117268

Saagi, Ramesh. (2017). Benchmark Simulation Model for Integrated Urban Wastewater Systems : Model Development and Control Strategy Evaluation.

Shaddel S., Bakhtiary-Davijany H., Kabbe C., Dadgar, F., Østerhus S. W., 2019. Sustainable Sewage Sludge Management: From Current Practices to Emerging Nutrient Recovery Technologies. Sustainability, 11, 3435; doi:10.3390/su11123435

SINANET, URL: http://www.sinanet.isprambiente.it/it/sia-ispra/fetransp.

Stillwell AS, Hoppock DC, Webber ME. Energy recovery from wastewater treatment plants in the United States: a case study of the energy-water nexus. Sustainability 2010;2:945–62. https://doi.org/10.3390/su2040945.

Zhang, X., Liu, Y., 2022. Circular economy is game-changing municipal wastewater treatment technology towards energy and carbon neutrality. Chemical Engineering Journal 429. https://doi.org/10.1016/j.cej.2021.132114.

5. Emerging contaminants in drinking water and wastewater

Contaminants of emerging concern (CECs) are natural or synthetically occurring substances that are not commonly and regularly monitored in the environment and having known or suspected undesirable effects on humans and the ecosystem (Rodriguez-Narvaez et al. 2017). Although their concentration is at trace level, they have a high potential of contaminating groundwater and surface waters and pose a risk to human health. Most types of the emerging compounds are often persistent to the conventional wastewater treatments (Samah et al. 2018). Emerging contaminants can be introduced into aquatic environments via CSO or wastewater discharges (Del Rıo et al., 2013; Montserrat et al., 2013; Passerat et al., 2011). These contaminants may also be mobilized from sediments during storm events and end up in the water (Del Rıo et al., 2013). Among the category of CECs, microplastics (particles being less than 5 mm in their longitudinal orientation) are constantly increasing in aquatic and terrestrial ecosystem and very little data are available for freshwater systems. Several studies highlighted that rivers and effluents are major pathways for plastics of terrestrial sources, but here is still a knowledge gap related to microplastics abundance and dispersion in freshwater. Moreover, data quality is ambiguous since standards are missing for sampling, processing and analysis.

The occurrence and fate of microplastics in the urban water cycle (UWC), which includes drinking water production and distribution, sewers system and wastewater treatment is the current challenge to critically analyse in order to plan and take actions and adequate mitigation measures. Both drinking water treatment plants (DWTPs) and wastewater treatment plants (WWTPs) have not been specifically designed to remove emerging contaminants such as microplastics (MPs). However, WWTPs and DWTPs are crucial environmental and technological infrastructures to reduce MPs in the environment. Both drinking and wastewater treatment plants (DWTPs and WWTPs) are dealing with large volumes of water and wastewater, and the amount of microplastics that pass through these facilities has serious implications, both for DWTPs providing water for people and for WWTP effluents being either discharged into the environment or used as agriculture fertiliser or irrigation. Therefore, there is a growing need to better understand the concentration, typologies and fate of microplastics in water treatment plants and to prevent the release of microplastics into household taps and the environment.

In line with the Circular Economy Action Plan and the European Strategy for Plastics in a Circular Economy, the general scope of the LIFE Blue Lakes project is the prevention and reduction of plastic waste in the Italian and German lakes through an integrated approach, combining governance, training, information and awareness-raising activities.

Another issue that needs to be addressed is the presence of Cyanobacteria in lakes water reservoir. Occurrence of cyanobacterial blooms has been increasingly detected in lakes and reservoirs due to anthropogenic emissions of nutrients and climate change. The presence of toxins from cyano-Harmful Algal Blooms (HABs) in drinking water reservoirs may represent serious health risks for the human population and thus further investigations are required.

5.1 Development of a protocol for MPs determination in WWTPs and DWTPs

Appropriate sampling is the first step to properly collect MPs and to minimize random errors and, therefore, to avoid wrong estimation of MPs in the environment. To date, there is no standard sampling method for MPs in drinking water and wastewater. Due to the heterogeneity and variability of the water matrix, it is important to collect representative samples of short- and long-term variability to identify temporal distribution and effective occurrence, relevance and fate of MPs. Sludge samples obtained from processes in WWTPs contain much more particle material than wastewater. In this regard, different methods are required, such as directly after the solid-liquid separation units or in the different points of the sludge treatment line. Sampling in DWTPs also require a different methodology compared to wastewater, such as the necessity of filtration of higher volumes of water.

5.2 Materials and methods

The activities carried out included an assessment of the sampling methods and protocols in order to optimize the routine sampling that environmental protection agencies or water utility operators should follow and implement on site. On that basis, an analytical protocol was developed for microplastics (MPs) sampling and characterisation in DWTPs and WWTPs. The protocol includes methodologies already described in scientific literature and directly experienced with analysis of MPs/MFs in those matrices (Magni et al., 2019; Pittura et al., 2021). Literature research was carried out to evaluate the most appropriate ranges of sampling volumes, choose between grab or composite samples and select the sampling points. For the investigation of MPs in drinking water treatment plants (DWTPs) higher volumes (e.g., 1000 l) of water should be sampled compared to wastewater. Composite sampling is recommended using steel buckets and sieves (e.g., 50 µm mesh size). As concern wastewater matrix, best practices suggest continuous composite sampling with as much volume of wastewater as possible, using steel buckets and sieves (50 µm mesh size). Volumes usually range between 30-300 l.

The protocol was developed and followed during the sampling activities performed under the LIFE BLUE LAKES project, concerning the pilots in the wastewater and drinking water treatment plants located in Garda and Castreccioni lake districts.

Depending on water characteristics and operative plant conditions, different sampling system can be used: sieves battery, b) cartridge filters or c) automatic system (prototype).

5.2.1 Sampling with sieves battery

Sieves Batteries (Figure 73) are made up by steel sieves of 5 mm, 2 mm and 50 µm mesh size (ISO 3310-1:2000). They are used in case of grab samplings, e.g., for wastewater, since this procedure is feasible only for limited amounts of volumes. A pumping system is required to convey the water flow to the sieves battery. The pumping system is made in non-plastic components, to avoid plastic contamination. In particular, the pump is in steel, and the connections are made in copper or brass. Filter the wastewater sample, e.g., 25 litres, through the steel sieves battery of 5 mm, 2 mm and 50 µm mesh size (ISO 3310-1:2000): rinse the particles on 2 mm and 50 µm sieves into glass jars with deionized water and subsequently filter onto cellulose nitrate filters (Sartorius Stedim Biotech, Ø 47

mm, 8 µm pore size) using a vacuum pump. After filtering, remove the 5 mm mesh sieve: this sieve is used in battery with the 0.05 mm to exclude any plastic larger than 5 mm that may be present in the sampling phase. The material held on the 5 mm sieve must not be recovered. Use the spray bottles and / or manual pressure pump with deionized water to collect the material held on the 0.05mm mesh sieve. The recovered material must be stored in glass jars closed with a lid and then with parafilm. If the smaller glass jars are sufficient to hold the sample, they can be used in place of the 1 L jars. Identify the sample by writing directly on the jar or on white tape / packs attached to the container. The preserved sample will then be processed later in the laboratory. To control any contamination, place a small jar and fill with deionized water up to ¾ of the volume. Leave the jar open during all the time in which the water is passed through the sieves and during the recovery operations of the material from the sieve. Close the jar with the lid and then with the parafilm.



Figure 73: Procedure for recovering samples collected by pumping and sieving battery

5.2.2 Sampling with cartridge filter

Cartridge filters (Figure 74) are preferably used when there is the possibility to connect the filtering system directly to the plant pipes. Direct filtration allows to obtain composite samples during the filtration time and can process bigger amounts of volumes. The filtering device consists of a metal container inside in which there is a cylindrical filter with a mesh of 50 µm. The filtering cartridge is made of stainless steel micro-stretched sheet. The filter area is 0.034 m2. The connections between the cartridge filter and the plant pipeline are made with copper tubes to avoid any plastic contamination.

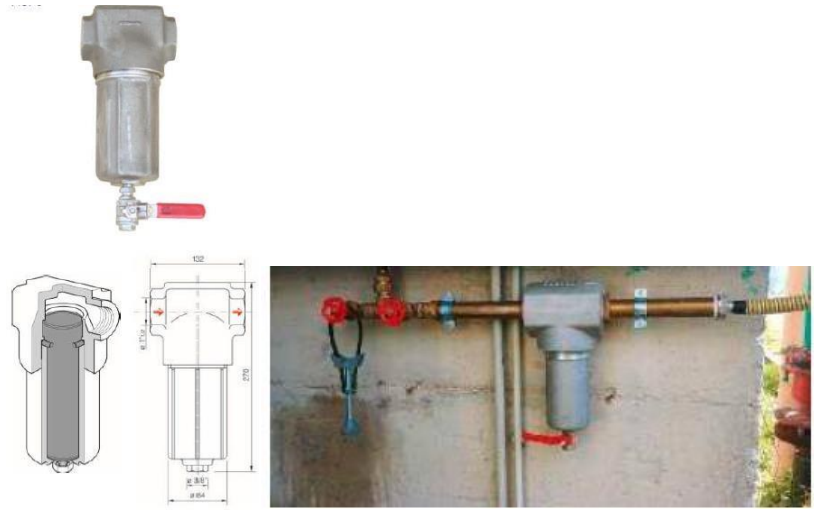


Figure 74: Cartridge filter.

Connect the filter to sampling tap of the DWTP (made of copper) via specific piping and connections made of brass. Adjust the flow rate of the water and record it manually. Calculate the necessary time to filter, e.g., 1000 L water, through 50µm steel stainless filter in situ. Once the filtration is completed, place the filter in a steel bowl or in glass beakers and rinse the filter with deionized water to recover the particles retained on the filter. If necessary, make use of washers and/or manual pressure pumps that could facilitate / optimize the operation. Place an empty jar filled with deionized water and leave open during the washing step for each sample. This will be used as control to represent any contamination from air. Once the filter is completely cleaned, pour the washed water into a glass jar and rinse the bowl into the jar to recover all particles. Close the jar with its lid and then using parafilm. Name the samples and each control jar, then bring to the laboratory for processing.

5.2.3 Sampling with automatic prototype

For a better quantification of MPs in wastewater, a prototype of autosampler (Figure 75) has been designed and built, in order to optimize sampling procedures. The automatic sampling system, designed and developed under the project LIFE BLUE LAKES, consists of a timed sampling pump, a cartridge filtration system (mesh size up to 25 µm, filtering area 1000 cm^2), an electromagnetic flow meter and control and data recording plc. The device allows to acquire a composite sample (24h) and to filter higher amount of water, until the filter is not completed clogged. The flowrate measurements will permit to know exactly how much volume is filtered. Moreover, the automatic system is designed to be upgraded with an additional cartridge filter of 25 µm to detect lower sizes MPs.

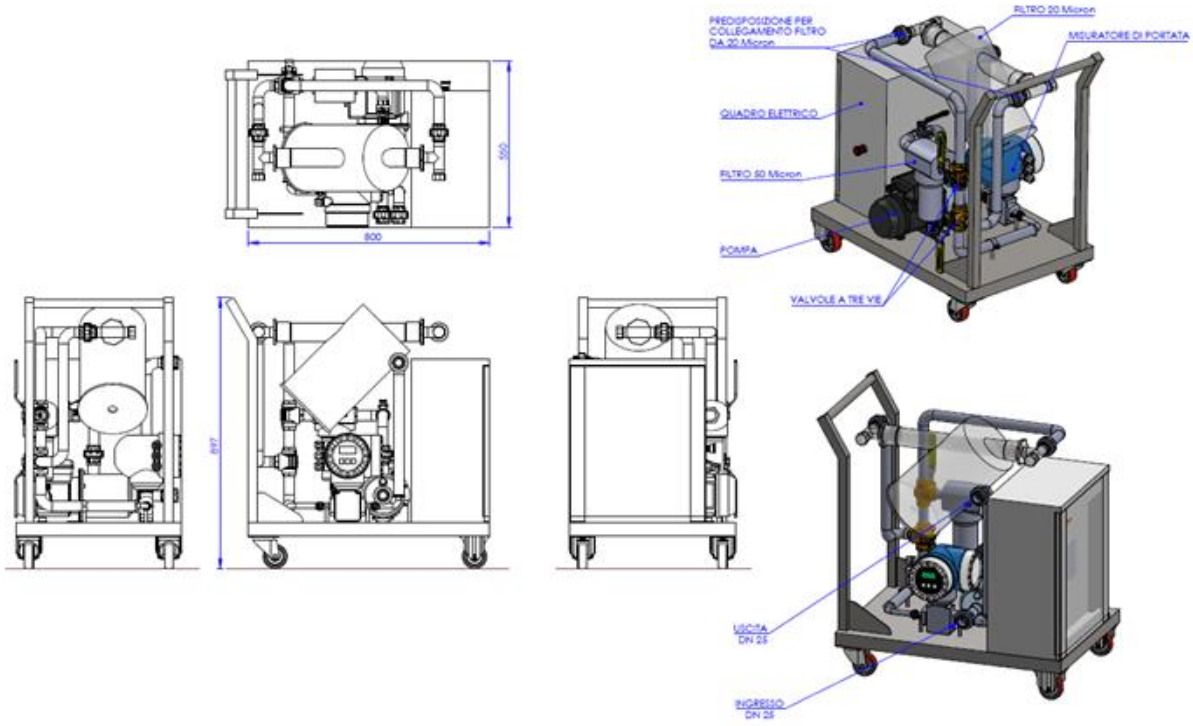


Figure 75: Prototype of automatic sampler for MPs – Skid

Flowrate and pressure can be set by the regulation of the apposite valves. The device can pump up to 50 litres/minute in normal working conditions, with a pressure on the filter of 1-2 bar. The device

can be set to work under time or combined time and flowrate controls. When it is set on time control, the pump is activated only at specific time intervals, with cycles of filtering and cycles of pause. In this way it is possible to perform averaged samples. On the other side, when the device works under time and flowrate controls, the pump automatically stops its filtering/pause cycles once the flowrate reaches a pre-defined lower threshold, meaning that the filter reached a definite clogging level.

5.2.4 Sludge samples

In case of sludge samples, grab measurements are performed and thus the sieving battery is used. Before the filtration, sludge samples are collected using automatic samplers or steel bucket and maintained in tanks until the laboratory processing. Samples are stored at -20°C if not immediately processed.

5.2.5 Characterisation

After the recovery phase of water samples, cellulose nitrate filters are used (Ø 47 mm, 8 µm pore size) to filter with a vacuum pump. Obtained filter is recovered in a petri dish, covered with 15% H2O2 and maintained at 50 °C overnight to remove organic matter. If necessary, filtration of a single sample is performed onto more than one filter. A direct vacuum filtration is preferred; however, a density separation can be applied for complex samples: it is carried out in 250 mL cylinders, stirring the samples with saturated NaBr salt solution (density 1.4 g cm^3) for 30 min and leaving to settle the mixture overnight.

As concern sludge samples, a minimum of 4 litres is usually processed for analyses of MPs and MFs. Sample, distributed in glass beakers, is dried in stove at 50 °C and subjected to a first organic matter digestion with a 15% H2O2 solution at a temperature of 50 °C. Time of this first treatments depends on nature of sludge (e.g., content of water). Then, a density separation procedure is carried out in 250 mL cylinders, stirring the samples with saturated NaBr salt solution (1.4 g cm^3) for 30 min (Frias et al., 2018) and leaving to settle the mixture overnight. The supernatant was then vacuum filtered onto cellulose nitrate filter (Ø 47 mm, 8 µm pore size) and filters were treated with 15% H_2O_2 solution for removing the residual organic matter.

Filters resulted from the extraction procedure is visually examined using a stereomicroscope (GZ808/810 Optech with Optech IS 4K-8 digital camera). All items resembling plastic and fibres were manually collected using a tweezer and transferred onto a clean cellulose acetate membrane (0.45 µm pore size) located on a microscope slide that is subsequently used as support for the µFT-IR analyses (Fig. 6). Sorted items were categorised in fibres and particles, which were furtherly divided into lines, fragments, film, spheres and glitter. Items are measured using an image analysis software and are categorized in size classes that are identified in the range between 5 mm (i.e., the maximum dimension of microplastics for definition) and 20 µm (i.e., the limit size for identification using the FTIR microscope system Spotlight 200i). Identification of polymer is performed by µFTIR spectroscopy in attenuated total reflectance mode, using a Spotlight 200i FT-IR microscope system (PerkinElmer) equipped with Spectrum Two and driven by Spectrum 10 software.

5.3 Sampling campaigns

For the pilot actions foreseen in LIFE BLUE LAKES project, 3 drinking water treatment plants (DWTP) and 2 wastewater treatment plants (WWTP) were selected. The first DWTP was located in central Italy, near Lake Castreccioni, and is managed by the water utility ACQUAMBIENTE. The other 2 DWTPs, respectively Garda Molinet and Castelletto di Brenzone, were selected in Lake Garda district and are managed by the water utility AGS.

The plants were chosen because they are characterized by different configurations of treatment units and processes, including ozonation, sand filtration, activated carbon adsorption, membrane filtration and chemical disinfection.

The DWTP in Castreccioni Lake treats an average flow rate of 500 l/s and the layout includes: pre-ozonation pre-treatment, flocculation phase with PAC dosage, sand filtration, post-ozonation unit and activated carbon filtration (Figure 76).

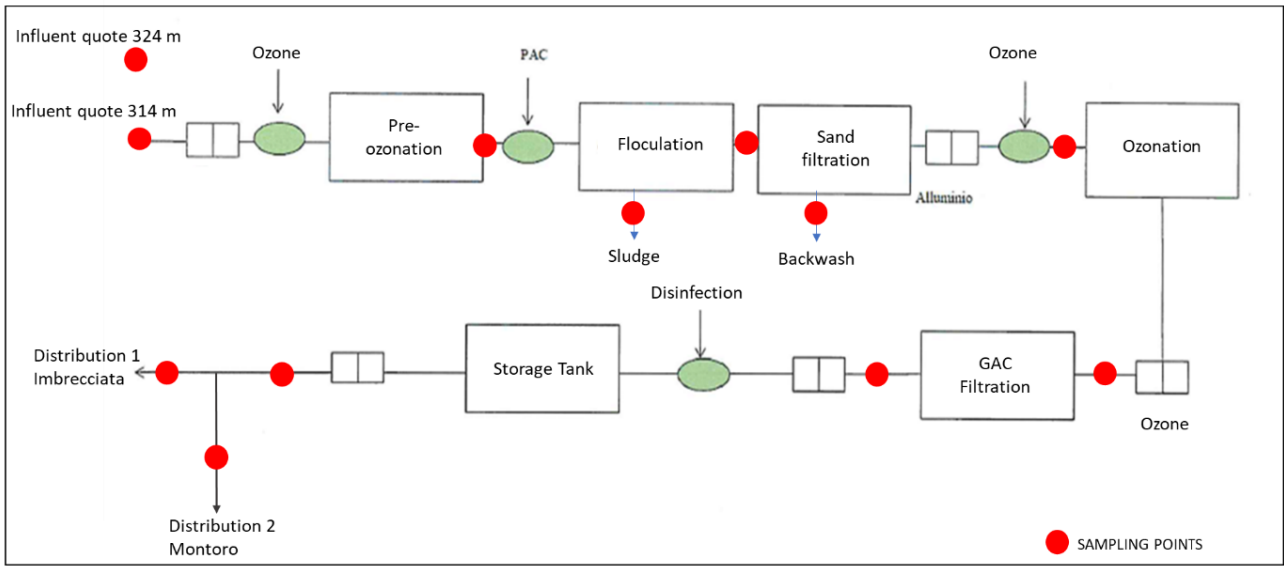


Figure 76: Layout of Castreccioni DWTP

In Garda territory, DWTP Molinet has maximum treatment capacity is 50 l/s and its layout consists of pre-oxidation with ozone, rapid multilayer filtration with sand and granular activated carbon and final chlorine disinfection (Figure 77). The DWTP located in Castelletto di Brenzone is characterised by a flowrate of 10 l/s and treats lake water, using gross filtration and membrane ultrafiltration unit (Figure 78).

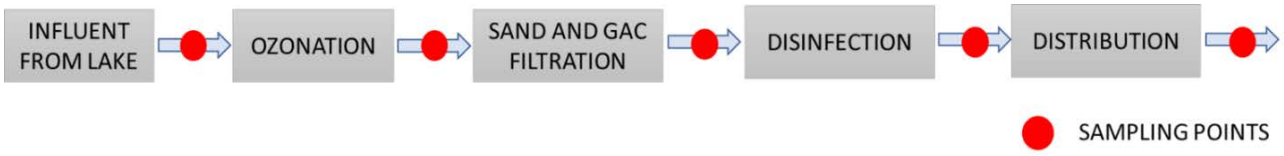


Figure 77: Layout of Garda Molinet DWTP

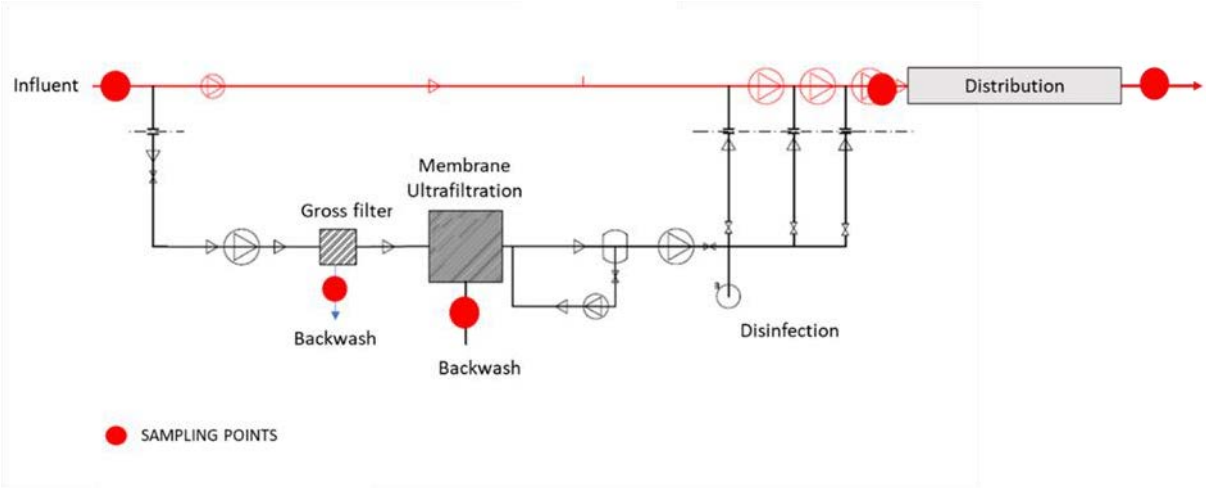


Figure 78: Layout of Brenzone Castelletto DWTP

For WWTPs, 2 plants were selected in Garda district, respectively Limone Tremosine and Peschiera del Garda WWTPs, managed by the water utility Acque Bresciane. They were identified for their different sizes (From 180000 to 330000 Population Equivalent) and configurations, respectively: pre-treatments, attached-growth biological unit, flotation and rotary tertiary filtration for Limone WWTP and sand removal, conventional activated sludge, coagulation, lamellar sedimentation, sand filtration, UV disinfection in Peschiera del Garda.

5.4 Results

The results of the sampling campaigns carried out for each one of the drinking water and wastewater treatment plants selected for the Blue Lakes project are reported in the following paragraphs. Data were presented as MPs, representing the sum of the microparticles and microfibres made of plastic polymers (MPPs and PMFs, respectively).

5.4.1 Castreccioni DWTP

Results of the sampling campaigns performed for Castreccioni DWTP are summarised and discussed in the following figures. MPs concentration for the three sampling campaigns were shown in Figure 79, for the different units of the DWTP layout and in Figure 80 for backwash and sludge.

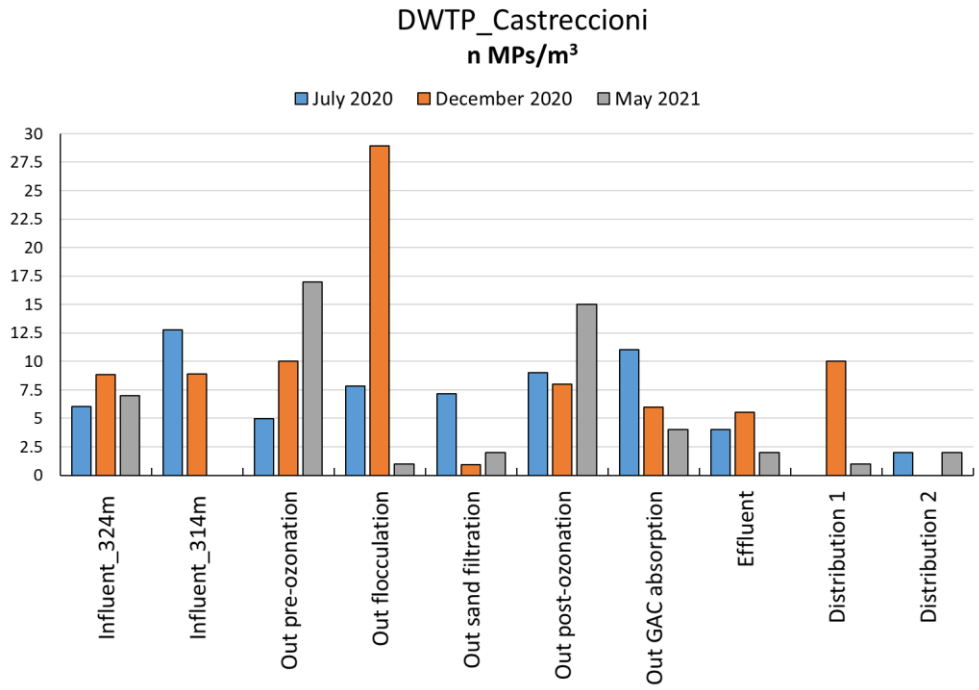


Figure 79: MPs abundance in waste and treated waters of Castreccioni drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m^3).

Influent measured concentrations are on average in the range of 0 – 13 MPs/m^3, specifically around 7 ± 1 MPs/m^3 for the intake at 324 m and about 7 ± 7 MPs/m^3 for the quote at 314 m, showing a higher variability for the lower quote. After the pre-ozonation process, an increase in the MPs concentration could be observed, with mean values of 11 ± 6 MPs/m^3. These results are in line with the literature research, since the ozonation process was found to be a possible cause of MPs release. In fact, the ozonation is characterised by an elevated oxidation effect on the organic matter. Generally, MPs could be attached to solid particles and therefore are more easily removed in the settled sludge, residual screening and degritting. Differently, some chemical processes enhancing the solid oxidation, determine release phenomena of MPs in the main water line. Possible correlations between the ozone dose and the indirect effect on MPs are probably expected and need to be furtherly investigated. The following flocculation presented variable trend of 13 ± 15 MPs/m^3, with higher concentration for the first two campaigns and significant reduction of MPs during the last sampling of May 2021. This finding is in line with some of the literature works analysed, which observed an increase in MPs concentration after coagulation-flocculation processes. Possible correlations between the type of dosed reagents and the indirect effect on MPs are probably expected and need to be furtherly investigated. The effluent from sand filtration was characterised by a decrease in MPs, with mean concentrations of 3 ± 3 MPs/m^3, even if after the post-ozonation process a new increase was observed, with values in the range of 11± 4 MPs/m3. However, the effluent from the following GAC adsorption process decreased to the final effluent values of about 4 ± 2MPs/m^3. Similar concentrations were measured in the two distribution points, each of one was characterised by values in the range of 4 ± 6 MPs/m^3 and 3 ± 3 MPs/m^3, respectively. Notwithstanding the really low detected value, the final data in the distribution network were found to be comparable or lower than the effluent from the DWTP, suggesting possible further settling phenomena in the distribution system.

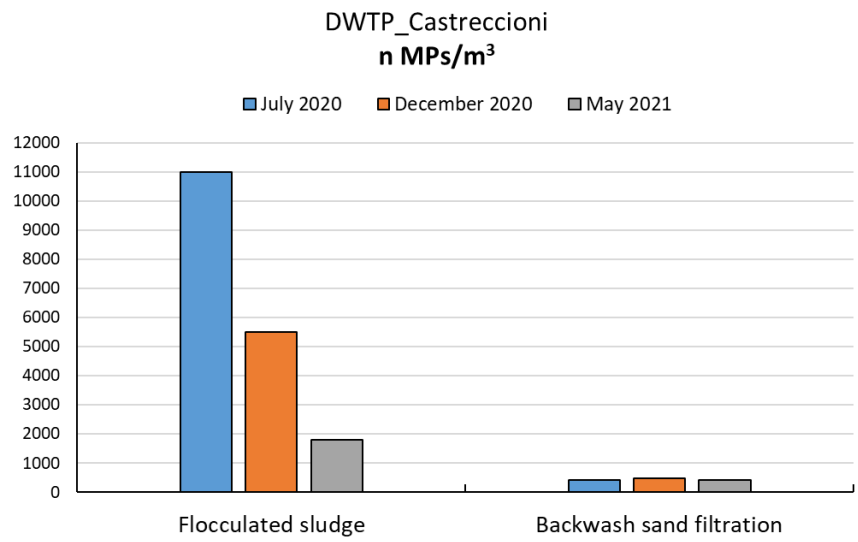


Figure 80: MPs abundance in sludge and backwash water of Castreccioni drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m3).

Flocculated sludge is characterized by high variations in MPs concentration in the range of 6100 ± 4629 MPs/m^3. In the same way, also backwash high values and high variability were measured, about 431 ± 36 MPs/m^3. No evidence was found about the influence of seasonality on MPs concentration.

Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (Figure 81 and Figure 93), their size (Figure 82) and their composition (Figure 83), at the different steps of Castreccioni DWTP (A), and measured in the backwash and in the sludge samples (B).

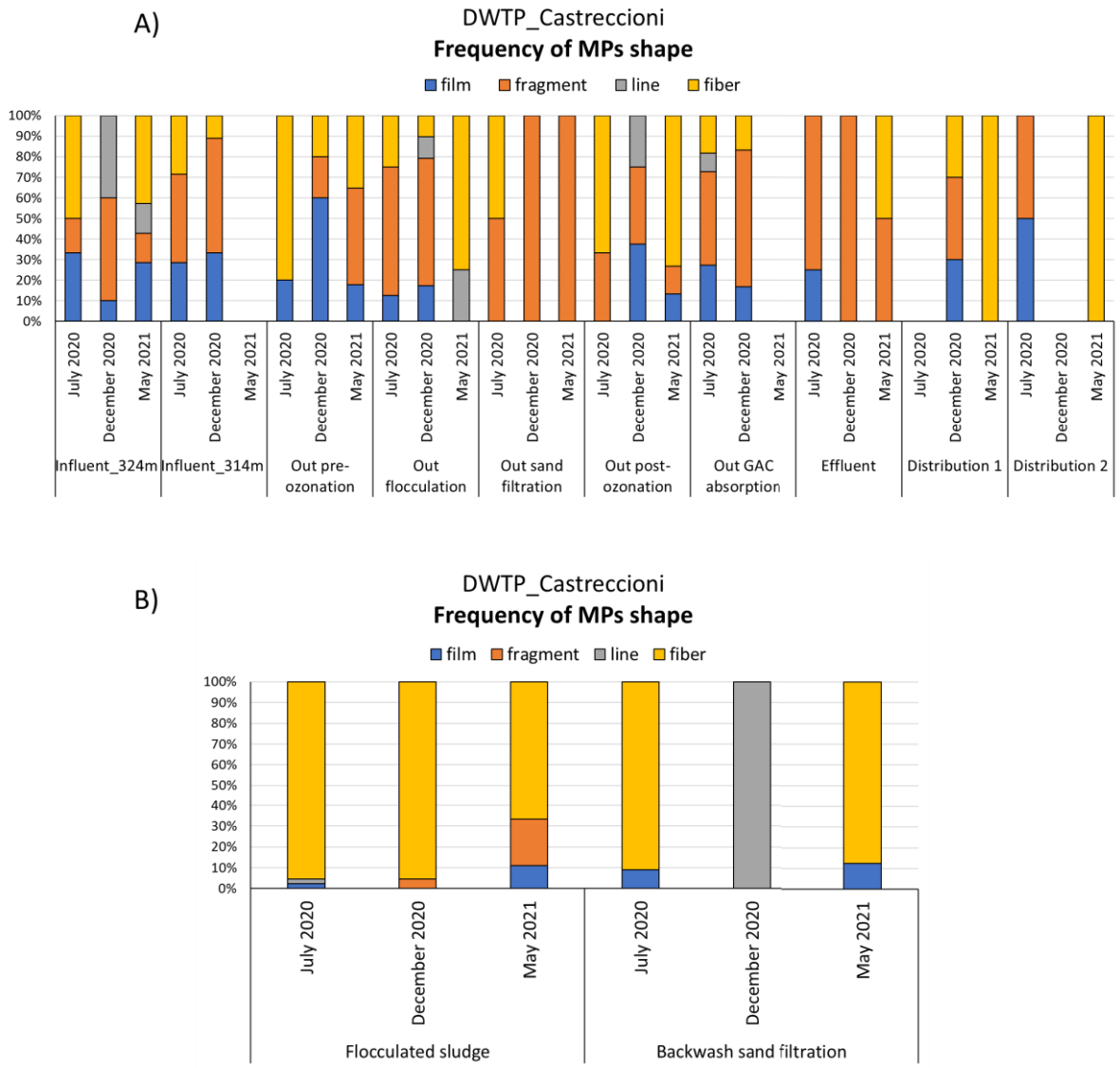


Figure 81: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) sludge line and backwash waters of Castreccioni drinking water treatment plant during the three-sampling campaign.

As concern particles shape, MPs are distributed between film, fragments, lines and fibres along the treatment line, while in backwash and flocculated sludge fibres are the most present.

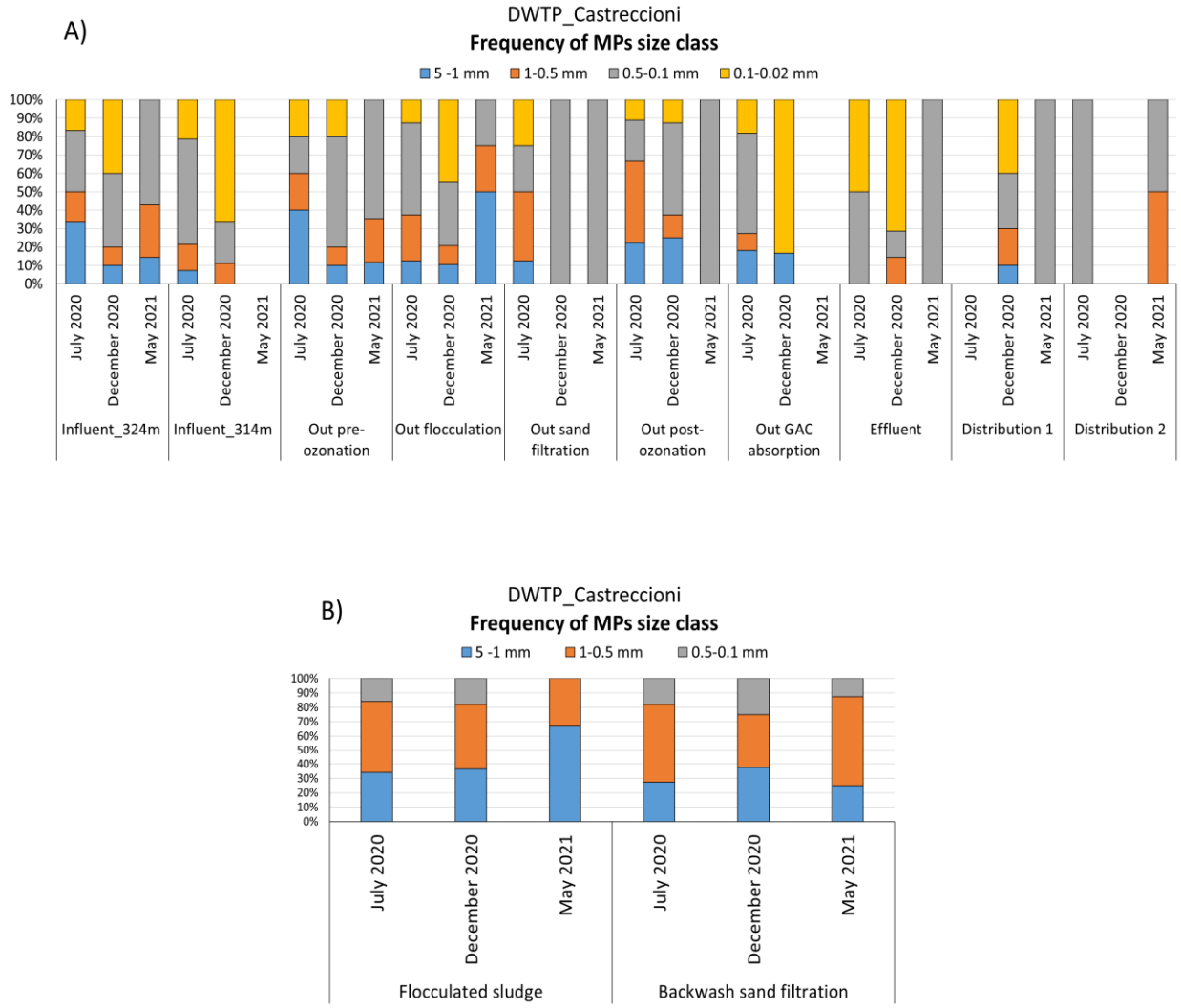


Figure 82: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) sludge line and backwash waters of Castreccioni drinking water treatment plant during the three-sampling campaign.

Regarding MPs dimensions, shapes were differently distributed in the influent, while in the effluent the presence of lower particles (0.02 – 0.1 mm) increased, mainly due to the removal of the bigger size MPs by the treatment units.

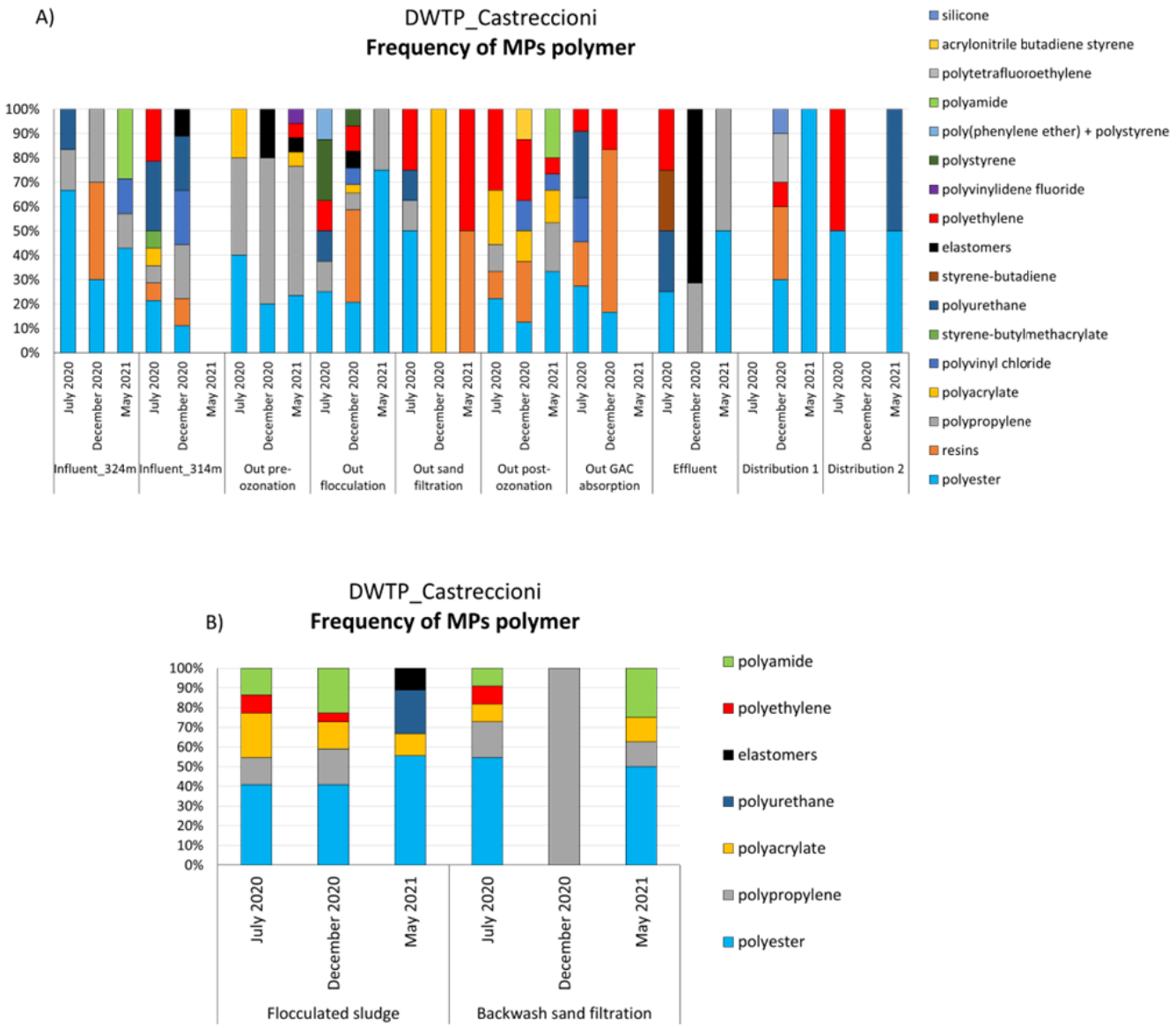


Figure 83: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) sludge line and backwash waters of Castreccioni drinking water treatment plant during the three-sampling campaign.

Considering chemical characterisation, MPs were constituted by a wide variety of material types. Influent MPs mainly presented poly(phenylene ether) and polystyrene, resins, polypropylene and polyurethane. The number of total typologies detected in the different sampling periods for each sampling point are reported in Table 43. The influent at the lower quote of 314 m was characterised by the highest variations, suggesting a correlation with particle density.

Table 43: Number of different plastic typologies detected during the sampling campaigns in Castreccioni DWTP.

	July 2020	December 2020	May 2021
Influent_324m	3	3	4
Influent_314m	7	6	0
Out pre-ozonation	3	3	6
Out flocculation	6	8	2
Out sand filter	4	1	2

Out post-ozonation	5	6	6
Out activated carbon	5	3	n.a.
Effluent	4	2	2
Distribution 1	1	5	1
Distribution 2	2	0	2
Backwash	5	1	4
Sludge	5	5	4

Statistical indexes were obtained for each sampling point, from the aggregation of the data from the three sampling campaigns. Results are graphically reported in Figure 84. The boxes represent the variability of the measurements in each point during the three different sampling periods. In particular, the extremes of the boxes correspond to the first and third quantiles, while the vertical lines reach, respectively, the minimum and the maximum values detected. The median values along the three samplings are reported as the blue lines into the boxes. It can be generally observed that the mean values in the influent are lower than 10 MPs/m^3. Highest variations could be detected in the different operative units, especially for ozonation and flocculation processes. However, the DWTP is able to provide good water quality in the effluent, with mean values below 5 MPs/m^3 and limited variability.

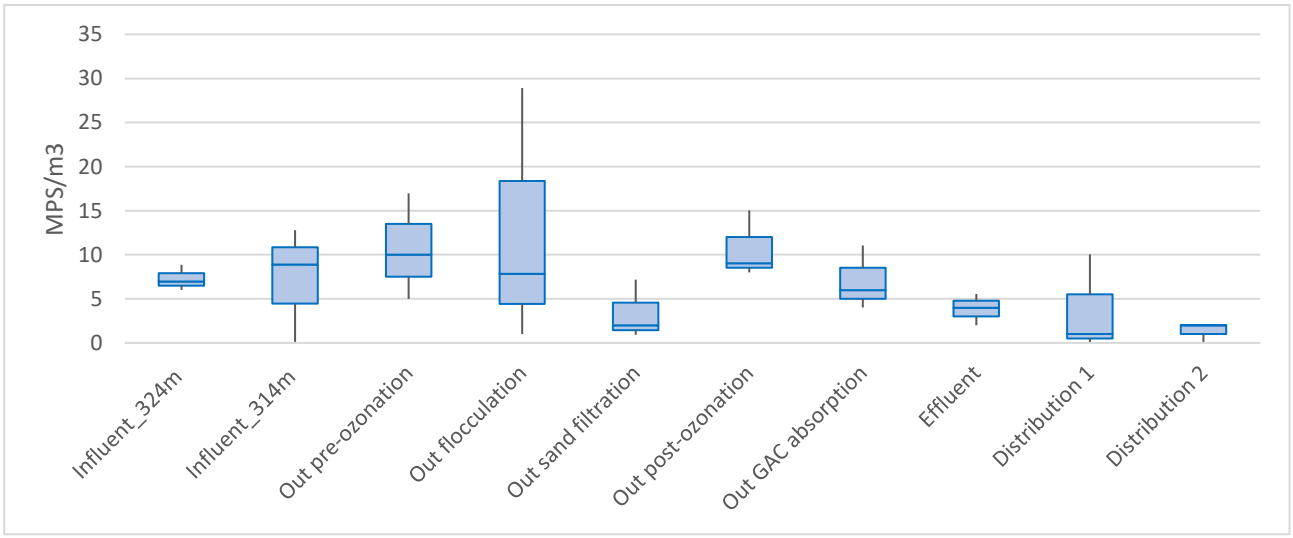


Figure 84: Distribution of MPs occurrence in Castreccioni DWTP

From Figure 85 it can be observed that backwash and sludge are generally characterised by expected higher concentrations.

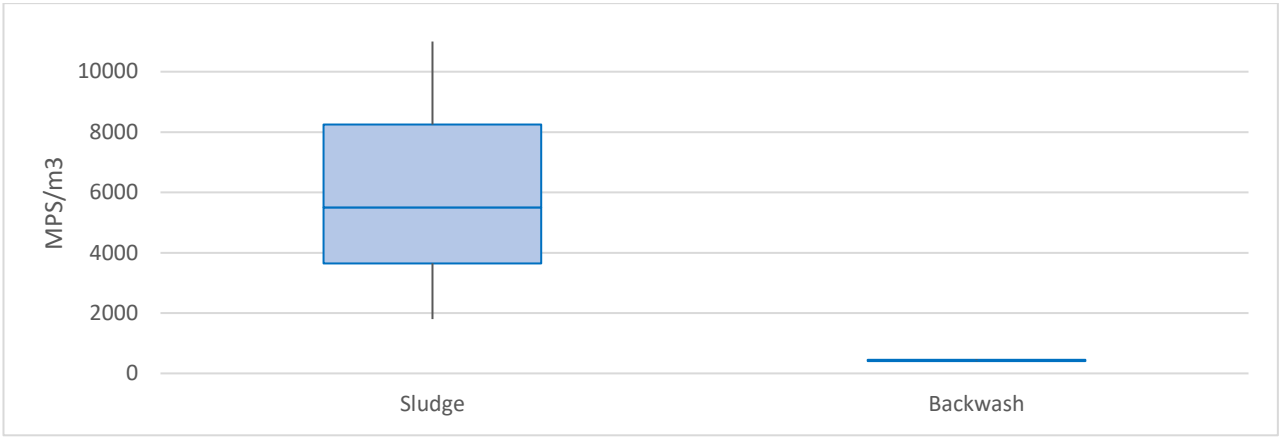


Figure 85: Distribution of MPs occurrence in sludge (left) and backwash (right) of Castreccioni DWTP

Figure 86 shows the relative decrement and increment of MPs, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of units' role in MPs fate.

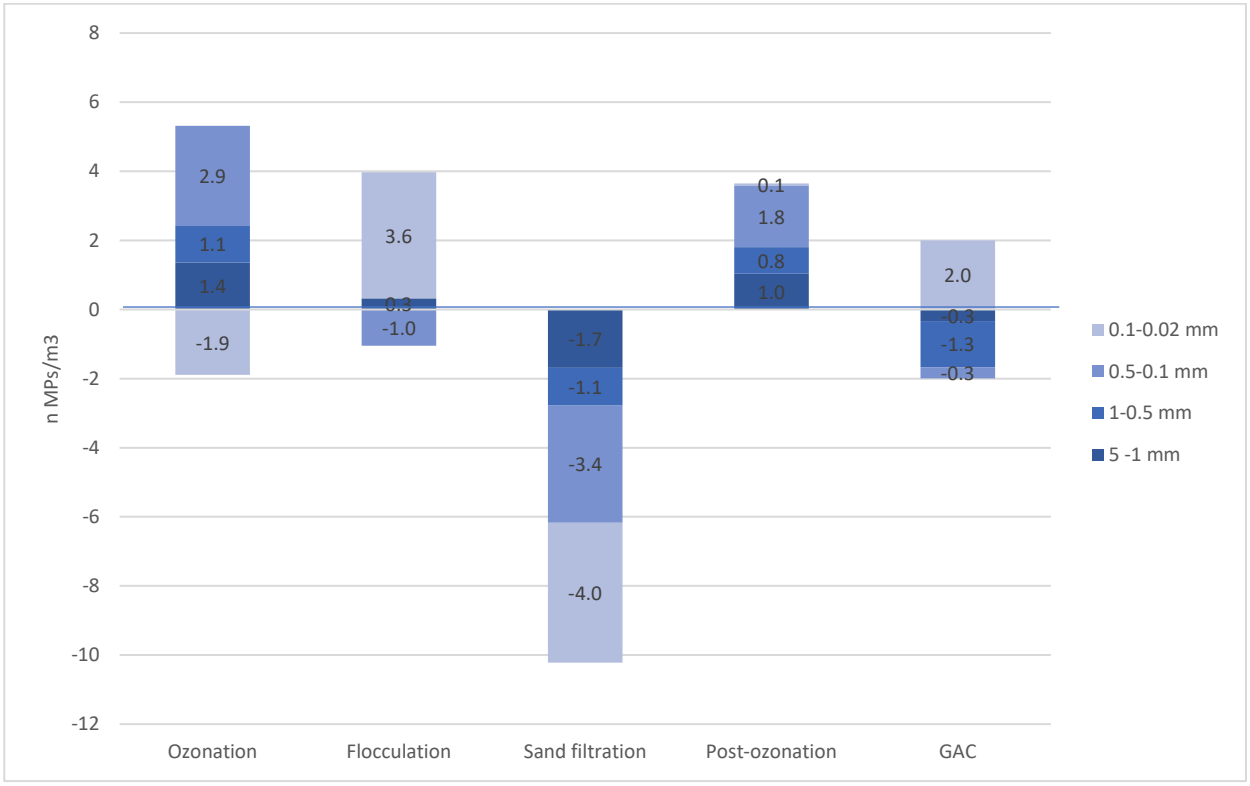


Figure 86: Decrement or increment of MPs in the treatment units of Castreccioni DWTP

It can be observed that the pre-ozonation unit was characterised by an increment on medium-high size particles (0.1 – 5 mm). Flocculation unit showed an increment in low size particles (0.02 – 0.1 mm) during the July 2020 sampling campaign, while a decrement of medium size particles (0.1 – 1 mm) was observed in the last sampling campaign of May 2021. Sand filtration is characterised by decrement of medium-low particles size (0.02 – 0.5 mm). In particular, sampling campaign of December 2020 showed the highest values. Post-ozonation unit was affected by a slight increase in MPs, mainly of medium-big size. Finally, GAC adsorption was able to reduce the medium-high sizes (0.1 – 5 mm), while an increase was observed for the MPs of lowest dimensions. This semi-

qualitative evaluation highlights the higher effect in MPs reduction linked to the physical processes, more than the chemical ones. Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

5.4.2 Garda Molinet DWTP

Results of the sampling campaigns performed for Molinet DWTP are schematised in the following figures. Figure 877 shows MPs concentrations along the different treatment units for the three sampling campaigns performed.

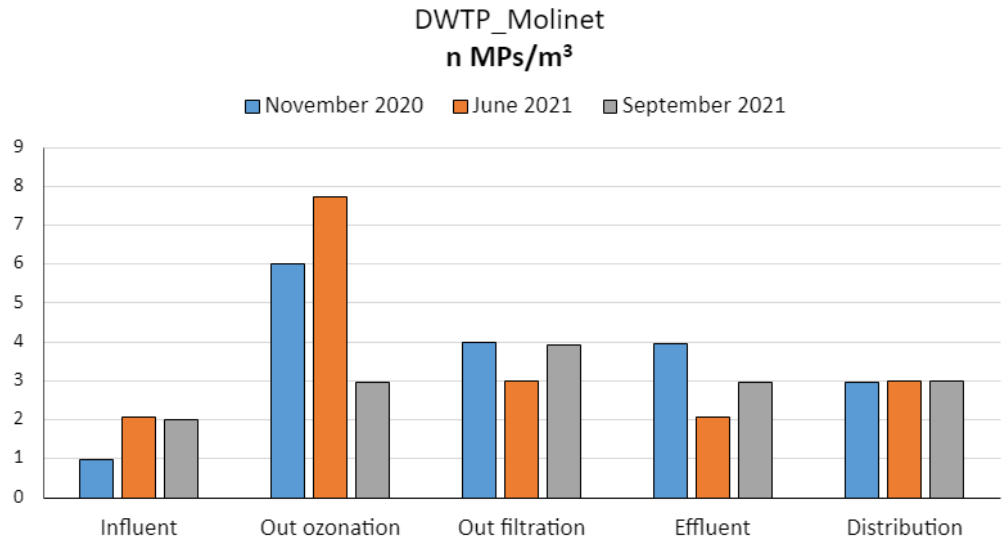


Figure 87: MPs abundance in waste and treated waters of Molinet drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m^3).

It can be observed that the MPs concentrations in the different treatment units were quite similar in all the different sampling periods, except for the ozonation unit, which presented the highest variability. Influent MPs concentration is about 1 – 2 MPs/m^3 and due to microparticles with shape of film or fragment (Figure 18A). Similar to Castreccioni DWTP, in the effluent from the ozonation process, an increase in MPs concentration was observed, with values of about 6 ± 2 MPs/m^3. The highest increment was identified in June, while in the last sampling campaign of September 2021 only a slight increase was detected. These results are in line with the findings already discussed for Castreccioni DWTP and in the literature research, as detailed in Chapter 6, since the ozonation process was found to be a possible cause of MPs release. Possible correlations between the ozone dose and the indirect effect on MPs are probably expected and need to be furtherly investigated. However, after the filtration unit, a reduction of MPs can be observed, with average effluent concentration values around 4 ± 1 MPs/m^3. In the final effluent, low concentration data were detected, of about 3 ± 1 MPs/m^3. Accordingly, the concentrations measured in the distribution point had an average of 3 MPs/m^3. Graphs reported in Figure 88 show the distribution of MPs characteristics, depending on their shape (A), size (B) and composition (C).

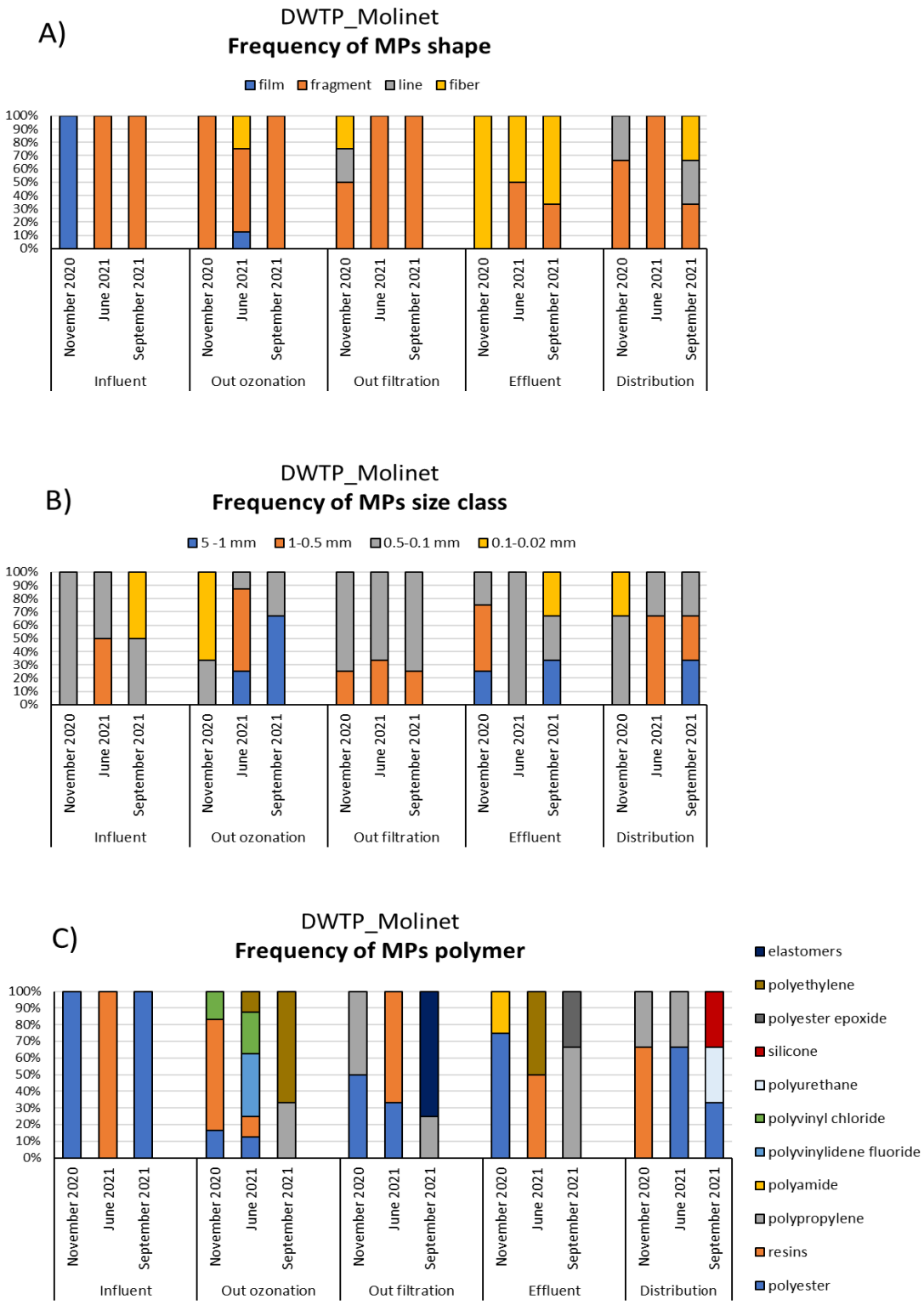


Figure 88: Characteristics of MPs extracted from waste and treated waters of Molinet drinking water treatment plant during the three-sampling campaign: A) relative contribution of shapes (%), B) relative contribution of size classes (%), C) relative contribution of polymers.

It can be observed that, while for the other steps MPs are mainly in form of particles, in the effluent a higher percentage of fibres was detected. As concern MPs size, it can be observed that in the

influent were found low-medium particles, while after the ozonation unit, particles of 1 – 5 mm were observed. This is in line with the high presence of fibres that was analysed, which are generally longer than fragments and films. It can also be noted that MPs detected in the influent were mainly made of polyester and resin. However, along the treatment layout, polyvinyl chloride, polyvinylidene fluoride, polyethylene, polypropylene, elastomers, polyamide and polyester epoxide were also found. The number of total typologies detected in the different sampling periods for each sampling point are reported in Table 44.

Table 44: Number of different plastic typologies detected during the sampling campaigns in Molinet DWTP.

	November 2020	June 2021	September 2021
Influent	1	1	1
Out ozonation	3	5	2
Out filtration	2	2	2
Effluent	2	2	2
Distribution	2	2	3

Figure 89 shows the distributions of MPs concentration for each point monitored in Molinet DWTP during the three sampling campaigns performed. It can be observed that on average, values are almost always less than 7 MPs/m^3. Moreover, it must be highlighted that the effluent from the ozonation process is the point affected by the highest variability.

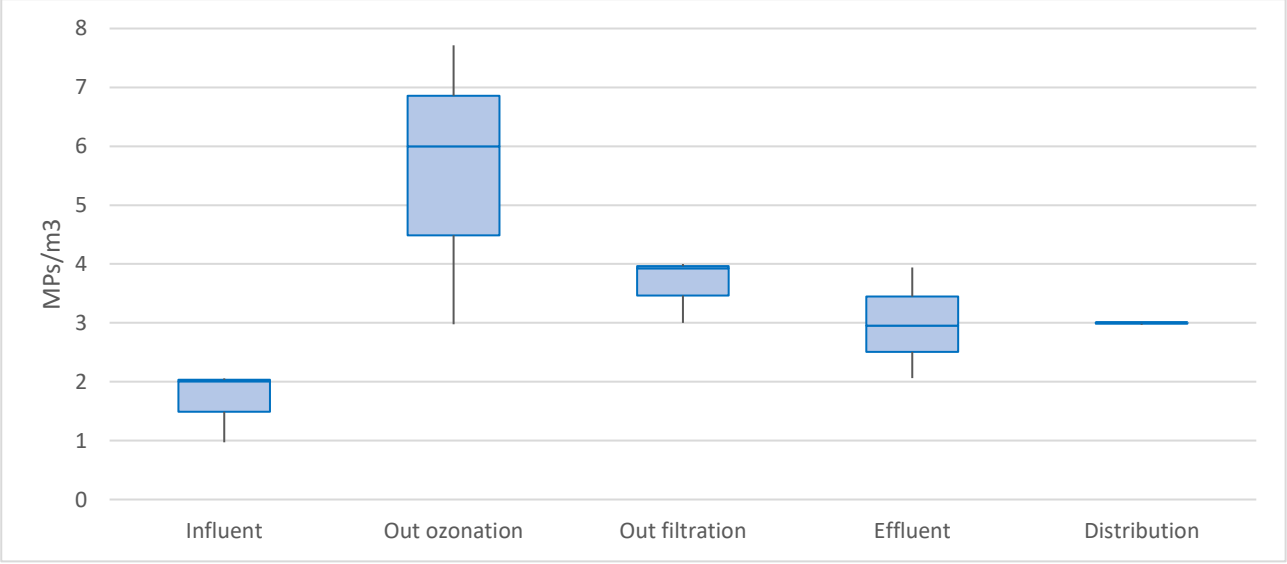


Figure 89: Distribution of MPs occurrence in Molinet DWTP

Influent and effluent concentrations are mainly characterized by low concentrations of MPs, even if an increase can be detected after the ozonation unit. However, globally the DWTP can provide a final effluent and distributed flow with MPs values lower than 3 MPs/m^3, with very limited variations, independently to the variability of the influent and the previous steps. Figure 90 shows the relative decrement and increment of MPs, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of the role of the different units in MPs fate.

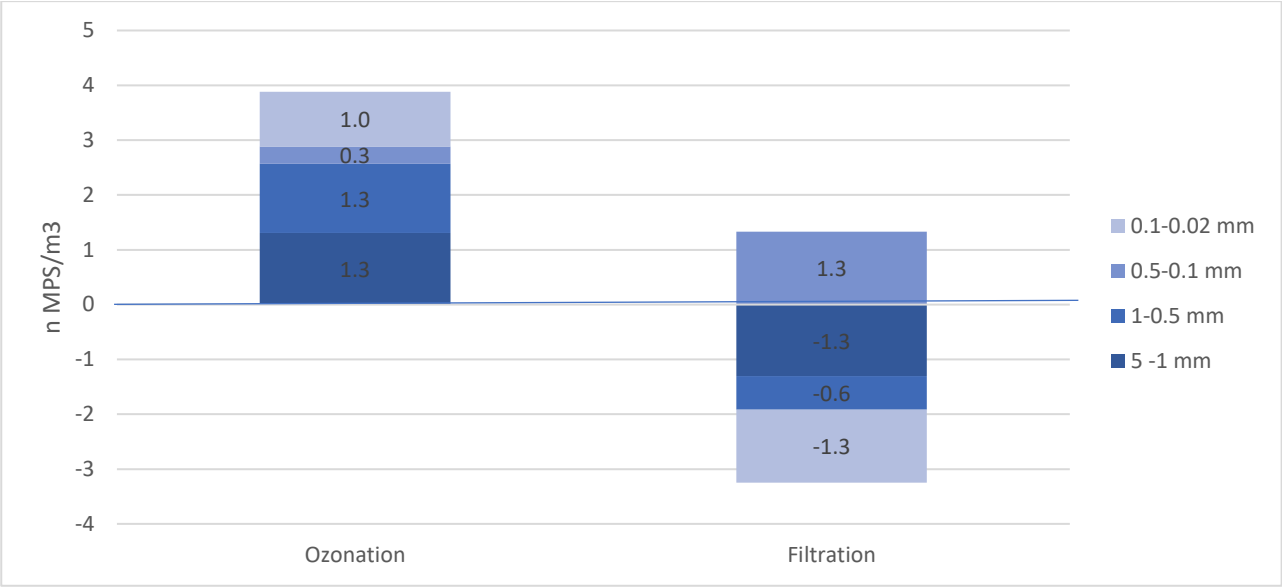


Figure 90: Decrement or increment of MPs in the treatment units of Molinet DWTP

For all the three sampling campaigns, an increase of MPs can be observed after the ozonation treatment, mainly due to low-medium size in November 2020 and to medium-big size in June and September 2021. For the filtration unit, a general decrement of MPs can be identified, even if particles in the range of 0.1 – 0.5 mm seem to slightly increase. The highest reductions were observed for particles of low dimensions (0.02 – 0.1 mm) in November 2020, while in June 2021 highest removals were performed for medium-high size particles (0.1 – 0.5 mm and 1 – 5 mm). Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

5.4.3 Brenzone Castelletto DWTP

Results of the sampling campaigns performed for Brenzone Castelletto DWTP are summarised in the following figures. Figure 91 shows MPs concentrations in the different units of Brenzone DWTP for the three sampling campaigns performed.

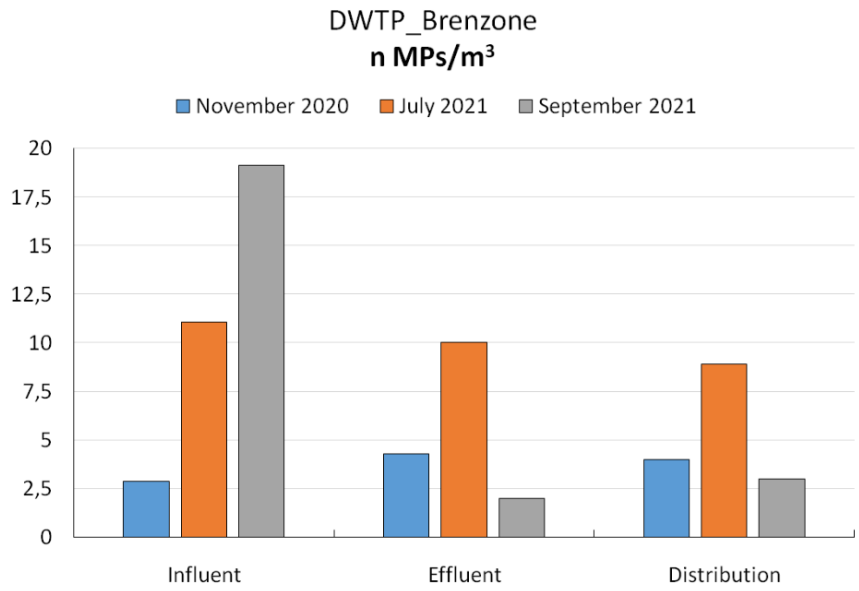


Figure 91. MPs abundance in waste and treated waters of Brenzone drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m3).

Influent concentrations were affected by high variability (11 ± 8 MPs/m^3). Accordingly, the effluent showed values of about 5 – 4 MPs/m^3, which were also similar to the ones detected in the distribution (about 5 – 3 MPs/m^3). The sampling campaign performed in the summer period (July 2021) was characterised by constant concentrations a from the influent to the distribution, while the sampling campaign of September 2021 showed the highest influent values, with a reduction of MPs in effluent and in the distribution point. It must be noticed that in the period 26 – 28 September 2021 a rainy event occurred (https://www.arpa.veneto.it/bollettini/storico/2021/0118_2021_PREC.htm). In fact, it is known that rain events may cause run-off in the catchment and overflows from sewer system, collecting and convey materials to the basin. Figure 92 shows concentrations measured in the backwash fluxes of the coarse filter and the ultrafiltration units. Concentrations are significantly higher (177 – 110 MPs/m^3 for the backwash of the coarse filter and about 68 – 19 MPs/m^3 for the backwash of the ultrafiltration), since backwash removes all the material that was accumulated during the operative working conditions of the filters.

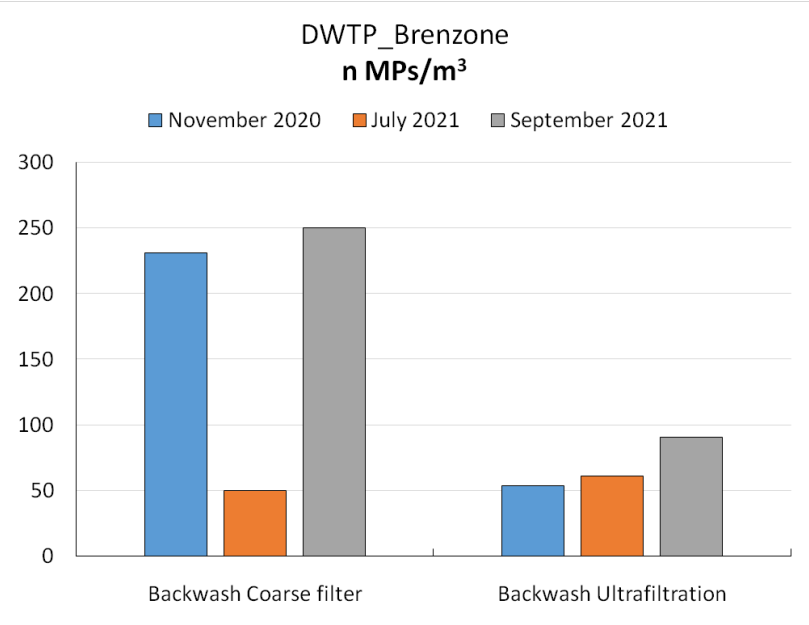


Figure 92: MPs abundance in backwash waters of Brenzone drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m3).

Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (Figure 93), their size (Figure 94) and their polymer composition (Figure 95), at the different steps of Brenzone Castelletto DWTP (A), and at the backwash fluxes (B).

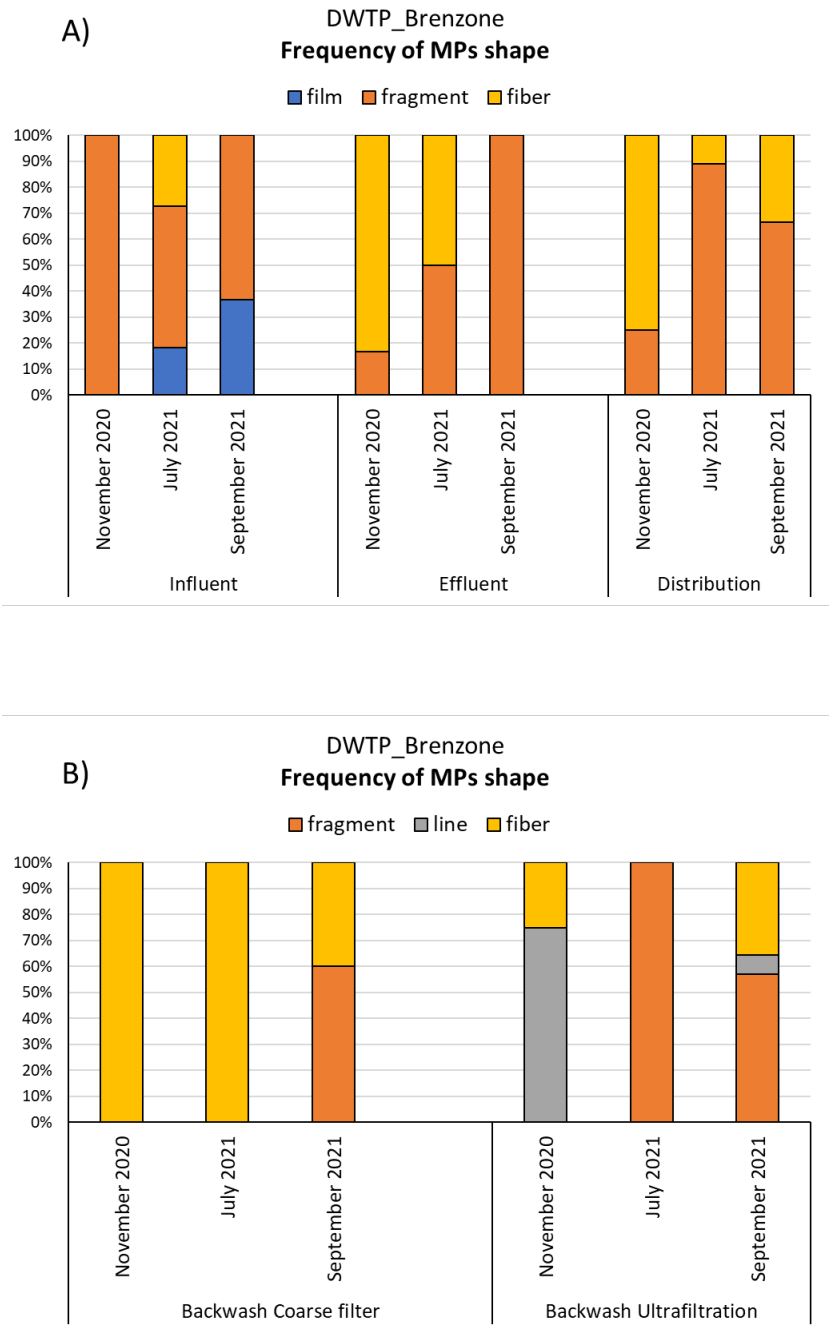


Figure 93: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) backwash waters of Brenzone drinking water treatment plant during the three-sampling campaigns.

It can be noticed that influent is mainly characterized by fragments, while fibers could be detected at the effluent.

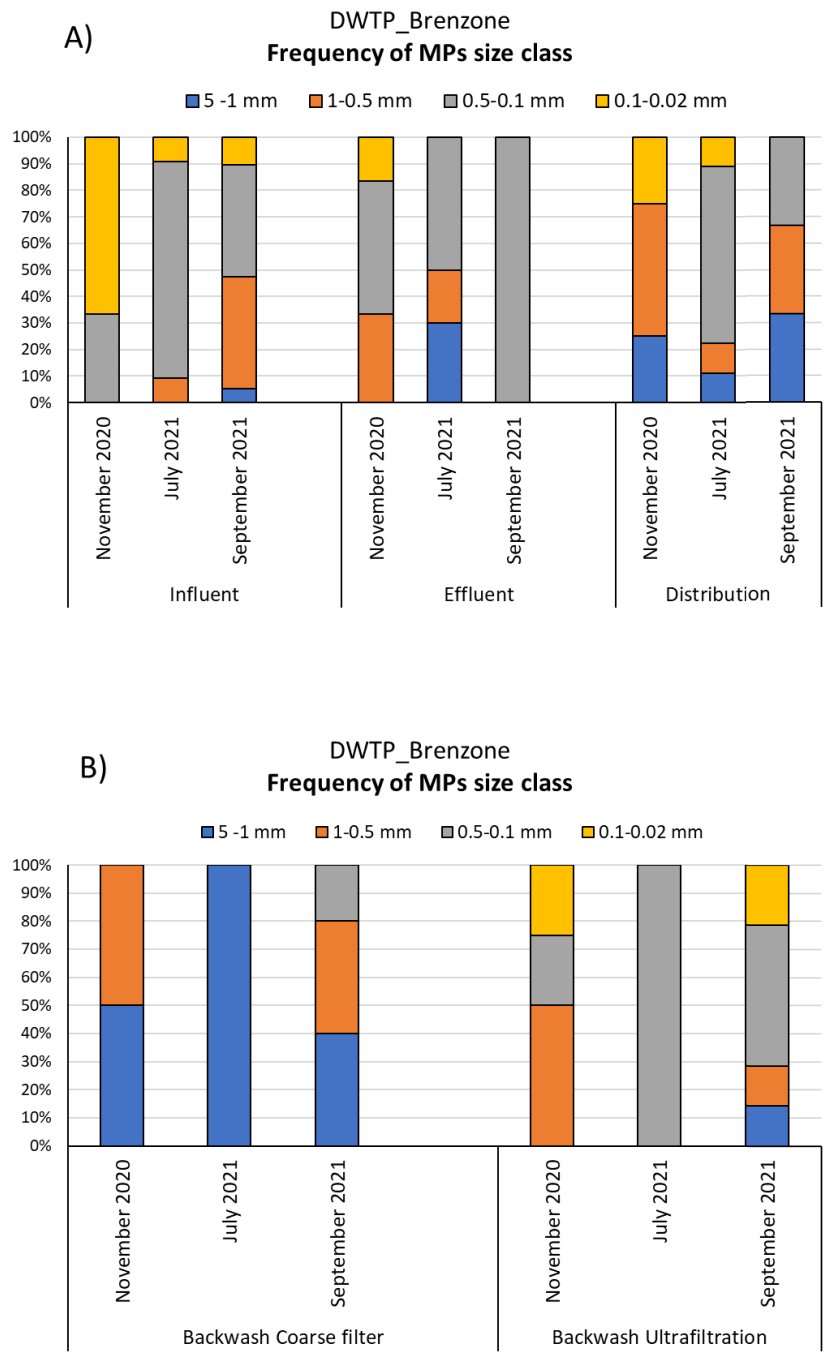


Figure 94: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) backwash waters of Brenzone drinking water treatment plant during the three-sampling campaigns.

Influent size distribution is mainly characterized by low-medium dimensions, even if on September 2021 sampling campaign, bigger size particles were detected also in the distribution.

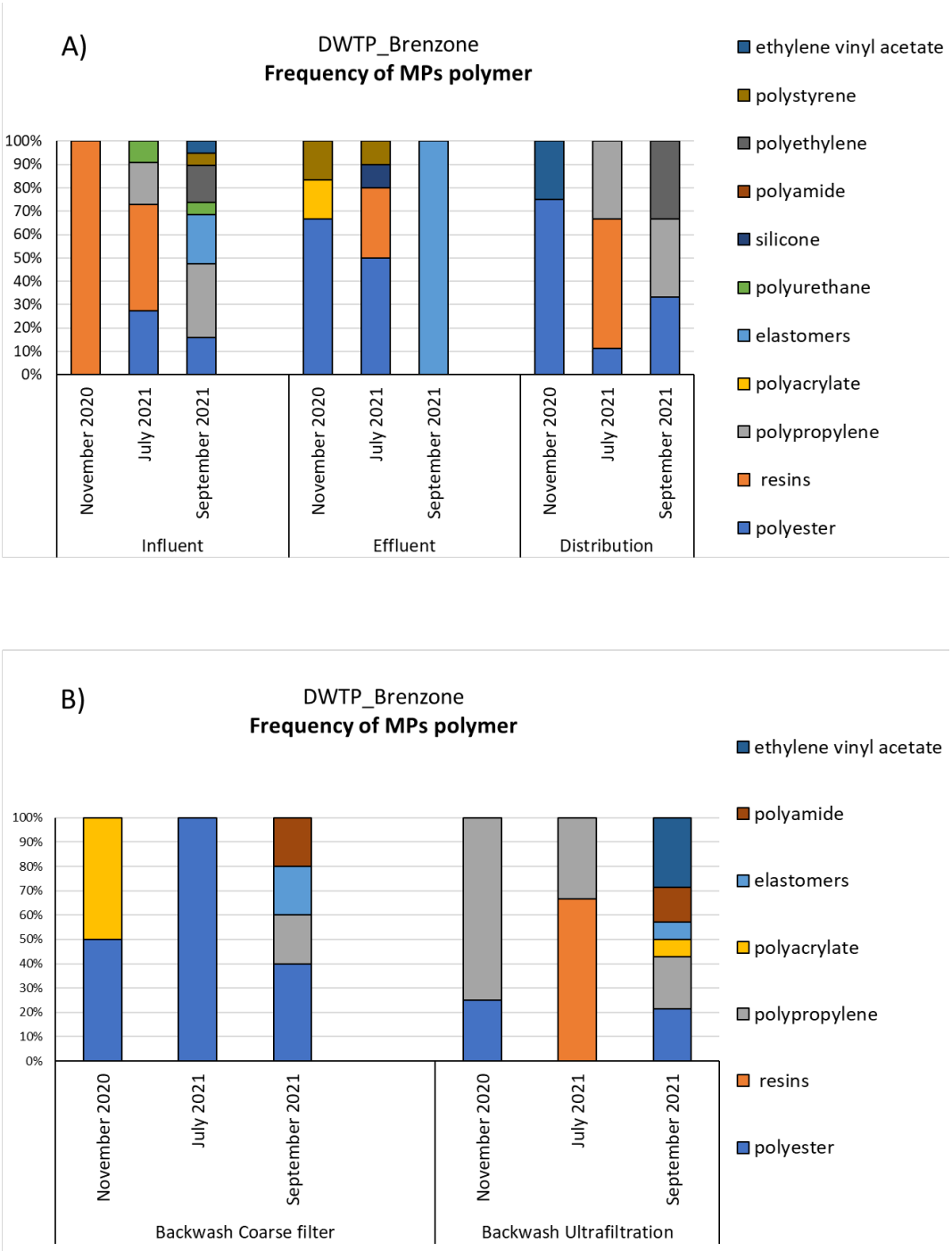


Figure 95: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) backwash waters of Brenzone drinking water treatment plant during the three-sampling campaigns.

As concern MPs characterization, in the influent polyester, resins, polypropylene, polyacrylate, elastomers, polyurethane, silicone, polyamide, polyethylene, polystyrene, ethylene and vinyl acetate were detected. It must be noticed that the widest variability occurred in correspondence of the September 2021 sampling campaign, that was probably affected by the rain events of the previous days. The number of total typologies detected in the different sampling periods for each sampling point are reported in Table 44.

Table 45: Number of different plastic typologies detected during the sampling campaigns in Brenzone Castelletto DWTP.

	November 2020	July 2021	September 2021
Influent	1	4	7
Effluent	3	4	1
Distribution	2	3	3
Backwash Coarse filter	2	1	4
Backwash Ultrafiltration	2	2	6

Figure 96 and Figure 97 report the distributions of MPs concentration for each monitored point in Brenzone Castelletto DWTP during the three sampling campaigns performed.

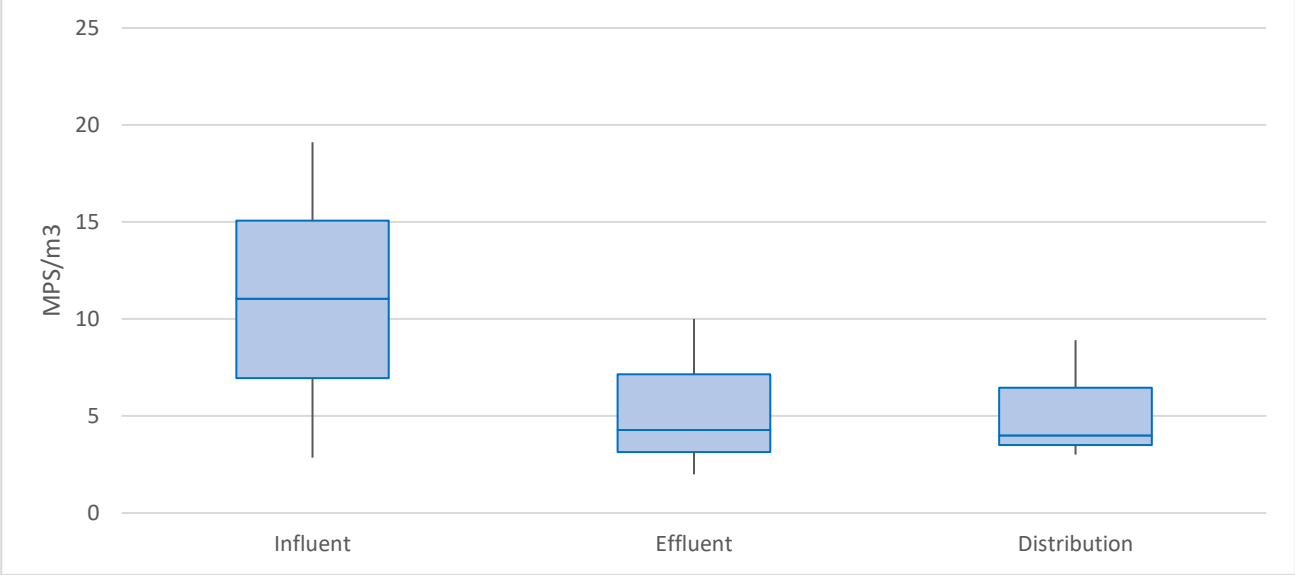


Figure 96: Distribution of MPs occurrence in Brenzone Castelletto DWTP

It can be observed that on average, values are almost always less than 15 MPs/m^3. Moreover, it must be highlighted that a net decrement can be observed in the DWTP. Influent and effluent concentrations are meanly characterized by low concentrations of MPs.

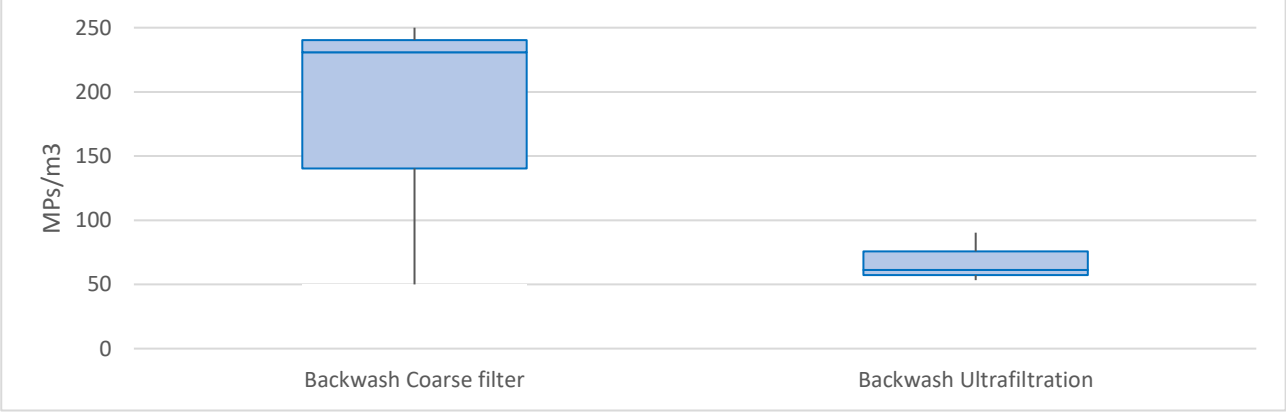


Figure 97: Distribution of MPs occurrence in backwash fluxes of Brenzone Castelletto DWTP

Backwash fluxes are characterized in general by higher distributions, since their effect is to remove the solid particles retained by the filtration units. Figure 98 shows the relative decrement and

increment of MPs, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of the role of the treatment units in MPs fate.

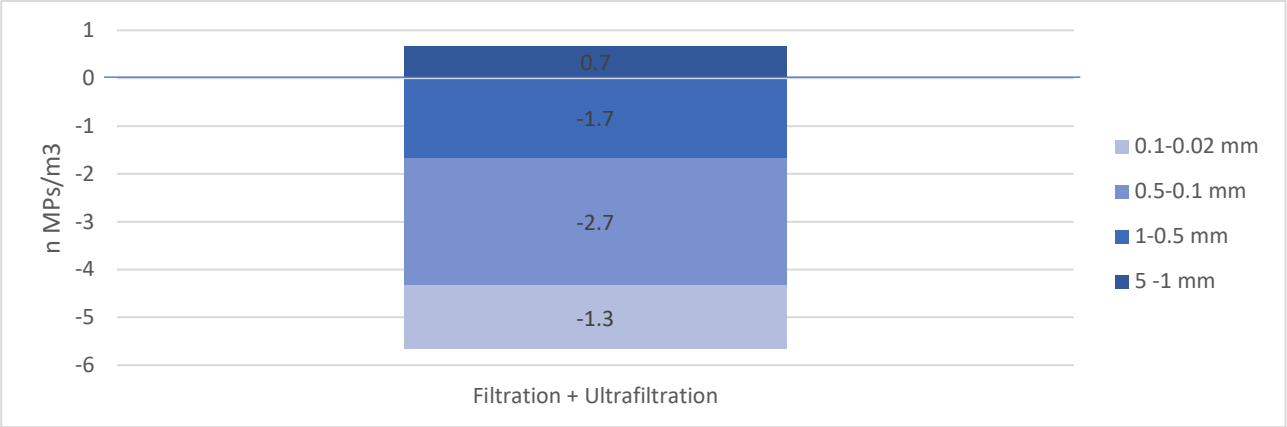


Figure 98: Decrement or increment of MPs in the treatment units of Brenzone Castelletto DWTP

Generally, decrements in the filtration units were observed for almost all the range sizes, even if some medium big size particles (0.1 – 5 mm) were detected in November 2020 and July 2021. The sampling campaign performed in September 2021 showed the best results, with decrement for all the size particles.

Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

5.4.4 Limone Tremosine WWTP

Results of the sampling campaigns performed for Limone Tremosine WWTP are summarised in the following figures. Figure 79 and Figure 80 show MPs concentrations in the treatment units for the three sampling campaigns performed, respectively, for the units of the WWTP layout and for the sludge grab samples analysed.

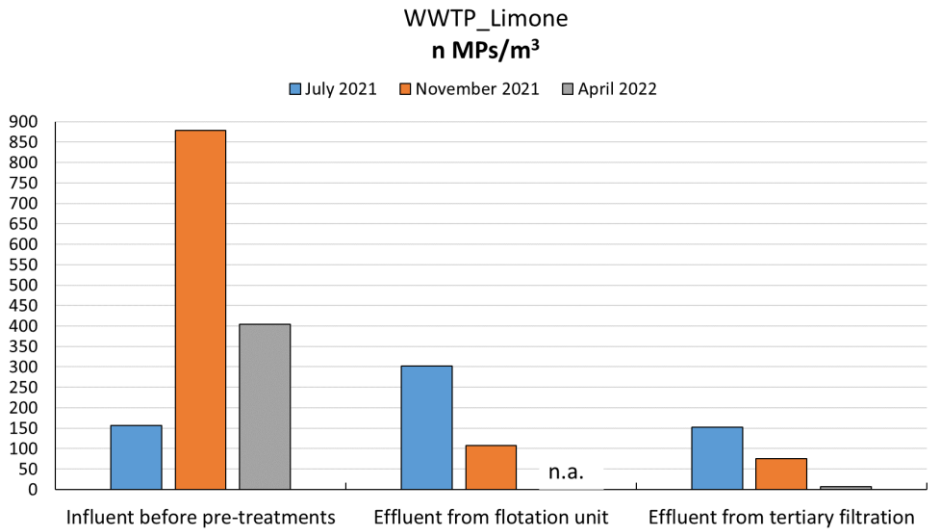


Figure 99: MPs abundance in waste and treated waters of Limone Tremosine wastewater treatment plant detected during the three-sampling campaigns. Data are given as number (n) of microplastics (MPs) per volume of sample (m3). n.a.= sample not available for that sampling campaign (April 2022).

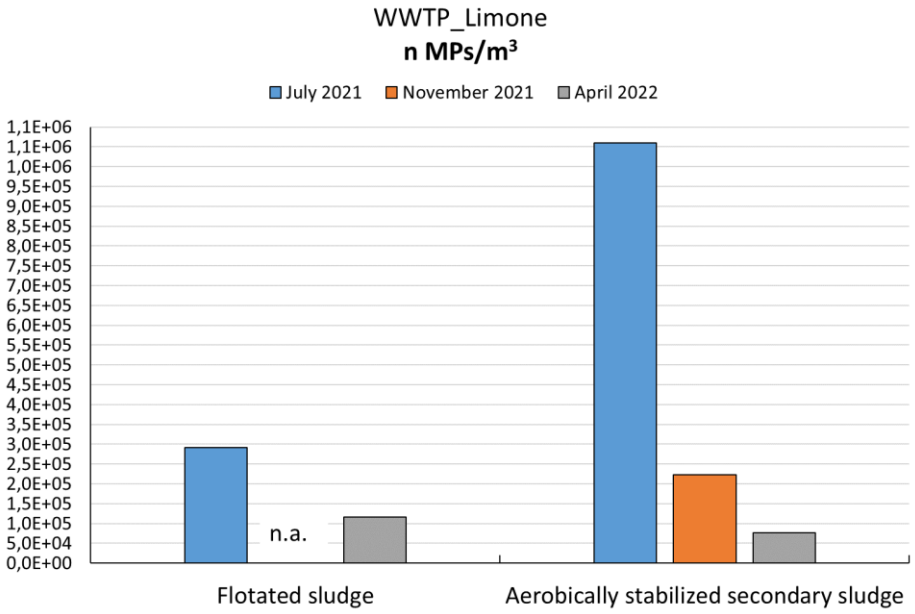


Figure 100: MPs abundance in sludge samples of Limone Tremosine wastewater treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m3). n.a.= sample not available for that sampling campaign (November 2021).

It can be observed that the influent is characterised by high variability of MPs concentration, in the range of 408 ± 367 MPs/m^3, with the highest values during the winter campaign and the lowest in summer. It must be underlined that during the winter campaign very low influent flows were observed in the WWTP, affected by touristic seasonality. Therefore, the influent collection was probably affected by the elevated presence of settled material on the bottom of the sampling point. In the first sampling campaign an increase was observed at the effluent from the flotation unit, after biologic treatments; however, concentrations decreased again at the final effluent after the tertiary filtration. On average, concentrations after the flotation units were measured of 205 ± 137 MPs/m^3, while in the final effluent mean values were of about 78 ± 72 MPs/m^3. MPs concentrations in sludge samples are generally higher (respectively, 203500 ± 123744 MPs/m^3 in the flotated sludge and 453083 ± 530540 MPs/m^3 in the stabilised sludge), due to the sedimentation of solid matter and accumulation effects. Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (Figure 93), size (Figure 94) and polymer composition (Figure 95), at the different steps of Limone Tremosine WWTP (A), and measured in the sludge (B).

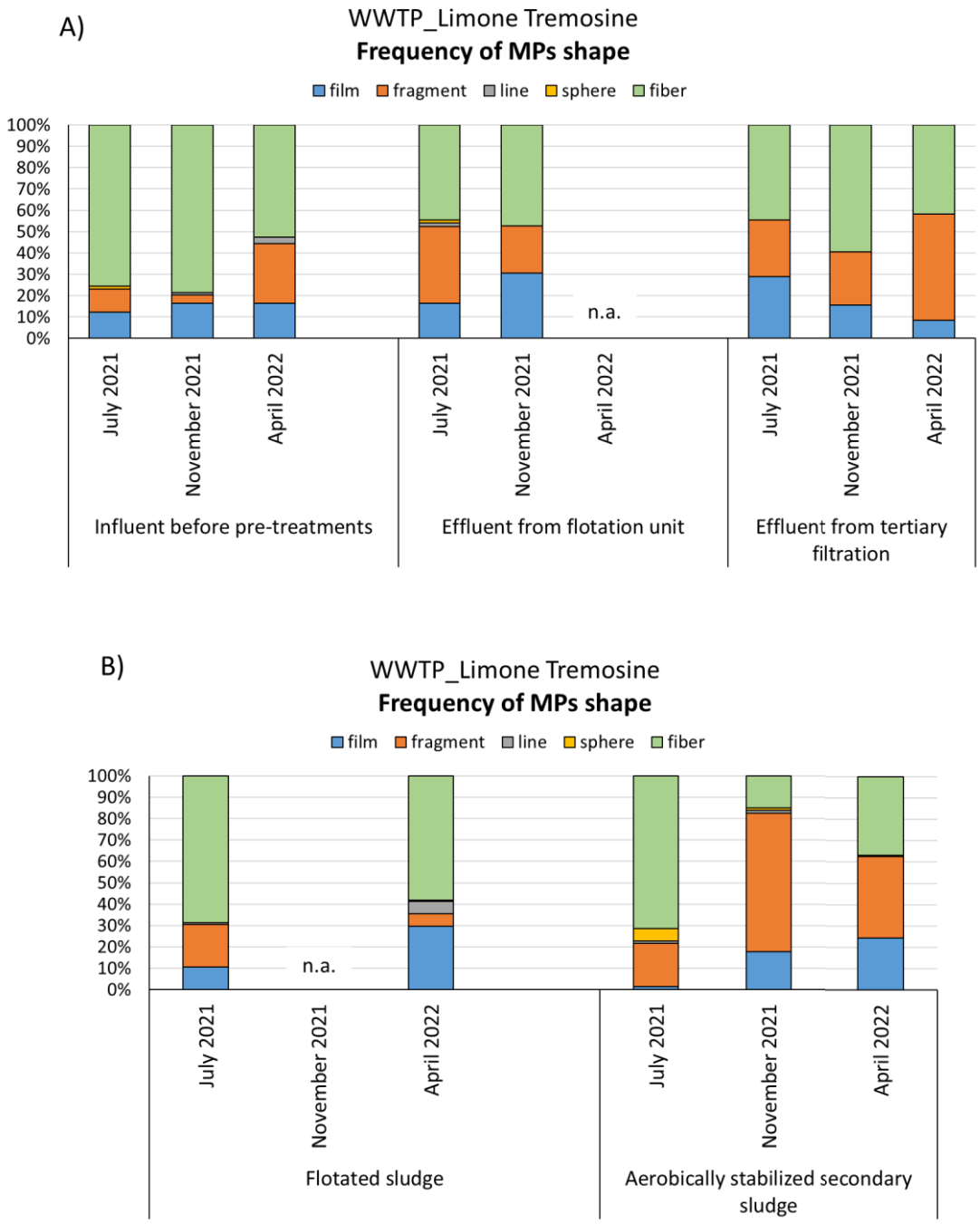


Figure 101: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) the sludge line of Limone Tremosine wastewater treatment plant during the three-sampling campaigns. n.a.= sample not available for that sampling campaign.

It can be observed that fibres are the most frequently detected shape, followed by fragments and films. The relative presence of the fragments, expressed in percentage, increased along the treatment line.

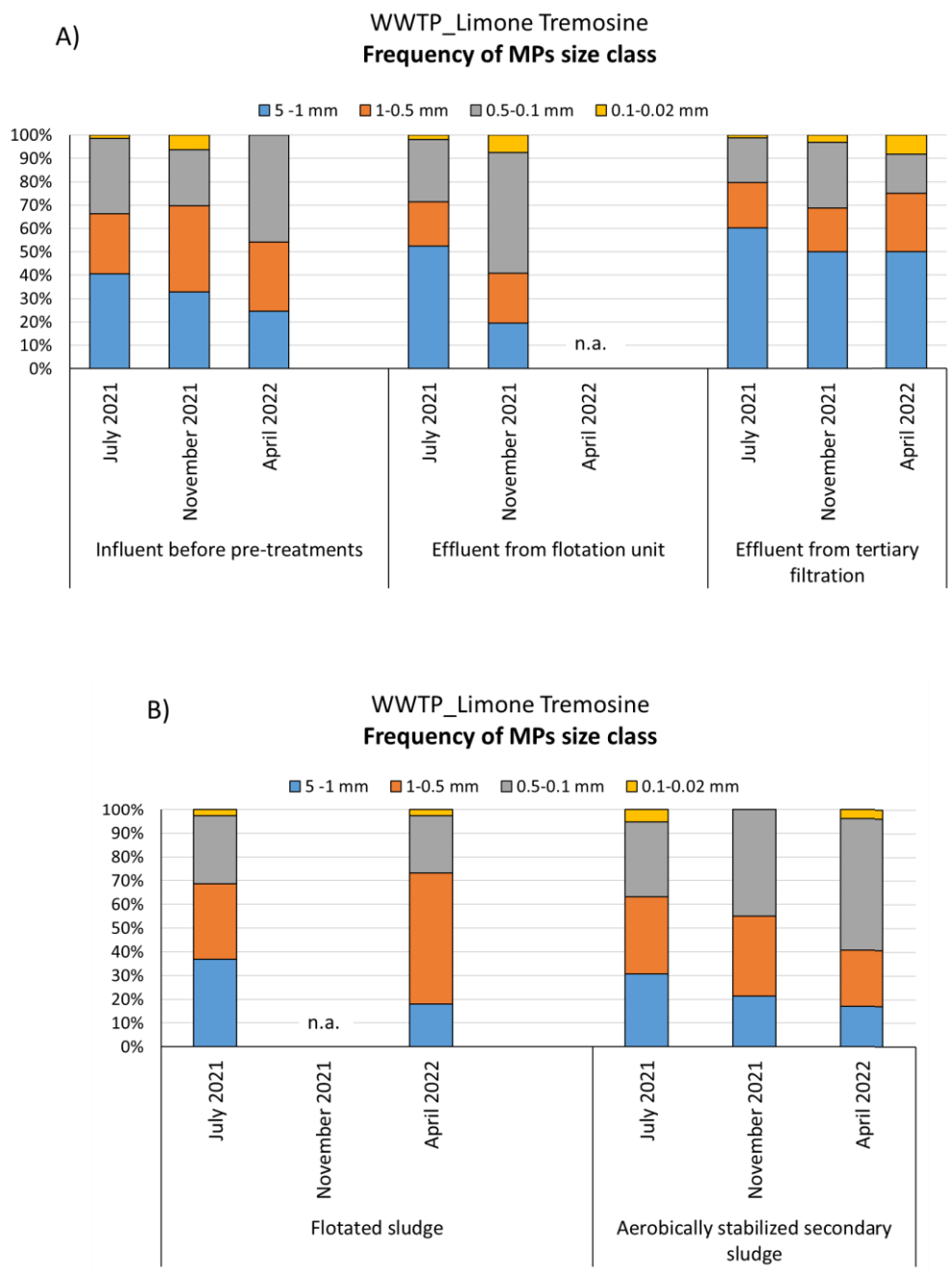


Figure 102: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) the sludge line of Limone Tremosine wastewater treatment plant during the three-sampling campaigns. n.a.= sample not available for that sampling campaign.

Considering particles dimension, it can be noted that medium-big size is the most frequent, according to the findings obtained for shape analysis, since fibres are usually characterised by the biggest sizes.

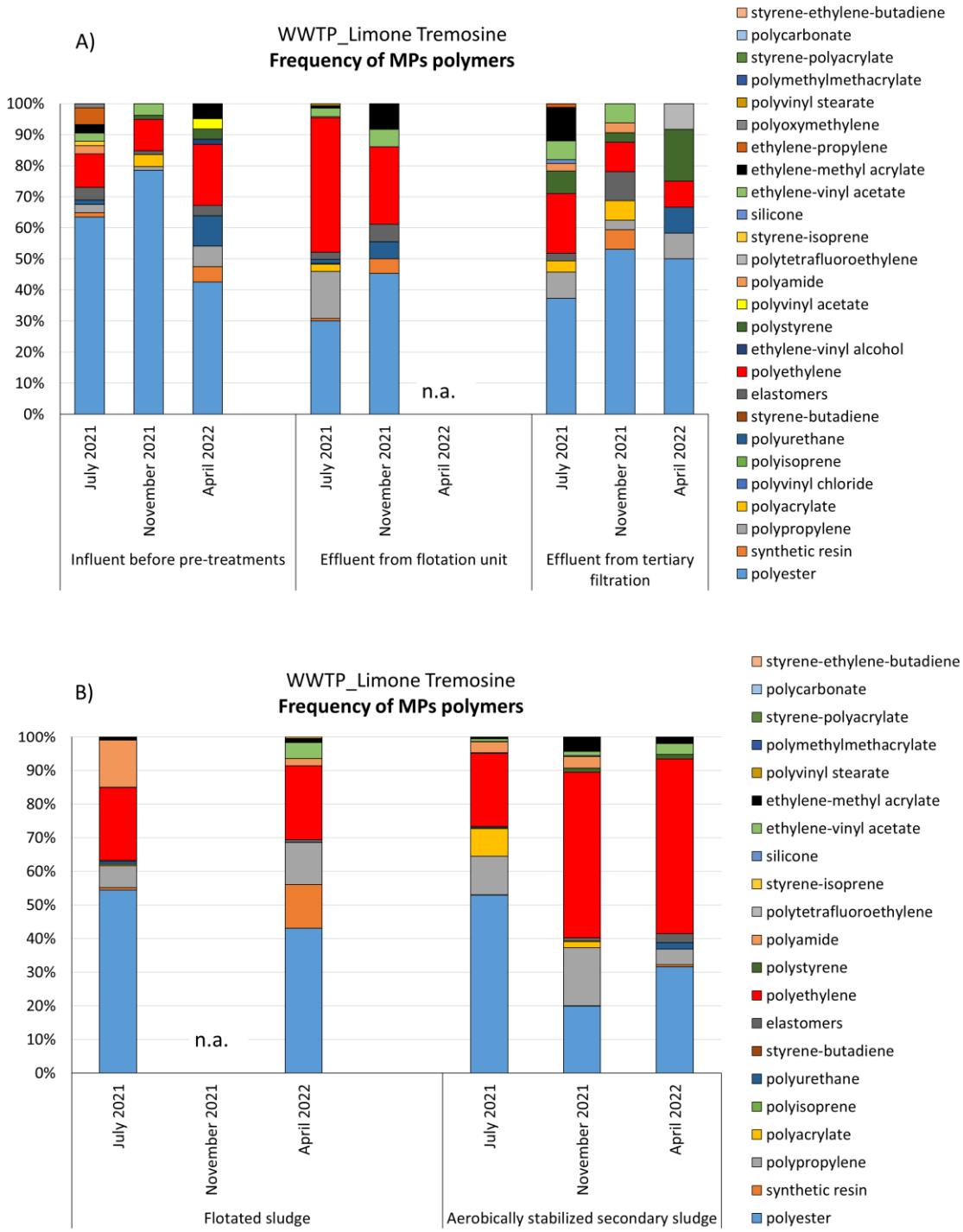


Figure 103: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) the sludge line of Limone Tremosine wastewater treatment plant during the three-sampling campaigns. n.a.= sample not available for that sampling campaign.

Analysing chemical characterisation, the most frequent particles are of polyester and polyethylene. Usually, polyester can be associated to synthetic fibres, while polyethylene can be found in fragments or films. Moreover, the relative presence of polyethylene, expressed as percentage, increased along the treatment line after secondary treatments. The presence of polyethylene is also detected in the sludge line. However, significant variations were observed in the treatment units, as the typologies and the relative percentages varied along the treatment steps. To better

investigate the possible origins of the MPs detected, a chemical characterisation was carried out on the carriers that are used as supporting material to the attached-growth biomass in the biologic treatment. It was found that the IR spectre can be attributed to high density polyethylene, as shown in Figure 104.

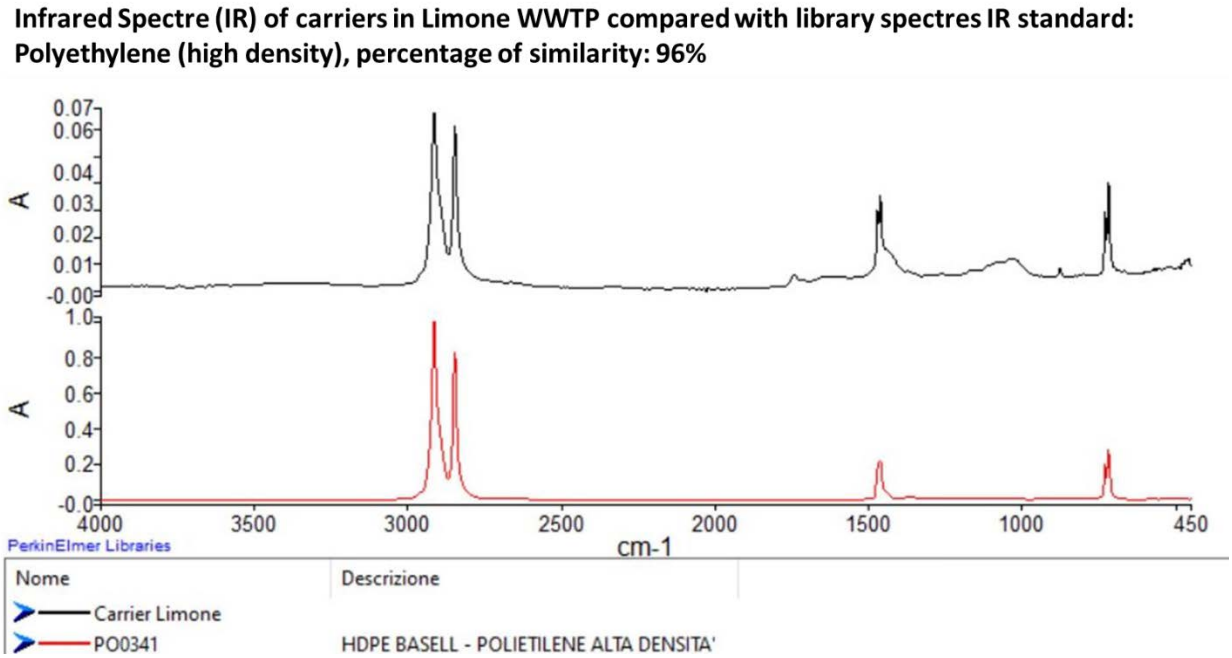


Figure 104: Characterization of the carriers in the biologic unit of Limone Tremosine WWTP

The number of total typologies detected in the different sampling periods for each sampling point are reported in Table 46.

Table 46: Number of different plastic typologies detected during the sampling campaigns in Limone Tremosine WWTP

	July 2021	November 2021	April 2022
Influent before pre-treatments	12	7	10
Effluent from flotation unit	12	7	n.a.
Effluent from tertiary filtration	11	9	6
Flotated sludge	12	n.a.	9
Aerobically stabilized secondary sludge	16	15	9

Figure 105 and Figure 106 show the distributions of MPs concentration for each point monitored in Limone WWTP during the three sampling campaigns performed. The boxes represent the variability of the measurements in each point during the three different sampling periods. In particular, the extremes of the boxes correspond to the first and third quantiles, while the vertical lines reach, respectively, the minimum and the maximum values detected. The median values along the three samplings are reported as the blue lines into the boxes.

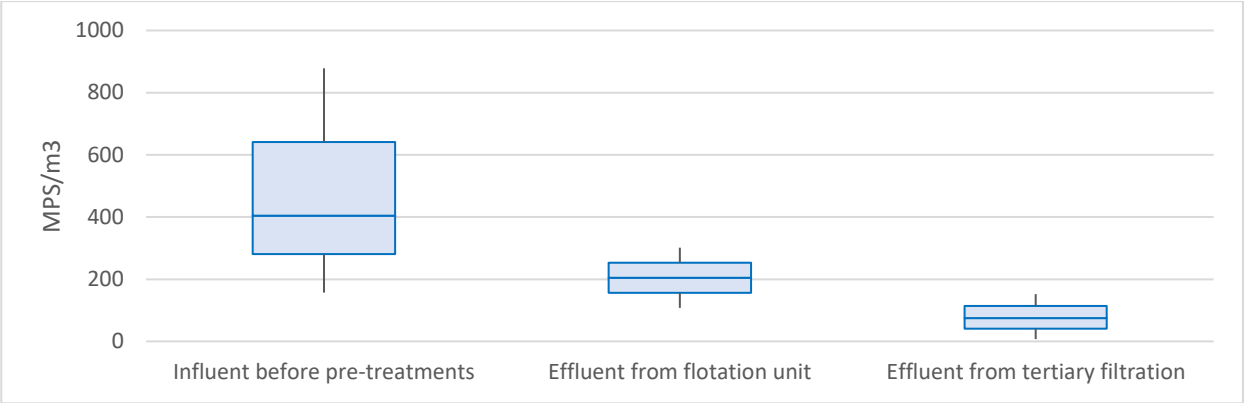


Figure 105: Distribution of MPs occurrence in Limone Tremosine WWTP

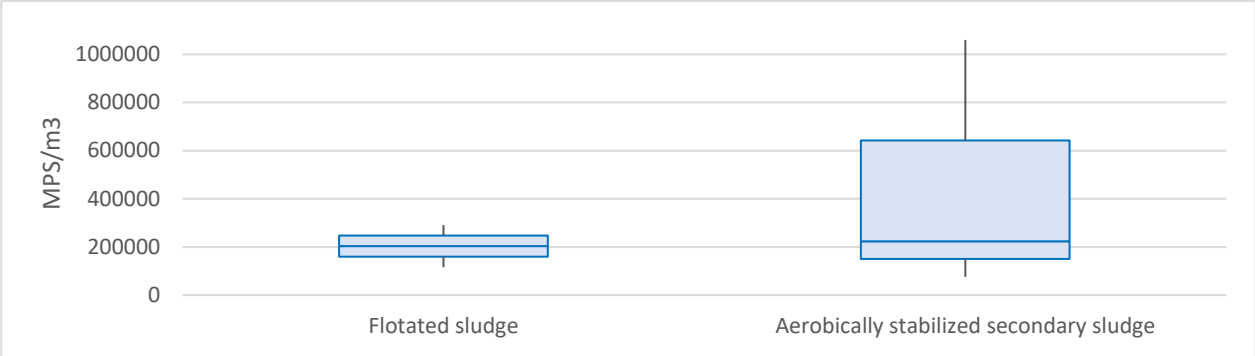


Figure 106: Distribution of MPs occurrence in sludge of Limone Tremosine WWTP

Figure 107 shows the relative decrement and increment of MPs, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of units role in MPs fate.

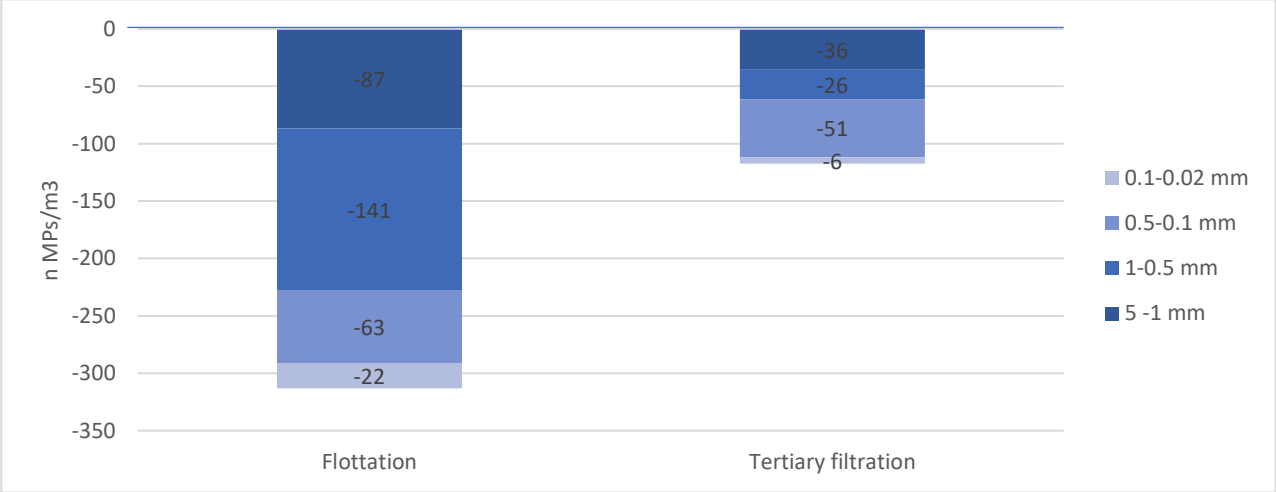


Figure 107: Decrement or increment of MPs in the treatment units of Limone Tremosine WWTP

Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

5.4.5 Peschiera del Garda WWTP

Results of the sampling campaigns performed for Peschiera del Garda WWTP are schematised in the following figures. Figure 108 and Figure 109 show MPs concentrations along the different treatment units for the three sampling campaigns performed, respectively, for the different units of the WWTP layout and for the sludge grab samples analysed. It must be noticed that results are plotted using a logarithmic scale, because of the very high measure detected at the influent on November 2021 sampling campaign.

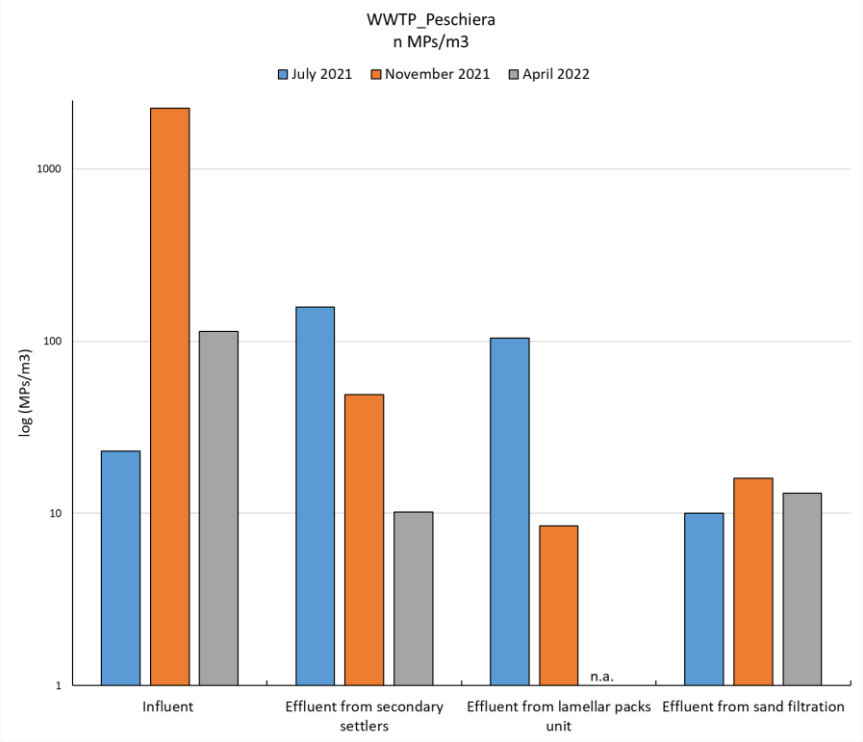


Figure 108: MPs abundance in waste and treated waters of Peschiera del Garda wastewater treatment plant detected during the three-sampling campaigns. Data are given as number (n) of microplastics (MPs) per volume of sample (m^3).

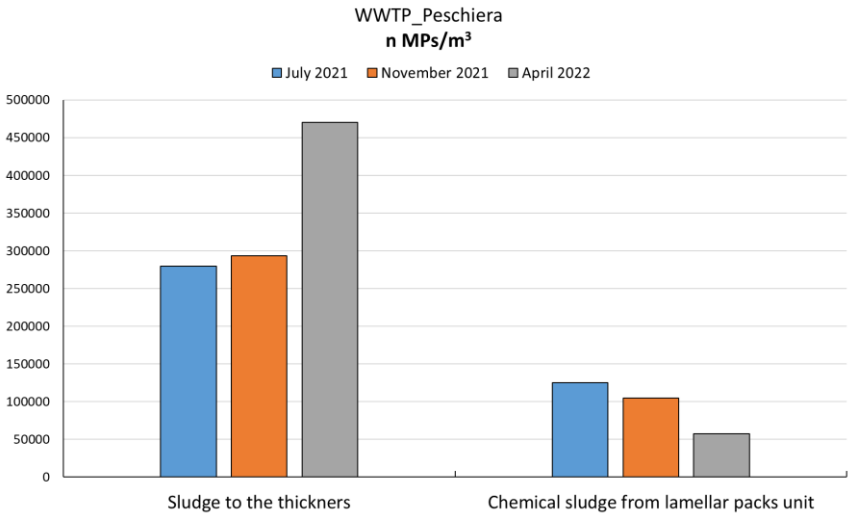


Figure 109: MPs abundance in sludge samples of Peschiera del Garda wastewater treatment plant detected during the three-sampling campaigns. Data are given as number (n) of microplastics (MPs) per volume of sample (m^3).

As concern samples collected in the water treatment line, a very high peak was detected in the influent on November campaign, while other concentrations were generally below 150 MPs/m^3. It must be underlined that during the winter campaign very low influent flows were observed in the WWTP, affected by touristic seasonality. Therefore, the influent collection was probably affected by the elevated presence of settled material on the bottom of the sampling point. It must be noted that the influent seems to be particularly diluted, perhaps from surface waters, and thus the impact from the anthropic contribution could be smoothed by the dilution. This hypothesis is in line with the low values of ammonia nitrogen found in the influent, which were on average at about 12 mg $N\text{-}NH_4$/l. Generally, a decreasing trend can be observed along the treatment train, with effluent concentrations lower than 10-15 MPs/m^3. However, only during the first sampling campaign of July, an increase was observed after the secondary treatments. As concern sludge line, sludge samples before the thickening units were generally characterised by higher concentrations and variability, in respect to chemical sludge. Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (Figure 93), their size (Figure 94) and their polymer composition (Figure 95), at the different steps of Peschiera del Garda WWTP (A), and measured at the sludge samples (B).

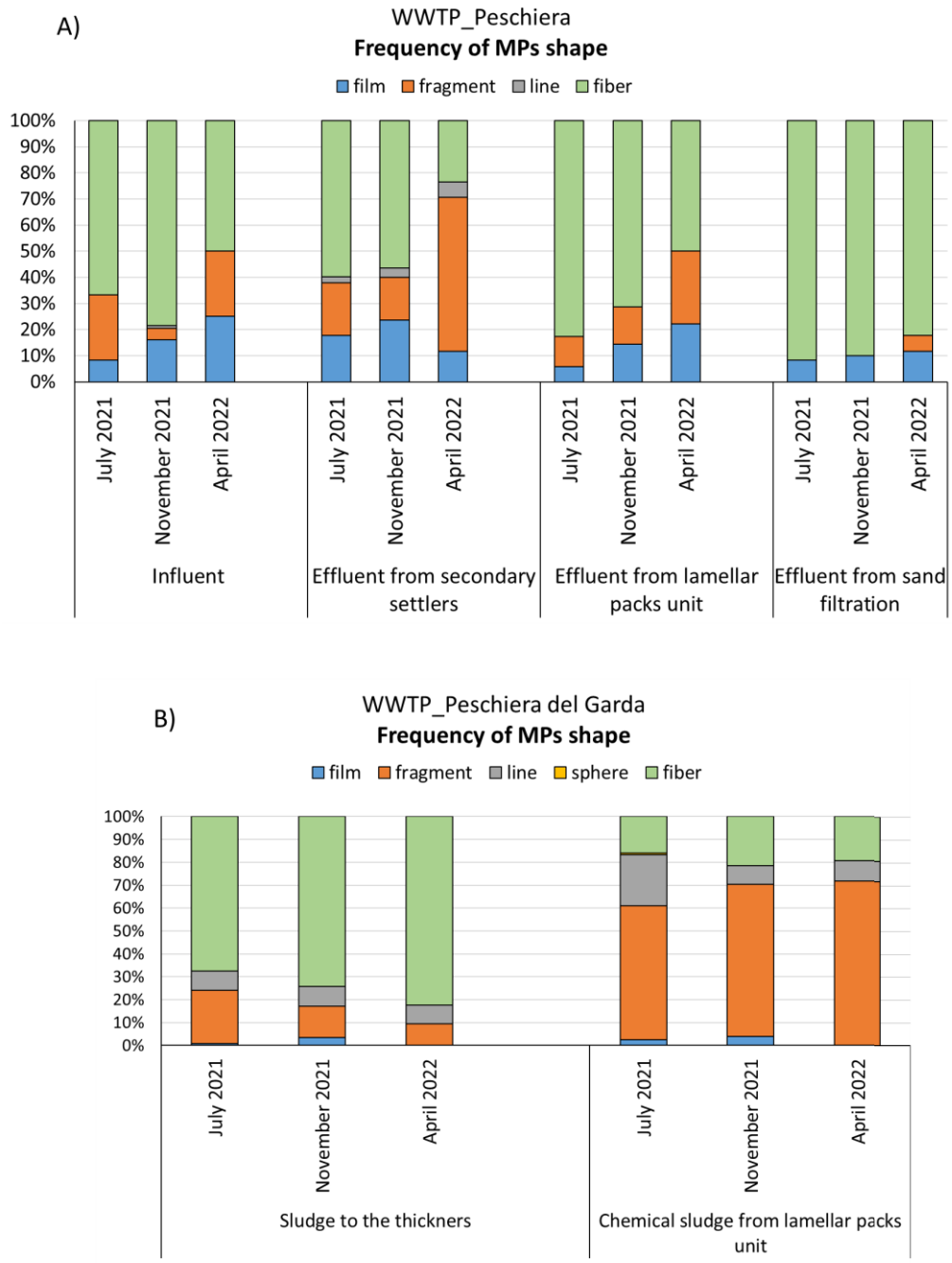


Figure 110: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) the sludge line of Peschiera del Garda wastewater treatment plant during the three-sampling campaigns.

It can be observed that fibres are the most frequent particles shape in all the water samples and in the sludge before the thickening unit. Chemical sludge is, on the other hand, characterised by the predominant presence of fragments.

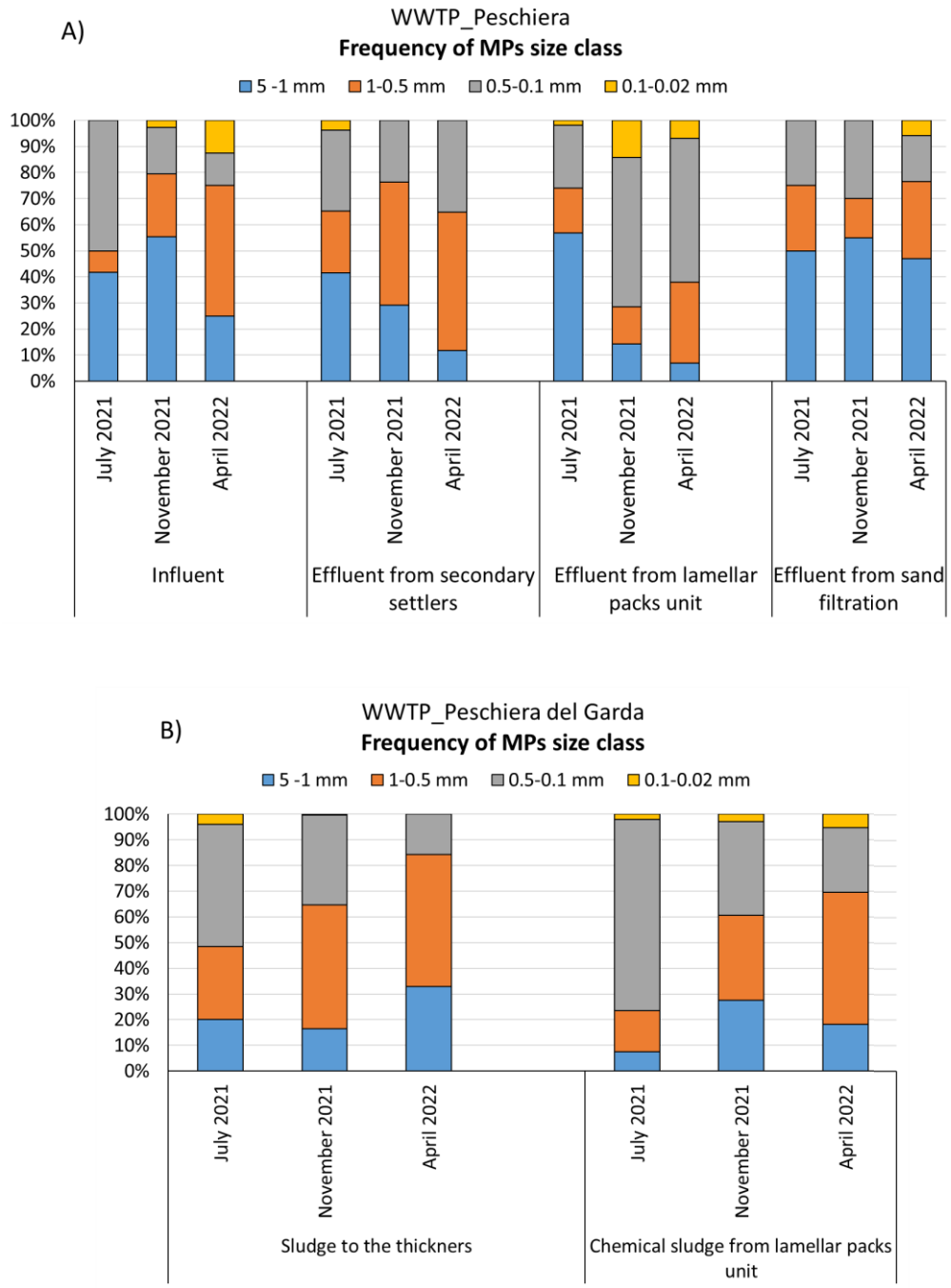


Figure 111: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) the sludge line of Peschiera del Garda wastewater treatment plant during the three-sampling campaigns.

Dimensions are quite well distributed, except for the lower size, which were less frequent.

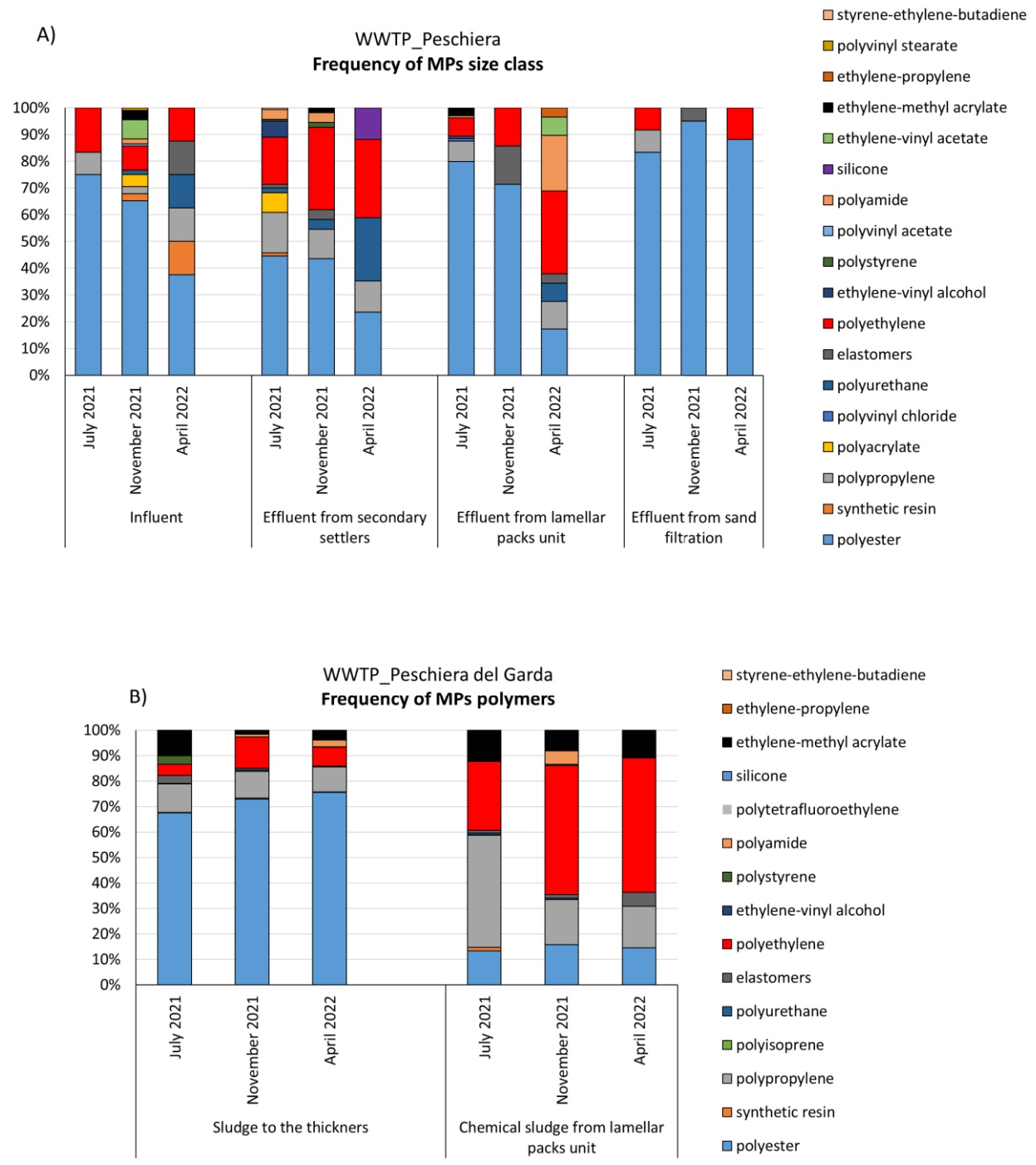


Figure 112: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) the sludge line of Peschiera del Garda wastewater treatment plant during the three-sampling campaigns.

The chemical characterisation is in line with the physical analysis of MPs shape. In fact, usually, polyester can be associated to synthetic fibres, while polyethylene can be found in fragments or films. Comparing these results with the characterisation obtained for Limone Tremosine WWTP, it can be noted that in Peschiera del Garda the percentage of polyester is generally higher and remains almost constant in all the different treatment steps. However, at the effluent a low amount of different plastic types was detected, mainly characterised by polyester and polyethylene. The number of total typologies detected in the different sampling periods for each sampling point are reported in Table 46Table 44.

Table 47: Number of different plastic typologies detected during the sampling campaigns in Peschiera del Garda WWTP

	July 2021	November 2021	April 2022
Influent	3	11	6
Effluent from secondary settlers	11	8	5
Effluent from lamellar packs unit	7	3	n.a.
Effluent from sand filtration	3	2	2
Sludge to the thickners	10	10	11
Chemical sludge from lamellar packs unit	10	9	6

Figure 113 and Figure 114 show the distributions of MPs concentration for each point monitored in Peschiera del Garda WWTP during the three sampling campaigns performed.

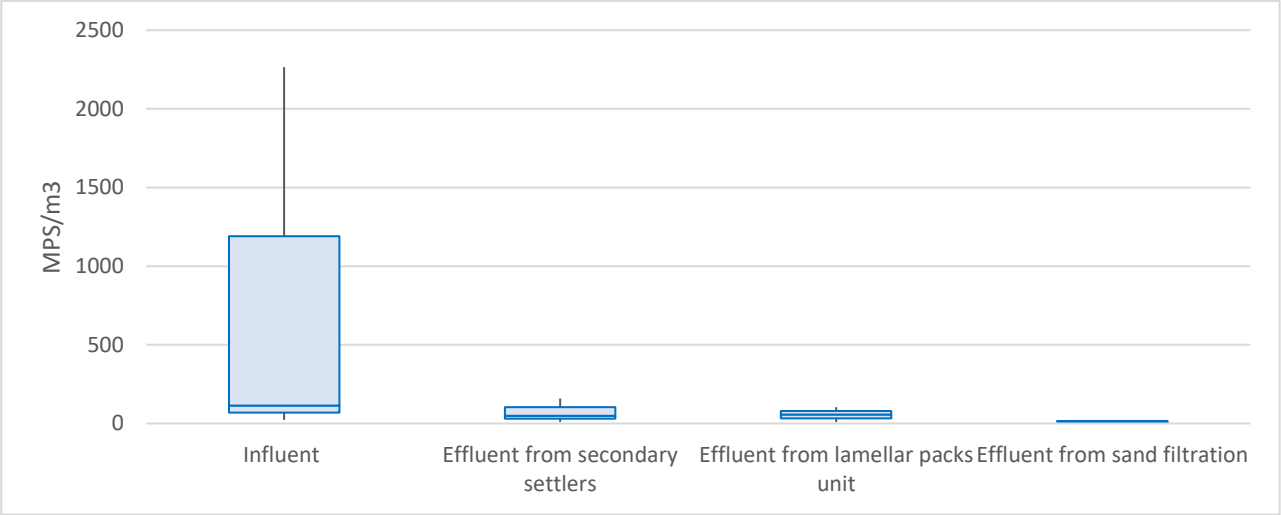


Figure 113: Distribution of MPs occurrence in Peschiera del Garda WWTP

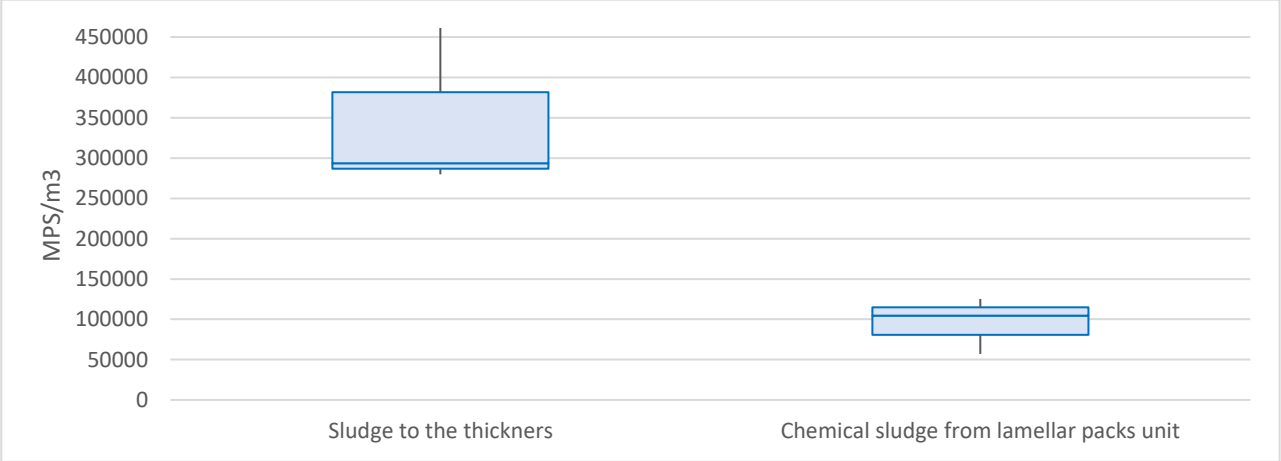


Figure 114: Distribution of MPs occurrence in Peschiera del Garda WWTP

Figure 115 shows the relative decrement and increment of MPs, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of units role in MPs fate.

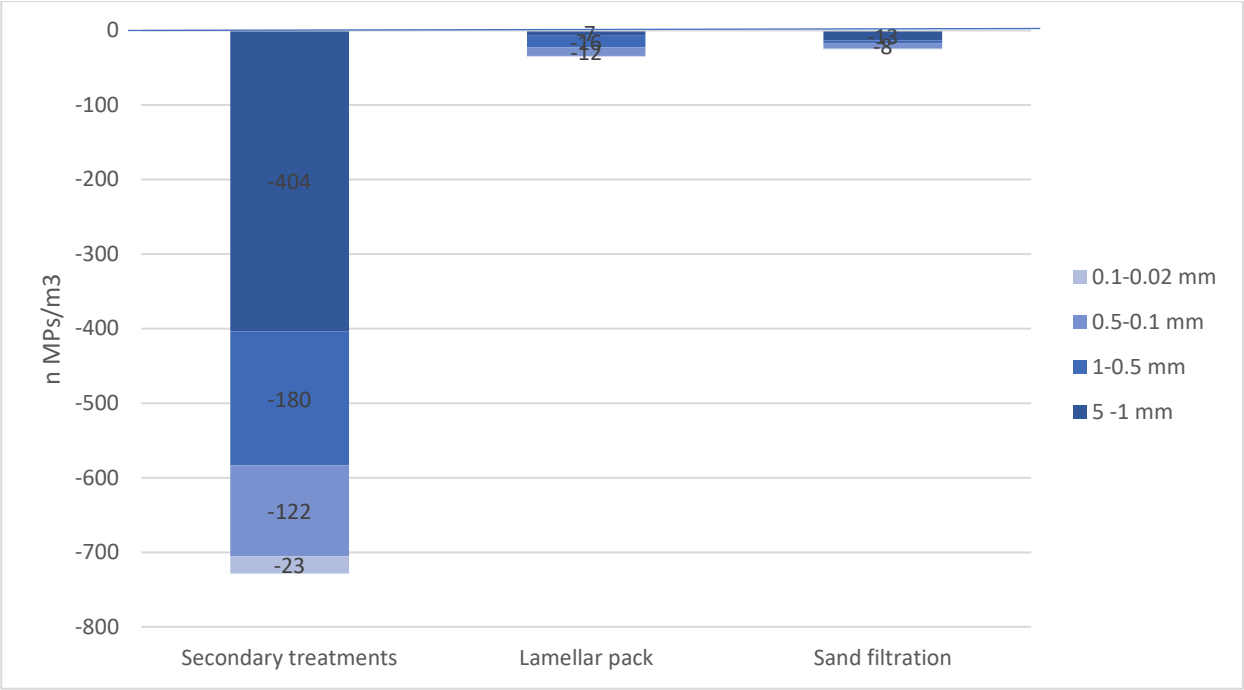


Figure 115: Decrement or increment of MPs in the treatment units of Peschiera del Garda WWTP

Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

5.5 Discussion and concluding highlights

MPs occurrence in drinking water and wastewater treatment plants varies in a wide range, depending on the specific site conditions, but also on the sampling procedures, the equipment used and the characterisation technique. Drinking water and wastewater must be analysed separately, due to the different concentrations expected, the different aims and the different characteristics of the different treatment units. Sampling methods, procedures to take into account the eventual environmental contamination, sampled volumes and mesh-size vary widely in literature research. Moreover, the analysis and the characterisation phases are essential to understand how many of the microparticles detected in the phase of screening were confirmed to be of synthetic origin. This is particularly essential for microfibres, since most of them can be attributed to a natural origin. For these reasons, a harmonised Protocol was first established to define the minimum volumes, the equipment required, the procedures to take into account environmental contamination, the characterisation phases and the results presentation. In particular, significative sampling volumes were considered according to the matrix and the characteristics, the mesh size was constant among all the sampling points, external contamination was eliminated by closed systems or by blank experiments, synthetic fibres were distinguished from natural fibres, and final results accounted only for plastic microparticles and plastic microfibres. Nonetheless, some variations were observed, maybe due to the discrete nature of the MPs, influent fluctuations, site-specific characteristics or environmental conditions. It is important to underline that, considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general

variability observed in the plants, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation. Observing MPs occurrence in drinking water (Figure 116), comparing literature research with the results obtained from the campaigns performed under LIFE Blue Lakes project, it can be noticed that the effluent quality form all the DWTPs analysed were on average lower than the ones detected in literature. Moreover, they were also lower than the concentrations detected in Bottle and tap water found in literature.

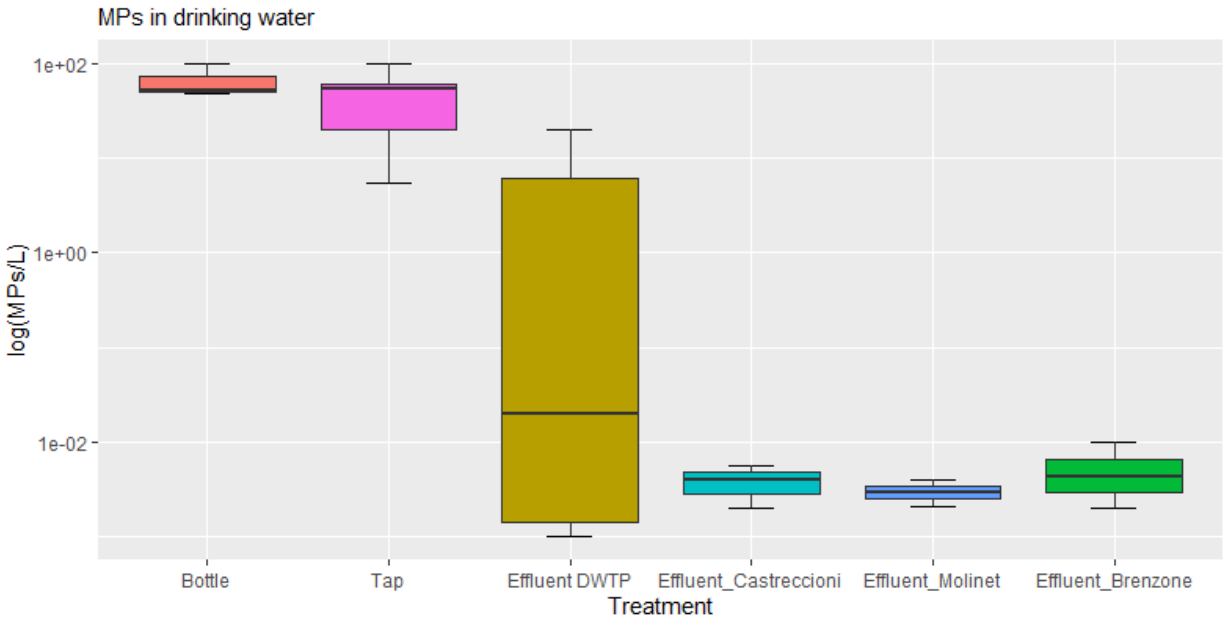


Figure 116: MPs occurrence in drinking water

Analysing the decrements/increments detected in the DWTPs, from Figure 117 it can be observed that globally a general decrement of MPs can be observed after the DWTPs. It must be underlined that, for drinking water treatments, it is not feasible to properly define a removal efficiency, due to the low concentrations detected and the different objectives of the treatment units.



Figure 117: MPs decrement or increment in DWTPs

Figure 118 shows MPs occurrence at the effluent from WWTPs. Both Limone Tremosine and Peschiera del Garda WWTPs could reach lower concentrations than the ones observed in the literature research.

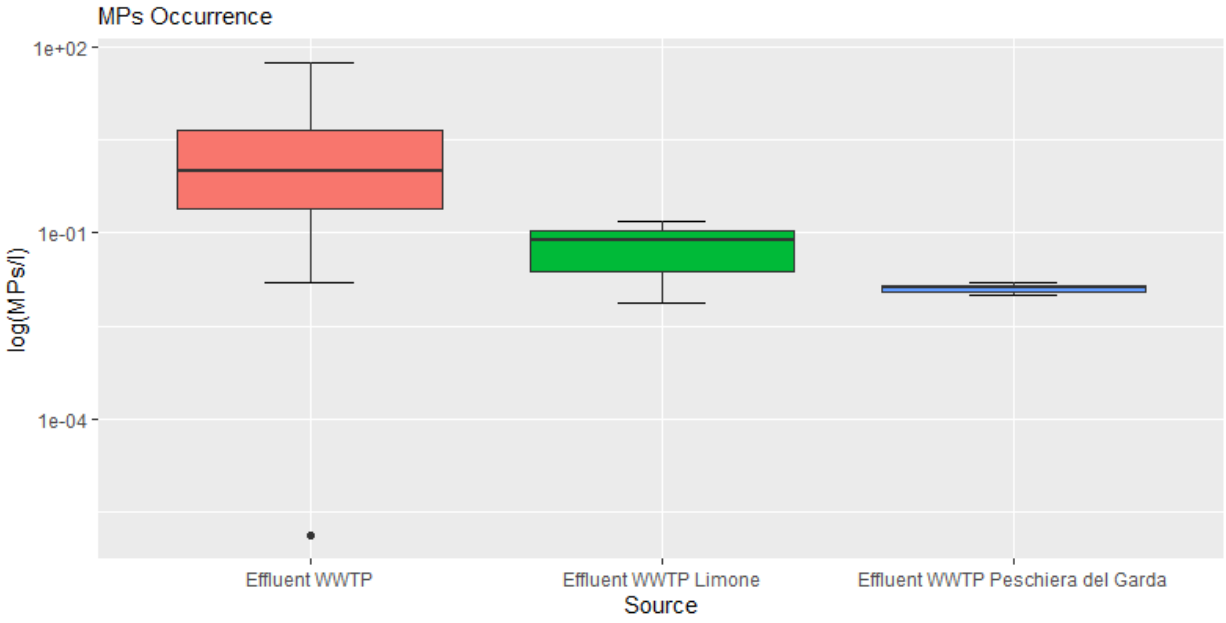


Figure 118: MPs occurrence in effluent wastewater

Analysing the decrements/increments detected in the WWTPs, from Figure 119 it can be observed that globally a general decrement of MPs can be observed after the WWTPs. As for the DWTPs, due to the relatively low values detected in the influent flows, it is not feasible to properly define a removal efficiency. However, it can be observed that WWTPs can anyway decrease the MPs concentrations, with decrements from 50% to almost 75%, on average.

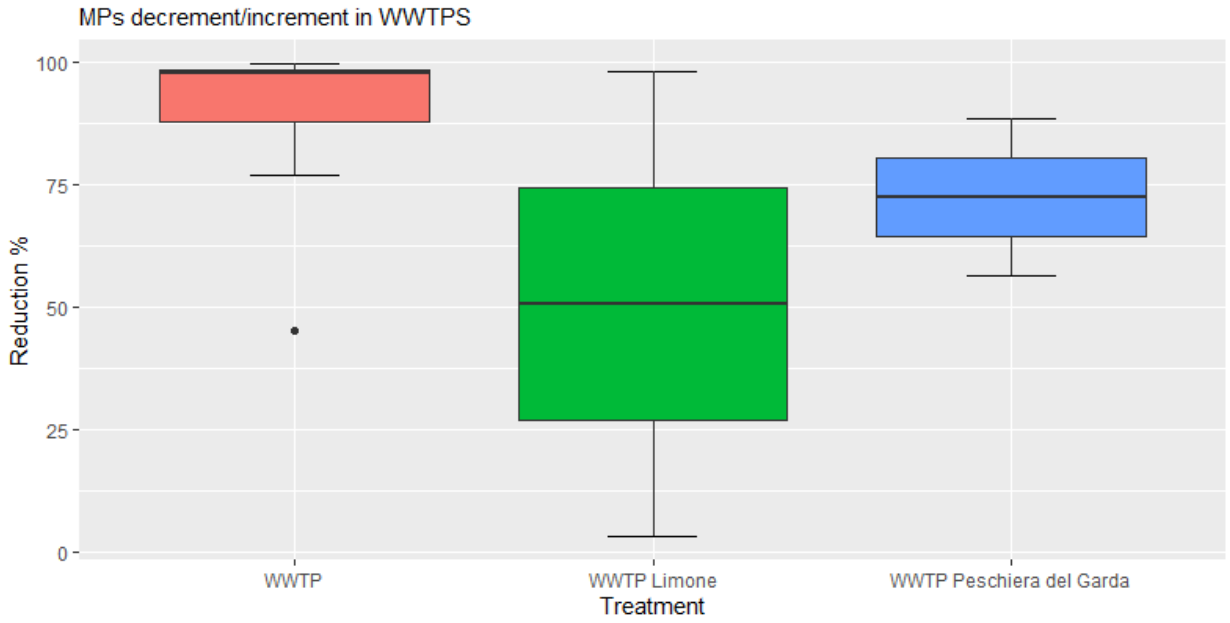


Figure 119: MPs decrement or increment in WWTPs

5.6 Knowledge transfer

The analytical protocol and the results obtained from the sampling campaigns are going to be disseminated among the professionals of the water sector, in order to spread a harmonised approach for MPs measurement and analysis for DWTPs and WWTPs.

To do so, specific training courses are organised to involve professional expertise working in the water sector, to inform and train them on the procedures and techniques for MPs sampling and characterization. The gained knowledge that allowed to define the Analytical and Technical Protocols will allow not only to inform and train about MPs detection and analysis, but also to optimise the design and operations of DWTPs and WWTPs with the goal to improve the removal of MPs from final effluents. The goal is to provide the participants the basics of wastewater and drinking water treatment processes and operative monitoring, optimisation and control mechanisms together with advanced practice in developing and managing technical applications for MPs removal. The courses will provide the participants the principles of water and wastewater treatment processes and operation control measures together with advanced practices in design and operation to improve the MPs control and removal in wastewater and drinking water treatment plants. The techno-educational project is structured to assure the participants the interaction with both academic and industrial/utility professionals, thus allowing theoretical lectures to be coupled with practical experiences. The course will include initial transversal training for all participants and following detailed modules on the basis of different training objectives. In particular, the course will contain two specific teaching modules: one for technical staff and operators employed in wastewater sector and water professionals and another one dedicated to technicians and analysts mainly involved in environmental monitoring and administrative management (i.e., employees from government bodies, environmental authorities, water authorities).

The main topics are structured as follows:

- MPs characterization and sampling in urban water cycle.

- Wastewater and drinking water treatment plants: main processes, technologies and MPs occurrence.
- Removal and fate of MPs in water and wastewater treatment.
- MPs mass balances and abatements in wastewater plant, in combined sewage overflows and in potabilization water plant
- Control and optimization of MPs removal in the different treatment units: management guidelines, optimal design configurations and diagrams for MPs minimization.
- Case studies and practical examples.
- Fundamentals of optimal operation and maintenance of urban water cycle infrastructures.
- Fundamentals of optimal flow scheme configurations in urban water cycle.
- Focus of health and risk associated to MPs and perspective in environmental and technical legislation
- Technical visit to UNIVPM labs.
- Technic and practice session of sampling in Castreccioni DWTP / Jesi WWTP / UNIVPM Pilot Hall in Falconara.
- Technic and practice session of sample processing, sorting and characterisation of MPs at UNIVPM labs.

The course is promoted through the main communication channels and social network, also with the support of Legambiente and Utilitalia, to maximise the diffusion among water professionals.



Figure 120: Leaflet of the first edition of UNIVPM training courses

The first edition is organised in Italy and targeted to Italian water utilities, while a second edition is planned to be targeted for an international audience, especially to German water utilities, since part of the activities of the project are carried out there.

5.7 Monitoring of Cyanobacteria bloom in drinking water

Harmful algal blooms (HABs) are becoming an aquatic stressor of great concern since the anthropogenic perturbations (e.g., climate change and eutrophication) increase continuously in coastal areas (Griffith and Gobler, 2020). Simply, biotoxins that are produced by many HAB species, called phycotoxins, can bioaccumulate in the tissues of bivalve shellfish in time and, when consumed by humans, can cause severe/fatal shellfish poisoning syndromes. Phycotoxins are also a serious threat to marine organisms including marine mammals, fish, sea birds, invertebrates, etc. (Huisman et al., 2018). Furthermore, the presence of toxins from cyanoHABs in drinking water reservoirs may represent serious health risks for the human population (Almuhtaram et al., 2018). The World Health Organization (WHO) set a guideline limit of 1 µg/L for the presence of microcystin-LR, one of the most harmful cyanotoxins known in drinking and recreational waters that inhibits protein phosphatase and causes liver failure and hepatic haemorrhage (WHO, 2020). Moreover, dissolved microcystin can be persistent in water for a long period without any change in its chemical structure, which makes it hard to degrade in natural ecosystems (He et al., 2021). Planktothrix rubescens is one of the most frequently detected cyanobacteria species in Europe with microcystins being the most frequent cyanotoxins in freshwaters (Bogialli et al., 2013). P. rubescens is a red-colored, filamentous cyanobacterium with gas vesicles, which can adjust its buoyancy and position in the water column to obtain its preferred low light environment. This metalimnic species can adapt to low underwater light climate, and usually forms deep-water maxima in the stratified lakes of temperate latitudes (Gallina et al., 2017). This has implications for planning monitoring activities, especially in the case of drinking water supplies, with offtakes typically at depths of some meters (Manganelli et al., 2016).

5.7.1 Materials and methods

The water intake of the DWTP in Castreccioni, which supplies drinking water from an oligo-mesotrophic lake with microcystin levels up to 10.3 µg/L, was systematically monitored over six bloom seasons. The water level of the lake varied between 332 and 343 m sea level during the monitoring period. The physical-chemical parameters were monitored at monthly intervals; while total coliform, E. coli, cyanobacteria, and microcystin were measured 4–5 times a month. Cyanobacteria was measured according to the protocol described in Lucentini and Ottaviani, 2011. Briefly, 1 L water sample taken from the water intake was fixed in Lugol's solution and an aliquot of 25 mL was sedimented and observed after 24 h in the Uterm¨ohl chamber under an inverted microscope for the quantitative determination of the cyanobacteria species. Cell dimensions were calculated using a transmitted light optical microscope with an immersion objective. Microcystin analysis was done via enzyme-linked immunosorbent assay (ELISA). The isolation of single filaments of the cyanobacteria P. rubescens was made from raw water taken from the water intake of the Castreccioni DWTP (water temperature = 10 ◦C, pH = 7.9, turbidity = 0.23 NTU), following the capillary pipette method (Hoshaw and Rosowski, 1973) under an inverted microscope (Zeiss Axiovert 135) equipped with phase contrast, at 200x magnification. The dominant cyanobacteria in the water intake, Planktothrix rubescens, was isolated and enriched to simulate cyanobacterial blooms in the lake.

5.7.2 Results and discussion

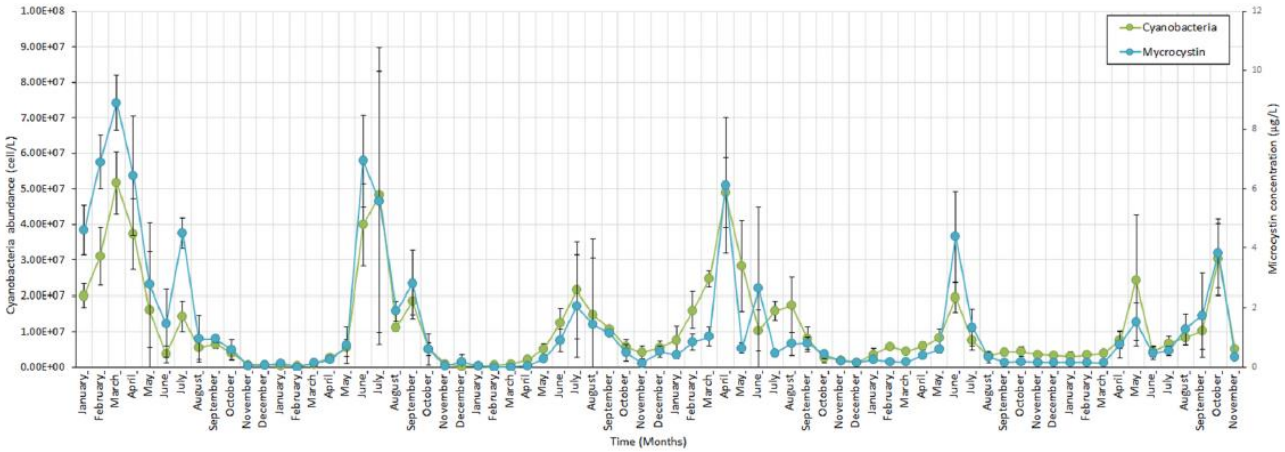


Figure 121: Cyanobacteria and Microcystin monitored trend

The cyanobacteria abundance and microcystin concentrations are presented in Figure 121. Generally, it can be observed that most of the peaks verify in correspondence of in March and April, which revealed a bloom. A second increase is observed in the early autumn. In some of the observed seasons, however, the bloom verified in August.

The historical data showed that the water temperature did not show extreme/large seasonal variation and it was not correlated either with cyanobacterial growth or microcystin concentration. Among all parameters, the cyanobacteria growth was negatively correlated with humidity and manganese and positively correlated with atmospheric temperature. No significant correlation was found between microcystin concentration and the climatic parameters.

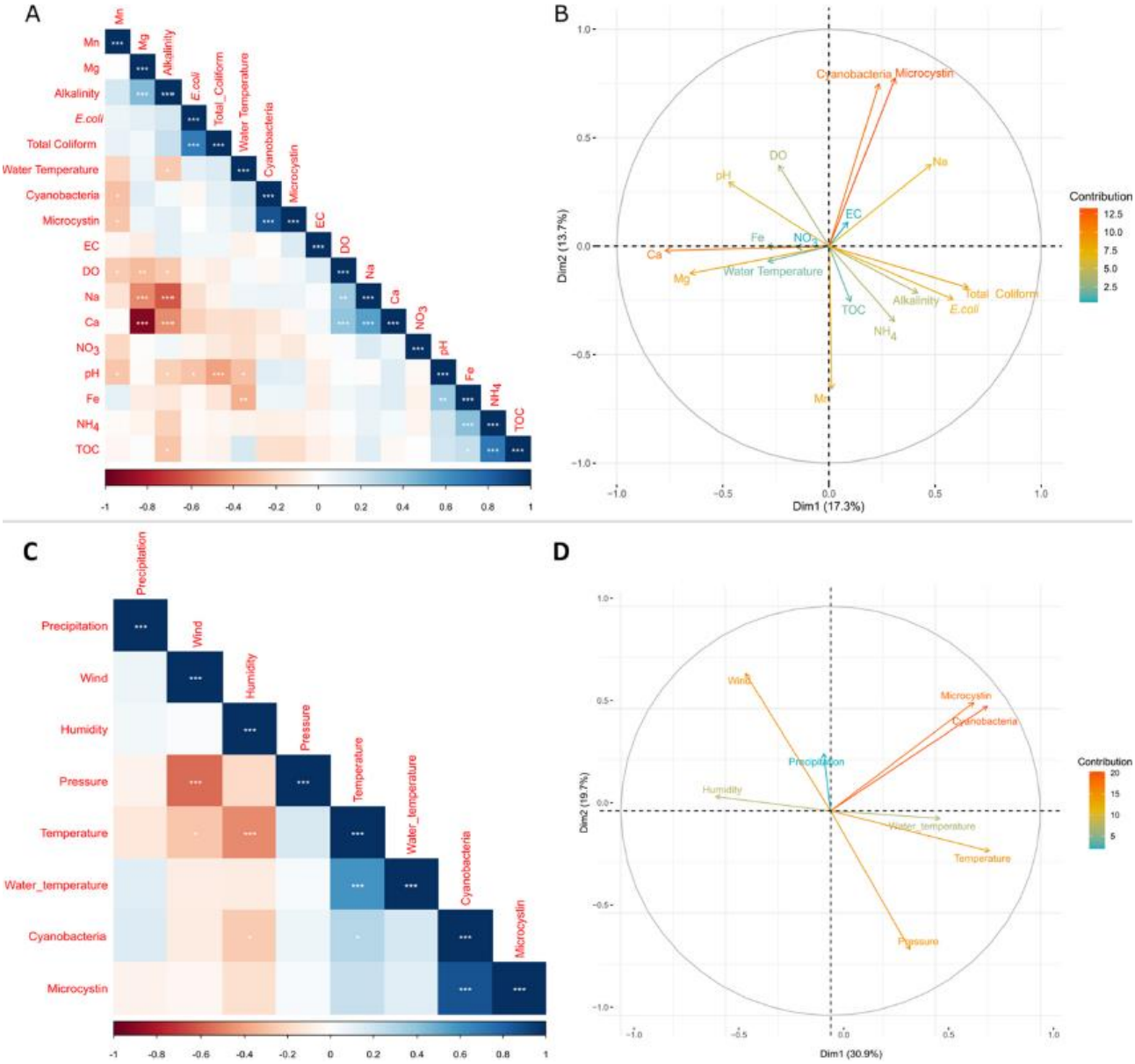


Figure 122: A) Pearson correlation between water quality parameters and cyanobacteria and microcystin occurrence throughout the monitoring period. Positive significant correlations are highlighted in blue while negative significant correlations are highlighted in red. Lower significant positive and negative correlations are highlighted in lighter and lightest blue and red colors, in proportion to the correlation coefficient (*p < 0.1, **p < 0.05, ***p < 0.001). B) **Variable-factor map.** C) **Pearson correlation between climatic parameters and cyanobacteria and microcystin occurrence throughout the monitoring period.** D) **Variable factor map.**

Polymer(chitosan)-enhanced microfiltration (PEMF) and ultrafiltration (PEUF) were further tested as an alternative microcystin removal approach from dense cyanobacteria-rich flows.

The microfiltration was characterised by fibre membrane filters of 0.45 µm, while the ultrafiltration unit consisted in Merck Millipore Amicon™ stirred cells of 10 kDa.

A chitosan solution of 1 mg/mL was added to improve the removal efficiencies of the filtering units. Dosage, mixing velocity and contact time were selected after a series of experiments.

The PEMF and PEUF were separately applied to enriched P. rubescens culture (PC) (microcystin = 1.236 µg/L) as well as to the sand filter backwash water (SFBW) of the DWTP where microcystin concentration was higher than 12 µg/L. The overall microcystin removal rates from the final effluent

of PC (always <0.15 µg/L) were between 90.1-94.7% and 89.5–95.4% using 4 and 20 mg chitosan/L, respectively. Meanwhile, after the PEMF and PEUF of SFBW, the final effluent contained only 0.099 and 0.057 µg microcystin/L with an overall removal >99%. The presented results are the first from the application of chitosan to remove P. rubescens as well as the implementation of PEMF and PEUF on SFBW to remove cyanobacterial cells and associated toxins.

References

Akyol, Ç., Ozbayram, E. G., Accoroni, S., **Radini, S.**, Eusebi, A. L., Gorbi, S., Vignaroli, C., Bacchiocchi, S., Campacci, D., Gigli, F., Farina, G., Albay, M., & Fatone, F. (2021). Monitoring of cyanobacterial blooms and assessing polymer-enhanced microfiltration and ultrafiltration for microcystin removal in an Italian drinking water treatment plant. *Environmental Pollution*, *286*. https://doi.org/10.1016/j.envpol.2021.117535

Almuhtaram, H., Cui, Y., Zamyadi, A., Hofmann, R., 2018. Cyanotoxins and cyanobacteria cell accumulations in drinking water treatment plants with a low risk of bloom formation at the source. Toxins 10. https://doi.org/10.3390/ toxins10110430.

Bogialli, S., Nigro Di Gregorio, F., Lucentini, L., Ferretti, E., Ottaviani, M., Ungaro, N., Abis, P.P., Cannarozzi De Grazia, M., 2013. Management of a toxic cyanobacterium bloom (Planktothrix rubescens) affecting an Italian drinking water basin: a case study. Environ. Sci. Technol. 47, 574–583. https://doi.org/10.1021/es302260p.

Carr, S. A., Liu, J., & Tesoro, A. G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. Water Research, 91, 174–182. https://doi.org/10.1016/j.watres.2016.01.002

Cherniak, S. L., Almuhtaram, H., McKie, M. J., Hermabessiere, L., Yuan, C., Rochman, C. M., & Andrews, R. C. (2022). Conventional and biological treatment for the removal of microplastics from drinking water. Chemosphere, 288. https://doi.org/10.1016/j.chemosphere.2021.132587

Gallina, N., Beniston, M., Jacquet, S., 2017. Estimating future cyanobacterial occurrence and importance in lakes: a case study with Planktothrix rubescens in Lake Geneva. Aquat. Sci. 79, 249–263. https://doi.org/10.1007/s00027-016-0494-z.

Griffith, A.W., Gobler, C.J., 2020. Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. Harmful Algae 91, 101590. https://doi.org/ 10.1016/j.hal.2019.03.008.

Hoshaw, R.W., Rosowski, J.R., 1973. Methods for microscopic algae. Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, pp. 53–68.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16, 471–483. https://doi.org/ 10.1038/s41579-018-0040-1

Johnson, A. C., Ball, H., Cross, R., Horton, A. A., Jürgens, M. D., Read, D. S., Vollertsen, J., & Svendsen, C. (2020). Identification and Quantification of Microplastics in Potable Water and Their Sources within Water Treatment Works in England and Wales. Environmental Science and Technology, 54(19), 12326–12334. https://doi.org/10.1021/acs.est.0c03211

Jung, J. W., Kim, S., Kim, Y. S., Jeong, S., & Lee, J. (2022). Tracing microplastics from raw water to drinking water treatment plants in Busan, South Korea. Science of the Total Environment, 825. https://doi.org/10.1016/j.scitotenv.2022.154015

Koelmans, A. A., Mohamed Nor, N. H., Hermsen, E., Kooi, M., Mintenig, S. M., & de France, J. (2019). Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. In Water Research (Vol. 155, pp. 410–422). Elsevier Ltd. https://doi.org/10.1016/j.watres.2019.02.054

Leslie, H. A., Brandsma, S. H., van Velzen, M. J. M., & Vethaak, A. D. (2017). Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environment International, 101, 133–142. https://doi.org/10.1016/j.envint.2017.01.018

Magni, S., Binelli, A., Pittura, L., Avio, C. G., della Torre, C., Parenti, C. C., Gorbi, S., & Regoli, F. (2019). The fate of microplastics in an Italian Wastewater Treatment Plant. Science of the Total Environment, 652, 602–610. https://doi.org/10.1016/j.scitotenv.2018.10.269

Magnusson, K., & Norén, F. (2014). Screening of microplastic particles in and down-stream a wastewater treatment plant. www.ivl.se

Manganelli, M., Stefanelli, M., Vichi, S., Andreani, P., Nascetti, G., Scialanca, F., Scardala, S., Testai, E., Funari, E., 2016. Cyanobacteria biennal dynamic in a volcanic mesotrophic lake in central Italy: strategies to prevent dangerous human exposures to cyanotoxins. Toxicon 115, 28–40. https://doi.org/10.1016/j. toxicon.2016.03.004.

Mason, S. A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., & Rogers, D. L. (2016). Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environmental Pollution, 218, 1045–1054. https://doi.org/10.1016/j.envpol.2016.08.056

Michielssen, M. R., Michielssen, E. R., Ni, J., & Duhaime, M. B. (2016). Fate of microplastics and other small anthropogenic litter (SAL) in wastewater treatment plants depends on unit processes employed. Environmental Science: Water Research and Technology, 2(6), 1064–1073. https://doi.org/10.1039/c6ew00207b

Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S., & Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Research, 108, 365–372. https://doi.org/10.1016/j.watres.2016.11.015

Mintenig, S. M., Löder, M. G. J., Primpke, S., & Gerdts, G. (2019). Low numbers of microplastics detected in drinking water from ground water sources. Science of the Total Environment, 648, 631–635. https://doi.org/10.1016/j.scitotenv.2018.08.178

Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. Environmental Science and Technology, 50(11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416

Oßmann, B. E., Sarau, G., Holtmannspötter, H., Pischetsrieder, M., Christiansen, S. H., & Dicke, W. (2018). Small-sized microplastics and pigmented particles in bottled mineral water. Water Research, 141, 307–316. https://doi.org/10.1016/j.watres.2018.05.027

Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T., & Janda, V. (2018). Occurrence of microplastics in raw and treated drinking water. Science of the Total Environment, 643, 1644–1651. https://doi.org/10.1016/j.scitotenv.2018.08.102

Pivokonský, M., Pivokonská, L., Novotná, K., Čermáková, L., & Klimtová, M. (2020). Occurrence and fate of microplastics at two different drinking water treatment plants within a river catchment. Science of the Total Environment, 741. https://doi.org/10.1016/j.scitotenv.2020.140236

Simon, M., van Alst, N., & Vollertsen, J. (2018). Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. Water Research, 142, 1–9. https://doi.org/10.1016/j.watres.2018.05.019

Talvitie, J., Heinonen, M., Pääkkönen, J. P., Vahtera, E., Mikola, A., Setälä, O., & Vahala, R. (2015). Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. Water Science and Technology, 72(9), 1495–1504. https://doi.org/10.2166/wst.2015.360

Talvitie, J., Mikola, A., Koistinen, A., & Setälä, O. (2017). Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Research, 123, 401–407. https://doi.org/10.1016/j.watres.2017.07.005

Wang, Z., Lin, T., & Chen, W. (2020). Occurrence and removal of microplastics in an advanced drinking water treatment plant (ADWTP). Science of the Total Environment, 700. https://doi.org/10.1016/j.scitotenv.2019.134520

World Health Organization. (2019). Microplastics in drinking-water.

Yuan, Z., Nag, R., & Cummins, E. (2022). Human health concerns regarding microplastics in the aquatic environment - From marine to food systems. Science of The Total Environment, 823, 153730. https://doi.org/10.1016/J.SCITOTENV.2022.153730

Ziajahromi, S., Neale, P. A., Rintoul, L., & Leusch, F. D. L. (2017). Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. Water Research, 112, 93–99. https://doi.org/10.1016/j.watres.2017.01.042

General conclusions

The thesis in exam aimed to present technical solutions addressed towards a circular approach of urban water systems, approaching the themes of water reuse, safe bathing, sustainability assessment and contaminants of emerging concern.

The theme of water reuse was faced starting from a detailed analysis on risk management plans and assessment methodologies, focusing on the EU Regulation on water reuse and the related technical guidelines. In the context of DWC project, risk assessment for water reuse in Peschiera Borromeo WWTP was performed in progressive steps both with semi-quantitative and with quantitative approaches. Results showed that treatment disinfection units and modern irrigation techniques allowed the reduction of health risks and could be implemented in a multi-barrier approach to reach the acceptability levels suggested by the health organisations. Drip irrigation techniques could reduce health risks for workers and local communities and medium-good treatment performances resulted to be effective to minimise the risks under the acceptability thresholds.

Tests on innovative sensor for microbial determination showed that good levels of performances could be reached in wastewater matrix, with a correlation coefficient of 0.8, bias of -0.35 log_{10} units and a precision of 1.81 log_{10} units, even if in freshwaters performances could reach up to 0.9 of correlation coefficient, -0.003 log_{10} units of bias and from 0.03 to 0.58 log_{10} units of precision.

As a preventive measure for risk minimisation in bathing waters, the development of an early warning system for the prediction of microbial concentration in a Paris bathing site was investigated. ProSe deterministic model was used to obtain simulated data on the future configuration of the sewer system in Paris, while a statistic model was implemented to obtain predictions on *E.coli* concentrations in the bathing site of Pont d'Iena, in the city centre of Paris. Results showed good level of agreement between the two models, with a correlation coefficient of 0.77, meaning that they could be integrated and implemented together for bathing water quality forecasting.

The sustainability of the circular approach to urban water management was evaluated and a methodology for parametric carbon footprint assessment of territorial wastewater service has been developed and tested in four Italian water utilities. It emerged that direct emissions linked to processes and indirect emissions to water body were the most impacting categories, accounting in the range of 17% – 36% and 21% – 71% on the territorial footprint, respectively. Moreover, mitigation strategies such as the use of renewable energy sources could contribute to the reduction of the emissions.

Another supporting tool is represented by the LCA, that was used to evaluate different scenarios and management strategies of Peschiera Borromeo WWTP. Implementing UASB reactor coupled with AnMBR technology in a water reuse scenario, the categories of water depletion and fossil fuel depletion were optimised.

The integrated approach on urban water management and the increased awareness on the associated health risks boosted the research towards the need to investigate on the presence and the impacts of emerging contaminants, such as microplastics. Since a standardised methodology for sampling and characterisation of microplastics in urban waters is still missing, a protocol was

developed and proposed to water utilities for the determination and the quantification of MPs in drinking water and wastewater connected to strategic lake water basins. The presence of MPs in DWTPs were on average in the range of 2 – 11 MPs/m^3, while at the treated effluent concentrations were as low as 3 – 5 MPs/m^3. Accordingly, also in WWTPs the detected MPs concentrations were in the range of 480 – 801 MPs/m^3, while at the treated effluent concentrations were reduced on average at 13 – 78 MPs/m^3. Results confirmed that DWTPs and WWTPs are important infrastructures to reduce MPs presence in environment, even if further research and more continuous monitoring are needed.

Monitoring revealed to be also fundamental for the detection of algal Cyanobacteria bloom in drinking water connected to lake intake.

Future research needs

Current wastewater treatment sector is going to be transformed to be more circular and face the society and environmental demands. Simultaneously, facilities are continuously updating their management using modern digital tools that help them to improve the process efficiency and cost savings. The transformation of the wastewater treatment sector from linear to circular can be boosted by the transition to digitalization, implementation of technologies for water reuse, growing awareness on the safety of reclaimed water, resource recovery and other sustainable practices (e.g., fit-for-purpose approach, fertigation). In this respect, wastewater reuse is a feasible option to reduce water scarcity issues, but it is still an underused practice. Despite its large potential benefits, wastewater reuse is still an under-used practice, also due to the risks associated to the pollutants contained in wastewater effluents. Many wastewater treatment facilities have not been designed to achieve reuse quality standards or consider CECs removal. Consequently, from a technical point of view, current WWTP should be implemented with new reclaimed water facilities and with the most convenient water reuse technologies that produce high-quality and safe reclaimed water with the lowest treatment costs and environmental impacts.

In recent years, modelling development gave a great contribution for the estimation of WWTP footprints, providing support tools for decision-making processes. However, a comprehensive tool that considers all the sectors of the WEFC nexus is still missing. In addition, besides several nexus studies at wide scale, ranging from global to regional to national, but with very coarse resolution, local specificities must be addressed when designing effective management plans because international and regional decisions have a direct effect at the local level.

Significant energy and resource recovery can be achieved in wastewater treatment and recycling as well as in sludge management using innovative technologies. However, the challenge here is to integrate WEFC nexus in the urban and peri-urban context considering all their key components as a whole. Although some remarkable efforts have been given to develop a large variety of models and tools to assess climate change impacts and ecosystem services and to provide relevant information to policy makers at a range of different levels, there is also a need for tools that focus on the holistic and integrated assessment of all sectors in the nexus. The nexus concept is indeed critical for achieving adequate water, energy and food securities which are naturally interconnected, but currently still regulated and governed by traditional sectorial approaches.

Annex: scientific publications related to the thesis

In this Annex, published papers linked to the PhD activity are reported, according to the order of appearance in the thesis.

- **Radini, S.**, Marinelli, E., Akyol, Ç., Eusebi, A. L., Vasilaki, V., Mancini, A., Frontoni, E., Bischetti, G. B., Gandolfi, C., Katsou, E., & Fatone, F. (2021). Urban water-energy-food-climate nexus in integrated wastewater and reuse systems: Cyber-physical framework and innovations. *Applied Energy*, *298*. https://doi.org/10.1016/j.apenergy.2021.117268
- Marinelli, E., **Radini, S.**, Akyol, Ç., Sgroi, M., Eusebi, A. L., Bischetti, G. B., Mancini, A., & Fatone, F. (2021). Water–energy–food–climate nexus in an integrated peri-urban wastewater treatment and reuse system: From theory to practice. *Sustainability (Switzerland)*, *13*(19). https://doi.org/10.3390/su131910952
- Foglia, A., Andreola, C., Cipolletta, G., **Radini, S.**, Akyol, Ç., Eusebi, A. L., Stanchev, P., Katsou, E., & Fatone, F. (2021). Comparative life cycle environmental and economic assessment of anaerobic membrane bioreactor and disinfection for reclaimed water reuse in agricultural irrigation: A case study in Italy. *Journal of Cleaner Production*, *293*. https://doi.org/10.1016/j.jclepro.2021.126201
- Marinelli, E., **Radini, S.**, Foglia, A., Lancioni, N., Piasentin, A., Eusebi, A. L., & Fatone, F. (2021). Validation of an evidence-based methodology to support regional carbon footprint assessment and decarbonisation of wastewater treatment service in Italy. *Water Research*, *207*. https://doi.org/10.1016/j.watres.2021.117831
- Akyol, Ç., Ozbayram, E. G., Accoroni, S., **Radini, S.**, Eusebi, A. L., Gorbi, S., Vignaroli, C., Bacchiocchi, S., Campacci, D., Gigli, F., Farina, G., Albay, M., & Fatone, F. (2021). Monitoring of cyanobacterial blooms and assessing polymer-enhanced microfiltration and ultrafiltration for microcystin removal in an Italian drinking water treatment plant. *Environmental Pollution*, *286*. https://doi.org/10.1016/j.envpol.2021.117535

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Urban water-energy-food-climate nexus in integrated wastewater and reuse systems: Cyber-physical framework and innovations

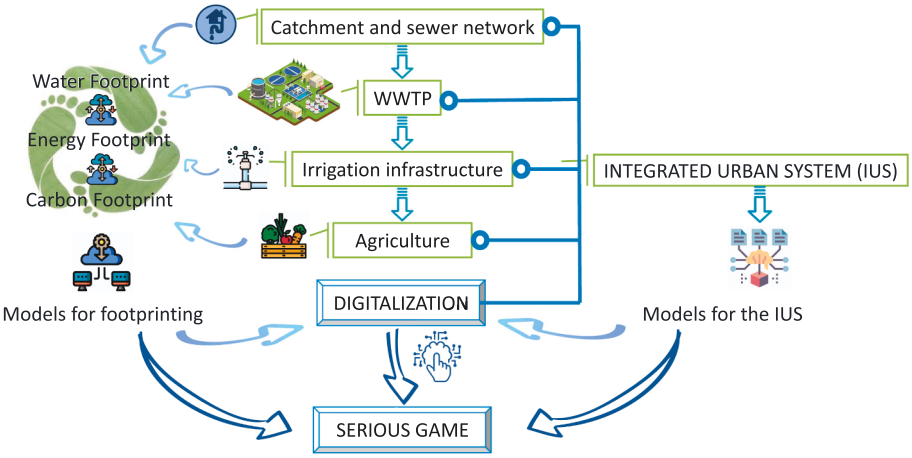
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HIGHLIGHTS

- WEFC nexus in urban/peri-urban reuse systems can support energy transition policy.
- Models and tools demonstrate decision support systems based on multi-sources data.
- A comprehensive tool that considers all the sectors of the WEFC nexus is still missing.
- Digital solutions can reduce the fragmentation of knowledge across the water value chain.

GRAPHICAL ABSTRACT



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Water-energy-food-climate nexus

ABSTRACT

Wastewater treatment is one of the major carriers of the water-energy-food-climate (WEFC) nexus, and although the relationship between water and energy is well recognized, there is still a lack of adequate analysis of the cyber-physical framework to address and assess urban and peri-urban WEFC nexus in an integrated approach. In this review paper, we deeply analyze and summarize the modelling tools and data that are currently used to quantify the nexus in wastewater treatment. Currently, comprehensive models and tools are missing that consider the interconnections amongst catchment, sewer network, wastewater treatment plant (WWTP), river and climatic system in a holistic approach and define relevant monitoring requirements and trustable

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information provision. Cyber-physical systems provide a technological ground for an efficient management of such integrated systems. The nexus approach in precision irrigation and smart agriculture is further discussed in the paper, highlighting the issue of water reuse and the engagement of different levels of stakeholders. Digital solutions and serious games addressing the nexus in urban and peri-urban water management are also presented to facilitate innovative practice aspects and to foster public involvement. Adaptable digital solutions can help to understand stakeholders' perception of water quality and its governance and to improve levels of awareness and collaboration between utilities, authorities, farmers and citizens. Finally, recommendations on the added value of currently used models, tools and possible digital solutions are given to WWTP and reclamation managers and/or operators to bring the WEFC nexus approach on the operative environment.

1. Introduction

There is a high interconnection between water and energy. Water is needed for most stages of energy production and transmission, and energy is crucial for water provision and treatment. This fundamental resource relationship is called the water-energy (WE) nexus [1]. The WE nexus assessment approach mainly focuses on the linkages between water and energy, but also on the network with other fields such as water-energy-food (WEF), water-energy-food-climate (WEFC), water-energy-pollution (WEP) and water-energy-food-ecosystem (WEFE) [2]. In fact, the challenges facing the secure supply of each sector are highly interconnected, mostly because of the high energy and water use in food production. In addition, there is the engaged connections of these three sectors with the broader ecosystems and climatic conditions [3].

Fragmented management of interrelated sectors as well as their consideration in isolation from the natural system hide a potential risk of not achieving the desired outcomes. This is caused by underestimated or even unexplored synergies, antagonisms and ripple effects. The shift towards integrated management (WEFC approach) requires a shift towards integrated approaches for systems analysis and assessment as well. So, the aim should be at using WEFC nexus, targeting the holistic systems analysis and revealing the multiple and complex interactions and feedback loops between technical and biological cycles. A multi-sectoral system analysis framework symbiotically managing different resources (e.g. water, energy) is needed for modelling both socio-economic (e.g. food) and non-economic (e.g. ecosystems, climate) sectors to investigate the complex interactions and interdependencies between the sectors in order to promote synergies and reduce antagonisms between them [4].

The WEFC nexus can be defined as a systematic approach on scientific investigation and design of coherent policy goals and instruments that focuses on synergies, conflicts and related trade-offs emerging in the interactions between water, energy, food and climate at bio-physical, cyber-physical, socio-economic, and governance level [5]. The assessment and optimization of the WEFC nexus is complex since it involves many variable factors with different consequences for the users of each resource (water, energy and land). Economic development requires energy, water, and land resources consumption. Agricultural practices for food production need considerable amounts of inputs, such as water, land, electricity, fertilizer, pesticides, as well as agricultural machinery, and cause major environmental changes, such as pollution and stresses on primary sources (Fig. 1). Hence, sustainable management of water, energy, primary resources and land use by balancing economic development and environmental pollution implies the characterization of WEFC nexus. A multi-objective analysis and programming is needed to better understand the interrelationships between the components of WEFC nexus, that are generally nonlinear and hard to quantify [6].

While the worldwide urbanization phenomenon is still on-going, about half of the world population still lives in rural areas. In the EU, about 30% of the population of former Central and Easter European countries (42 million people) lives in settlements of less than 2000 inhabitants, while this percentage is lower than 20% in the western part. Many other areas of the world still exhibit strong rural or peri-urban characteristics [7]. For instance, approximately 768 million people in China held rural status in 2010, corresponding to 57% of the total population. However, the domestic wastewater treated was only about 11% in provincial towns and less than 1% for rural villages [8].

In most rural areas of developing countries, the supply of water, energy and food are limited, which results in rural populations turning to natural systems for basic survival. As the nexus approach is mainly concerned with integrating these three connected resources, the approach is envisaged to provide pathways that transform rural livelihoods and ensure their resilience [9]. Given the huge differences between developed and developing countries in political structures, socio-economic conditions and financial capacities, the replication of a developed country's wastewater management structure is not appropriate nor viable in developing countries. Most of the developing countries suffer from political interference in environmental decisions such as site selection and other aspects related to construction and operation [10]. While many developing countries cannot afford expensive and complex WWTPs to construct and operate, this also becomes a challenge in rural areas with low population density [11]. Despite the lack of water and funding for a proper centralized treatment, decentralised systems are often common in small communities in developing countries. The implementation of a decentralized structure provides a long-standing and effective and cost-efficient solution for these communities [11,12].

The characteristics of water treatment and reuse systems are site specific and usually very different between rural and urban areas as well as between developed and developing regions. Rural areas, where non-point agricultural runoff dominates wastewater production and sewage systems are mainly absent, may require decentralized treatment systems for small communities that are rather limited potential for intentional

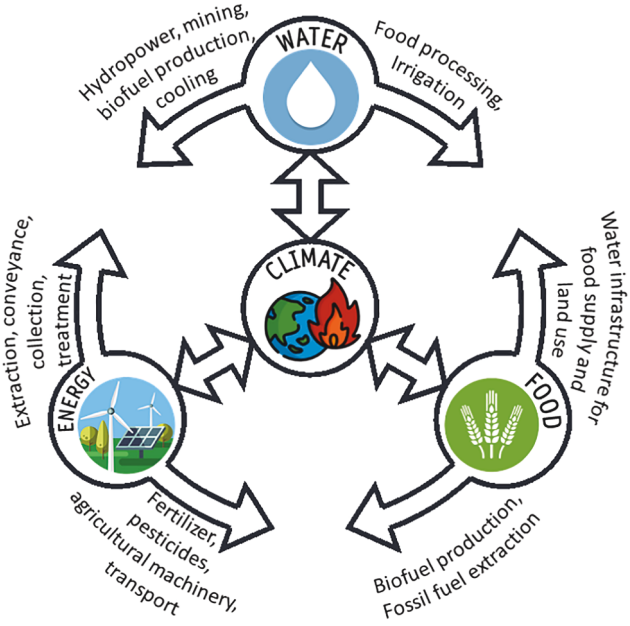


Fig. 1. Water-energy-food-climate (WEFC) nexus.

reuse mainly focused on agriculture. On the other hand, urban areas may rely on a combination of decentralized and centralized treatment plants, depending on the degrees of agglomeration and centralization of waterworks. Urban areas, therefore, have larger potential for intentional reuse, as smaller distances and existing sites for pipelines can more easily connect the locations for treatment and reuse. In fact, decentralised water reuse systems are attracting more interest in urban areas due to their modular design that can be implementable near the source of generation such as households, high-rise buildings, or parts of a city in response to demand [13]. In a recent study by Landa-Cansigno et al. [2], the impact of centralised and decentralised water reuse strategies was assessed in an integrated urban water system using integrated assessment framework of urban water metabolism and the WEP nexus. The authors found that decentralised water reuse strategies perform the best with respect to potable water saving, reductions of eutrophication and GHG emissions, while centralised strategies can provide the largest savings of energy use.

When addressing the water-related nexus, infrastructures are the key interfaces of urban resource use connecting production to consumption, energy to water and land use [14]. In the context of traditional management of urban water systems, all components are considered to be independent of each other in a fragmented manner. The Integrated Urban Water Management (IUWM); on the other hand, has been defined in model development, with integration of the whole system without neglecting important physical phenomena in each component and their interactions [15]. In sustainable IUWM, a significant effort needs to be undertaken to improve water and energy use efficiency, which can further help to reduce their associated environmental impacts [16]. At this point, ensuring cost- and resource-efficiency of wastewater treatment is important in order to ensure the reliability and financial sustainability of the service. WWTPs are great energy carriers and responsible for up to 26% of the GHG emissions of the whole water supply chain [17]. From an environmental and economic point of view related to climate change, any reduction in energy consumption and associated with GHG emissions is valuable [18]. Furthermore, nutrients and reuse potential of reclaimed water are valuable and can be reutilized in urban agriculture as a potential strategy to provide local communities. Hence, integrated wastewater treatment and reuse systems are a typical case of WEFC interactions both at city and farm levels, bridging the gap between food and climate sectors. The impact of water sector is expected to gain more importance in the future due to different factors such as more stringent water quality standards, increasingly urbanized population, higher living standards, more stringent energy efficiency requirements and climate change mitigation plans. Many of these needs are already acknowledged by the European legislations, in particular within the Water Framework Directive 2000/60/EU, Energy Efficiency Directive 2012/27/EU [19] as well as in the new EU Regulation on Minimum Requirements for Water Reuse 2020/741. In this regard, several methodologies are developed to quantify the nexus in urban wastewater and reuse systems using different footprinting tools and models (i.e. carbon, energy, water). In addition to individual footprinting approaches, where considerations on other system(s) are incorporated into the optimisation of a main system through footprint data, the aim of integrated footprints is to co-optimise multiple systems, considering these systems in objectives and decision variables, and incorporating similar levels of detail for their structural and/or behavioural characteristics [3].

Cyber-physical systems (CPS) can be used to respond to the needs of smart wastewater and reuse management. CPS is an emerging framework to control physical systems using digital tools (e.g., sensors) based on information and communication technologies [20,21]. The implementation of digital solutions, such as innovative sensors or knowledge- and data-driven analysis, can help to support decision making based on multi-sources data platforms, including real-time quality and quantity monitoring of wastewater treatment. In a typical CPS of an IUWM, subsystems are designed as complex network of interacting elements that are usually operated by different utilities. Therefore, the interoperability of these integrated systems is a major challenge [22].

This review on the WEFC nexus discusses existing tools and models that have been used to investigate the interdependencies and interlinkages between the different sectors of the WEFC nexus but also between the sectors and the natural environment while placing wastewater treatment at the center. The added value of this paper is that it further connects the WEFC nexus with smart agriculture and digitalized solutions in a more innovative platform with upgraded perspectives. The following sections were conceptualized as follows: The nexus concept in wastewater treatment is initially addressed and followed by the footprinting tools and models that are frequently used to quantify the nexus in WWTPs within the urban scale. Then, the nexus in precision irrigation and smart agriculture is discussed focusing on the reuse of treated water from urban wastewater treatment in peri-urban areas. Digital solutions and serious games addressing the WEFC nexus are further evaluated as an alternative approach for smart management to raise awareness and to reduce the fragmentation of knowledge across the water value chain amongst relevant end-users. In the last section, possible research and application routes are recommended to bring the nexus perspective to operators. Finally, main highlights are brought together with the concluding remarks.

2. Nexus in wastewater treatment

An urban water management considers water supply, wastewater treatment and disposal as an integrated system. The increasing demand for good quality water brings higher necessity for water extraction, conveyance, treatment and discharge, which in turn demands more energy use and associated greenhouse gas (GHG) emissions. More specifically, the main processes in urban water systems that consume the major share of energy are extraction, conveyance and storage, raw water treatment and distribution to consumers, pumping and wastewater collection treatment and disposal.

Urban water, in particular wastewater treatment, is often evaluated in the framework of the WE nexus [16,23] or WEF nexus [24,25] as well as considering climatic effects in terms of GHG emissions [26] as discussed latter, while often neglecting the whole picture. Today, many technologies are available to recover energy and resources (e.g., water, nutrients) from wastewater. The reuse in agricultural irrigation is the most common end-use for reclaimed water [27], which is discussed in Section 4. Recovered nutrients from wastewater can be valorised as soil amendments or fertilizers. Alternatively, nutrient-rich effluents can be useful for fertigation purposes [28]. When effectively designed and operated, wastewater can generate positive externalities through by-products like fertilizer and energy [29]. For instance, Gondhalekar and Ramsauer [30] applied the urban WEF nexus for climate change adaptation in a neighbourhood of Munich, Germany. The authors provided 26% saving of current freshwater supply by wastewater recycling and reuse coupled with rainwater harvesting as well as 66% of local demand for fruit and 246% of local demand for vegetables by intensive urban agriculture. On the other hand, energy-intensive nature of wastewater treatment and discharge creates an important part in sustaining urban water systems [31]. WWTPs are often considered great energy consumers and a typical case of the WE interactions as illustrated in Fig. 2. Wastewater treatment stages, such as the collection and conveyance of wastewater, physical and chemical treatment, biological treatment, disinfection, sludge treatment and disposal, require considerable amount of energy [23]. A conventional WWTP spends approximately 25 40% of its operating costs on energy consumption [32,33]. This energy demand takes place onsite (i.e. electricity required for pumping and aeration), and offsite (production and transportation of materials/chemicals) [34]. Regional and local characteristics are also highly effective on the urban nexus. In many European countries, domestic and industrial water cycles account for 1 3% of total electric energy consumption, and can reach up to 4 5% when water

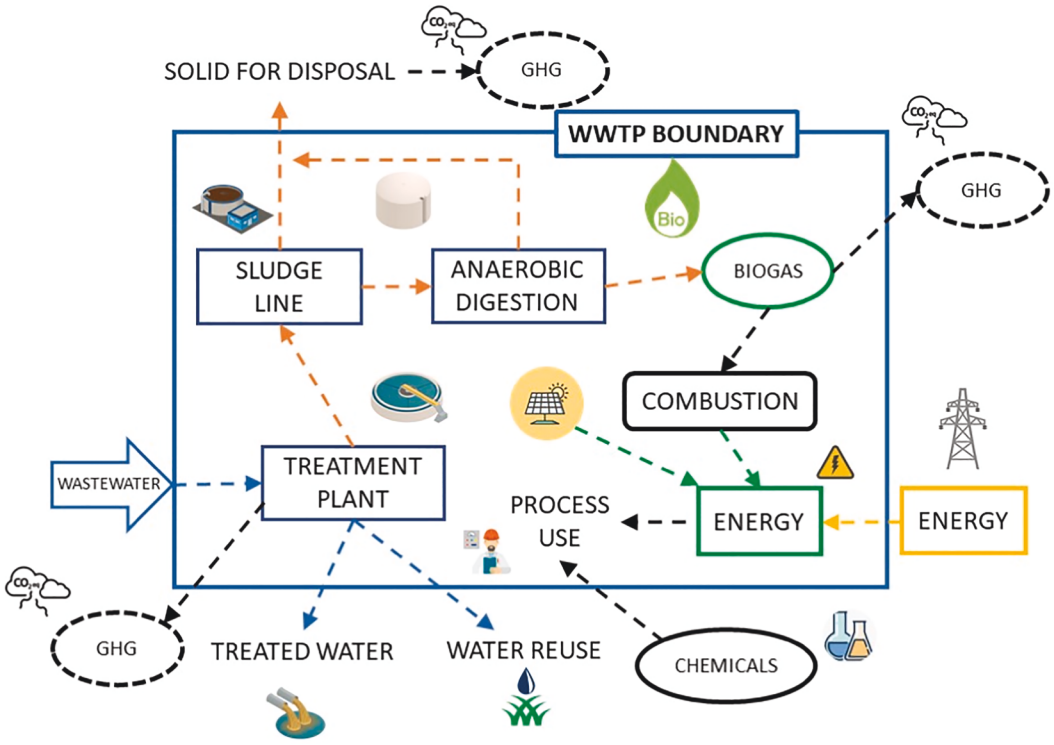


Fig. 2. A simplified scheme of the WEFC nexus in a wastewater treatment and reuse system.

management and agricultural demand are also included [34-36]. Other sources indicate 0.3–2.1 kWh/m^3 of power consumption for treated wastewater in the EU, and 0.41 to 0.87 kWh/m^3, in the U.S., depending on type of treatment, plant size and topography, etc [37].

Other criteria for the energy demand of a WWTP include the plant size, type of treatment process (e.g. aerobic or anaerobic), desired effluent quality, etc. [33]. The treatment scale and treatment process are key parameters influencing energy consumption in a WWTP. A conventional municipal WWTP commonly consists of primary, secondary, and tertiary (advanced) treatment stages. The wastewater collection and treatment are less energy intensive at the primary stage compared to the subsequent stages. The energy consumption for secondary treatments vary, depending on the technologies used and the geo-political context. For example, the average energy input of conventional activated sludge (CAS) treatment systems can assume values of 0.46 kWh/m^3 in Australian WWTPs, 0.269 kWh/m^3 for China, 0.33–0.60 kWh/m^3 for USA, and 0.30–1.89 kWh/m^3 for Japan [33,38]. Aeration system is the main contributor to the energy-consumption in the CAS treatment process. In the majority of medium and large WWTPs with CAS treatment, aeration is responsible for 50–60% of the total electricity consumption. In terms of other processes, sludge treatment contributes to 15–25% of the plant energy consumption, while the energy demand for secondary sedimentation is 15% including recirculation pumps [39]. Wastewater treatment with the use of membrane processes usually results the highest amount of energy consumption. Typically, the energy consumption of membrane bioreactors (MBR) are in the range of 0.37–2.5 kWh/m^3 [33].

Sludge treatment is indeed an energy-intensive process. The results from a research work conducted in 10 WWTPs in Greece, with an overall capacity of 15,000–4,000,000 population equivalents (PE), showed that sludge treatment units accounted for about 8% of the total energy consumption [39]. The final destination of treated sludge also influences the overall energy consumption. For instance, more than 14% of the sewage sludge is incinerated in many European countries, while the USA and Japan incinerate over 25% and 50% of the sewage sludge, respectively [40]. A research conducted in 985 Japanese municipal WWTPs demonstrated that the specific energy consumption of WWTP configurations applying CAS treatment coupled with sludge incineration was 0.38–1.49 kWh/m^3, which could be lower in plants using CAS system without incineration. Moreover, specific energy consumption of advanced wastewater treatment was between 0.39 and 3.74 kWh/m^3 [41]. Although the data on advanced wastewater treatment did not consider whether incineration is adopted or not, it seems that the incineration and advanced treatment process cause a relatively higher energy consumption in municipal WWTPs. A comprehensive assessment was performed on sludge processing in reference and upgraded WWTPs [42] showing that separate processing of primary sludge (wet oxidation) and secondary sludge (intensive stabilization processes) reduced the total sludge production by 45–49%. Innovative technologies can improve sludge management, while the same authors reported that dewatered sludge decreased from 45 to 56 g SS/(PE × day) in conventional plants to 14–49 g SS/(PE × day) in the upgraded ones.

Power consumption depends not only on the design and technology but also on the plant size (PE, organic, or hydraulic load). In general, the smaller the facility, the higher its specific power consumption, where the unit energy consumption is in reverse proportion with wastewater inflow. In the study of [41] showed a decrease in energy consumption from 2.07 kWh/m^3 to 0.44 kWh/m^3 for secondary treatment in oxidation ditch for inflow volume ranging from 100 m^3/d to 8500 m^3/d. An increase on the inflow from 600 m^3/d to 283,000 m^3/d, is accompanied by energy consumption varying in the range of 1.89–0.30 kWh/m^3 in a CAS process without sludge incineration [33].

Approximately 1% to 2% of the overall energy requirement is estimated to be attributed to wastewater utilities in a country level. Based on the report published by the US EPA, the energy requirements for wastewater treatment in the US is expected to increase by 20% in the next years; in parallel to what is expected in other developed countries [43]. In this way, more pressure is put on the climate considering the increased demand for energy in the form of electricity that promotes GHG emissions [44,45]. In fact, the compliance with the Water Framework Directive requirements in the UK wastewater utilities is expected to result in more than 110,000 tonnes of CO_2 emissions annually only

because of the increase in operational energy requirements [46]. According to the Energy Efficiency Directive, it is obligatory for large - water utilities (250 employees, income 43 million or yearly trading volume 50 million) to perform energy audits. For instance, a water utility in the UK paid £5.9 million to the Carbon Reduction Commitment in 2012 due to carbon emissions from energy consumption in addition to the annual electricity [47]. The water utilities endeavour significantly to be able to cope with the increasing costs and climate change challenges.

In the last decade, wastewater sector started to recognise wastewater as a resource and makes effort to recover materials and energy [48]. There is an increasing number of studies on WWTPs that develops innovative technologies for efficient recovery of valuable resources, such as chemicals, nutrients, bioplastics, enzymes and water [49 51]. It is estimated that wastewater contains 1.3 MJ/person/day (6.5 MJ/L) in terms of chemical energy, 20% of the phosphorous consumed and 10%-30% of nitrogen needed in agriculture. In this regard, economic, environmental and industrial incentives take place in the circular economy concept of the water sector [52].

In recent years, great efforts have been made to promote the sustainability of WWTPs via two main approaches: improving energy efficiency and resource recovery [53]. Electricity consumption is not considered the most significant contributor to the operational carbon footprint during the wastewater treatment. Wastewater treatment processes are highly energy demanding, with high operational cost, and significant sources of GHG emissions, whose reduction has been recently mandated by the EU and other countries policies [54] and raised as global concern [55,56]. CH_4, N_2O and CO_2 are the main GHGs emitted in WWTPs. Emissions are categorized as direct, if they are released during treatment processes, such as biologic treatment, sludge processing or biogas combustion. Emissions due to energy and chemical consumption, transport, waste disposal and treated water discharge are referred as indirect, because even if they are not emitted inside the plant boundaries, they are dependent on WWTPs activities or management decisions. In recent years, particular attention has given to N_2O emissions, as its Global Warming Potential (GWP) is 265 times higher than CO_2 [57]. The US EPA estimated that wastewater sector was responsible for 5% of global non-CO_2 GHG emissions in 2005, with a predicted increase by 27% by 2030 [58].

N_2O is also the most significant contributor to ozone depletion [59]. The main processes that contributes to N_2O formation in a WWTP include activated sludge systems and autotrophic oxidation of ammonia to nitrite/nitrate under aerobic conditions (nitrification/nitritation). It is also an intermediate during the reduction of nitrate/nitrite to nitrogen gas (heterotrophic denitrification/denitritation) by heterotrophic denitrifying bacteria under anoxic conditions. In general, biological nutrients removal (BNR) processes applied at WWTPs have different design and operation approaches such as varying number of compartments/zones for nitrification and denitrification, recirculation flows, flow-patterns, operation mode etc. It was reported that the contribution of direct N_2O emissions during biological nutrients removal to the operational carbon footprint of WWTPs can be up to 78% [60].

Another source for significant N_2O emissions is the biological treatment of high-strength wastewater streams. The anaerobic supernatant is formed during the treatment of primary and secondary sludge via anaerobic digestion following sludge dewatering. This stream is small in volume (1 2% compared to the mainstream line), but very concentrated in nutrients and is conventionally recycled back to the primary treatment. In this regard, biological technologies (i.e. partial-nitritation anammox) have been developed to treat side-stream high-strength streams in a cost and energy efficient way [61]. In these processes, favourable condition for N_2O generation can prevail (i.e. NO_2 accumulation, elevated NH_4 concentrations etc.). Schaubroeck et al. reported that biological processes treating high-strength streams can contribute to the total direct N_2O emissions by over 90% compared to the mainstream BNR processes [62].

N_2O emissions originated from biological nitrogen removal in WWTPs were extensively studied [63 65]. The outcomes of these studies pointed out that environmental and operational parameters (e.g. dissolved oxygen (DO), NH_4, NO_2, pH etc.) highly influence the magnitude and generation mechanisms of N_2O at WWTPs. Overall, N_2O emission dynamics vary temporally [66,67]. N_2O emission factors (EFs) estimated during monitoring campaigns in mainstream full-scale biological processes range between 0.0025% and 5.6% of the total nitrogen (TN)-load [17]. Vasilaki and colleagues [17] critically reviewed the quantified N_2O EFs in full-scale WWTPS in the last decade. Their survey showed that there is a significant variability in the monitoring strategy and sampling duration and frequency between different studies that can eventually impact the amount of the quantified N_2O EFs. For instance, discontinuous (i.e. via grab-samples) and continuous (i.e. via chambers and gas analysers) monitoring of full-scale wastewater treatment process yielded an average EF equal to 0.44% and 1.2% of the N-load, respectively. Additionally, Daelman and co-authors [60] suggested that short-term campaigns cannot accurately determine annual EF in the target system and the underestimation of actual emissions is highly possible. The relative error of the estimated annual N_2O emissions ranged between 22% and 35% (95% of the cases) by simulating a 50-days long N_2O sampling campaign.

Over the past few decades, energy recovery from wastewater has increased [68,69], and also been developed and applied in WWTPs. In fact, carbon neutrality and serious reduction in operation costs, and energy consumption can be achieved in energy self-sufficient WWTPs [33,69,70]. Up to date, many models and tools have been developed and integrated to determine the footprints of the WWTPs as discussed in the following section. These methodologies can help to take an action towards a more sustainable and energy-efficient urban wastewater management.

3. Modelling tools and data currently used to quantify the nexus

WWTPs generate significant amounts of operational data originated from heterogenous sources such as sensors and laboratory analyses. In order to comply with regulatory discharge requirements, WWTPs have been using various monitoring techniques for so long to reduce pollution risks and control/optimise treatment processes. Several process parameters such as temperature, pressure, flowrate, DO are conventionally monitored online in WWTPs. SCADA systems or remote-control tools are often applied to collect and manage all the data acquired online. Several studies have applied data-driven techniques to the variables conventionally monitored in WWTPs to predict latent variables and key performance metrics that cannot be quantified via sensors; more detailed overview of this topic can be found in another study [71]. Commonly-used data-driven methods for the classification and regression practices are based on (i) linear methods for dimensionality reduction and regression, (e.g. principal component regression (PCR), partial least squares regression (PLS)) and (ii) non-linear supervised learning techniques (e.g. artificial neural networks, support vector machine (SVM) methods) [71,72].

The optimisation of the performance of WWTPs can be possible by using multivariable and multi-objective approaches that enables the correlation of energy consumption or operational costs with system performance [73,74]. For instance, the benchmark simulation model 2 (BSM2) was combined with Life Cycle Assessment (LCA) to evaluate the sustainability of different operating strategies [75,76]. The key features, development steps, calibration, validation, optimisation and uncertainty to develop integrated urban water models were reviewed elsewhere [15]. In addition, the data limitations in WEF nexus methodology, methods, and tools were addressed [77].

Overall, the type of data required for a holistic assessment of the WEFC are:

fields within natural systems with very good protocols and metadata (i.e. related to weather and climate);

fields within natural systems that are underdeveloped in terms of data requirements and reporting (i.e. ecosystem services);
human systems in which challenges with both (i) accessibility, data ownership, trust, inter-organizational competition, security and privacy issues for data-sharing and (ii) lack of knowledge on which data are required and how they can be used, different philosophies in data importance, acquisition techniques prevail.

Water and carbon footprints of WWTPs are connected via energy footprints. However, there is still lack of availability of data on the long-term real-field WWTP. The study of Gu et al. [23] showed that on average 13.38 L of freshwater is required to produce 0.4 kWh of electric energy, used as input for treating 1 m^3 domestic wastewater, with a consequent emission of 0.23 kg CO_2 during the process. However, the reduction of Grey Water Footprint GWF due to wastewater treatment was 6.78 m^3/m^3 of sewage treated in Chinese WWTPs. One impact of wastewater treatment is an increase in GHG emissions that results from energy use in WWTPs. Energy modeling can help to analyse different WWTP management scenarios and choose the most suitable solution amongst various technology and operational options, before putting them into practice [23].

The International Water Association (IWA) developed the Activated Sludge Models (ASM) to simulate WWTP processes. During years, models have been upgraded and different versions are now available. Activated Sludge Model No. 1 (ASM1) describes biological process of carbon and nitrogen removal and provides the concentrations of pollutants and solids in biological reactors and in effluent. Activated Sludge Model No. 2 (ASM2) includes chemical precipitation of phosphorus, while Activated Sludge Model No. 2d (ASM2d) describes also denitrification due to phosphorus accumulating organisms (PAOs) [78]. Activated Sludge Model No. 3 (ASM3) was developed based on ASM1 and distinguishes decay processes of heterotrophic and autotrophic bacteria, includes a death-endogenous respiration process and considers the linkage between heterotrophs and nitrifiers. ASM3-BioP model was further upgraded to include phosphorus processes. Anaerobic Digestion Model No. 1 (ADM1) was developed to describe biological and physical-chemical processes in anaerobic digesters [79].

In order to integrate models with operative controls of WWTP and foster a plant-wide analysis, benchmarking tools were developed by combining process models (e.g., ASMs) with instrumentation, control and automation (ICA) equipment [80]. IWA developed Benchmark Simulation Models, creating a simulation environment in a defined plant layout, providing a generator of influent flows and loads, testing procedures and evaluation criteria. BSM1 [81] represents a virtual plant integrating biological process modelled with ASM1 coupled with a secondary settler and can evaluate different control strategies in a period of 14 days in different weather conditions. BSM1LT is a modification of BSM1, with a longer evaluation period (609 days), and simulates toxic events or problems on sensors and actuators [82]. BSM2 [83] has a more complete layout, adding a primary clarifier and a sludge treatment line to BSM1 consisting of a thickener, an anaerobic digester modelled with ADM1, and a dewatering unit. Dynamic behaviour of sensors and actuators is modelled considering the response time, noise and measuring interval of each instrumentation. The objectives for the monitoring strategies are to maintain the NO_3-N concentration in the second compartment of biologic reactor at a set point of 1 g/m^3 and the DO concentration in the fifth compartment at 2 g COD/m^3. For N-NO_3 control, the manipulated variable is the internal recycle flow rate (Qa), while for DO regulation the manipulated variable is the oxygen transfer coefficient in the fifth compartment (KLa,5). BSMs can evaluate performances of different scenarios considering two indices, describing treatment efficiency and costs. In particular, the Effluent Quality Index (EQI) represents the pollution removal efficiency in terms of TSS, COD, BOD_5, TKN and NO_X; while the Operational Cost Index (OCI) includes operating costs in a plant, such as energy for aeration, pumping and mixing, additional carbon source, sludge production, methane generation and the net heating energy required for anaerobic digestion [44].

ASMs generally require inputs of flows characterization, such as flow rates, concentrations and pollutant loads, operating parameters values, such as retention times or DO settings in aerated tanks, environmental conditions and volumetric and surface dimensions of the different treatment units. Models contain assumptions on kinetic variables and process parameters default values. BSMs integrate those data with typical parameters measured by online sensors. Regarding carbon and energy footprints, additional data are required, such as energy consumptions, chemical dosage, transports and the final destination of sludge and wastes. Integrated models are able to simulate influent flows from catchment and sewer network; however, open-access models, such as SWMM developed by EPA, can be used to obtain sewage water characterization from morphologic data, demographic information, land-use factors, rain flows, pipeline and furniture characteristics.

3.1. Carbon footprint

Carbon footprint is an evaluation of GHG emissions due to WWTPs activities. General guidelines on carbon footprint inventory are provided by ISO 14064-1:2018[1]. However, we still miss a unified methodology to assess carbon footprint from WWTPs [57,84]. One of the simplest ways to evaluate carbon footprint is using EFs that normalize the emissions with respect to a process parameter. Depending on the category, there are many databases that collect and update EFs. IPCC Guidelines provide EFs specific to different processes or activities and developed an online database, the EFDB[2], that can be freely consulted. On the other hand, more specific EFs can be evaluated at national level. For instance, ISPRA and SINANET developed national databases for the main activities in Italy [85], while the Swiss national GHG inventory has been updated under the UNFCCC in 2020, covering the years 1990 2018 [86].

According to IPCC an EF of 1.6% for of the total N-load is suggested [87]; to increase reliability of the EF estimates in large WWTPs, a country-specific EF is also proposed. Reliable EFs estimates require long-term sampling campaigns to capture the seasonal variability of N_2O emissions; however, this is rarely applied in practice due to the associated costs and labour demands.

Many Excel tools have been proposed, such as the Carbon Footprint Calculation Tool [88] developed in the project entitled Calculation of the CF from Swedish WWTPs (SVU 12 120). More sophisticated tools use models to simulate treatment processes. First, steady-state process models were developed to assess GHG emissions. In recent years, dynamic models are also applied to evaluate strategies for GHG emissions mitigation [80].

3.1.1. Carbon footprinting models

Procedures and equations to estimate GHG emissions from different sources or activities are reported in the IPCC Guidelines for National Greenhouse Gas Inventories. At national level, UKWIR developed a standardised workbook, the Carbon Accounting Workbook (CAW) [89], that can be followed for estimating operational GHG emissions.

Models can be used to integrate calculation in an automatic way. An example of steady-state model is represented by the diffusive emissions estimation model (DEEM), that uses ASM1 framework and includes the estimation of CO_2 and N_2O emissions due to nitrification process. Rodriguez-Garcia et al. [90] applied the steady state DEEM to Spanish WWTPs and implemented its results for LCA.

The Horizon2020 (H2020) project CF-FOOT-CTRL [3] developed a model in the same name of a software tool to evaluate the carbon footprint in WWTP. Simulations are performed using sub-models for the

[1] https://www.iso.org/standard/66453.html
[2] https://www.ipcc-nggip.iges.or.jp/EFDB/find_ef.php?root
[3] http://www.cfootcontrol.gr/

main stages of the treatment line. Primary treatments, as the primary clarifier, the gravity and mechanical thickeners and the dewatering unit, are estimated using mass balances based on the efficiency of each unit; the biological reactor is simulated using ASM1 and the secondary settler is schematized using a one-dimensional model. An anaerobic digestion sub-model was developed for the sludge line. For instance, in a C-FOOT-CTRL and H2020 SMART-Plant[4] combined approach: The software tool has three components, online measurements, database and dynamic model for carbon footprint estimation. The integrated mathematical model for the simulation of a WWTP consists of several sub-models, one for each treatment unit. Different sub-models were developed in order to simulate the processes in all stages, energy consumption and GHG emissions. More specifically, for the processes taking place in a bioreactor, the developed mathematical model is based on the activated sludge model no.1 (ASM1), while for the secondary settler a one-dimensional model was used. For anaerobic digestion, an anaerobic digestion sub-model was developed and in cases of pre-treatment, mass balances were used with respect to the efficiency of each unit such as primary clarifier, gravity and mechanical thickeners and dewatering unit. Besides the simulation of the processes within the plant units and the assessment of the effluent quality, the mathematical model is able to estimate biogas production of anaerobic digestion of sludge, oxygen consumption of aerated compartments of a bioreactor, energy consumption of each unit of the WWTP and direct and/or indirect GHG emissions.

The Activated Sludge Models for Nitrogen (ASMN) is a modified version of ASM1, in which the nitrification process is described in two steps and the denitrification in four steps. Guo et al. [91] modified the BSM2 model substituting ASM1 with ASMN and including the model proposed by Guisasola and co-authors [92] to predict CH_4 emission in the sewer system. The combined models were applied to a sewer system and to a WWTP, finding that N_2O emissions from WWTP were responsible for approximately 25% of the total system GHG emissions, while CH_4 emissions from the sewer system accounted for 8%. In terms of WWTP performance optimization, low DO results in CO_2 emissions reduction due to the aeration system, but increase of N_2O emissions from biological process [80].

The BSM2G model was developed by Flores-Alsina et al. [44] to implement the BSM2 in order to analyse GHG emissions from WWTPs. Model simulations are evaluated using the EQI and OCI indicators included in BSM2, in order to assess pollutant removal efficiency and related costs. [44] used the BSM2G to evaluate GHG emissions under different operative conditions in WWTPs. The BSM-e model developed by Sweetapple et al. [93] implements the BSM2, dividing the denitrification process in four steps, including N_2O stripping and CO_2 emissions. BSM2-e was used to evaluate strategies to reduce GHG emissions, adopting multi-objective optimization criteria. They found that the variance in total GHG emissions was mainly related to the changes in direct N_2O emissions.

In most cases, WWTPs performance are conditioned by economic aspects and oriented to the reduction of energy consumption, operating strategies to decrease sludge production and to increase biogas generation. In addition to operational costs, it is also crucial to consider environmental impacts when realizing these aspects since it allows to reduce direct and indirect GHG emissions [94 96]. Operating parameters (such as DO, temperature, influent C/N ratio, sludge retention time, nitrite concentrations in nitrification and denitrification stages) may have a high influence on N_2O emission. The treatment of GHGs in WWTPs can be another possible solution to reduce emissions. Industrial gaseous streams such as N_2O, CH_4, and CO_2 can be annihilated by various technologies available, while these technologies are still quite expensive and there is still a need to develop efficient low-cost technologies [97].

3.1.2. Carbon footprinting tools

Tools have been developed to facilitate carbon footprint estimation, with different levels of complexity depending on the type of the model applied. The choice of the complexity level, from the simplest that consider steady-state scenarios, to the more complex ones that perform dynamic simulations, is strictly related to the availability and the affordability of the input data, other than the quality of the requested output.

A ready-to-use tool for carbon footprint calculation consists in a MS Excel spreadsheet, The Carbon Footprint Calculation Tool (CFCT) dedicated for WWTPs, uses EFs provided by literature studies and IPCC Guidelines, together with Global Warming Potentials (GWPs), to evaluate the total CO_2 equivalent emissions related to wastewater treatment [88]. The tool requires input data for influent and effluent characterization, energy consumption, chemical dosage and supply, sludge and waste production and final disposal destination.

In addition, CF-TOOL-CTRL evaluates on-site and off-site emissions [98]. The tool estimates direct emissions due to biological, biogas production and consumption of chemicals, and includes indirect emissions due to energy consumption and sludge disposal.

3.2. Energy footprint

Energy footprint allows the evaluation of energetic efficiency of WWTPs and the individuation of the most consuming categories, considering that 25 40% of operating costs are attributed to energy consumption in conventional WWTPs [33]. Energy footprint is performed through the evaluation of consumptions, related to specific parameters that indicate the quantity of energy required to treat a certain amount of water or pollutant load. Energy footprint needs a common and shared methodology in order obtain benchmarks to compare different plants with different treatment capacity and operating in different conditions of loads, environment or regulatory context. Specific energy consumptions are generally used that refer to a process parameter.

ENERWATER[5] methodology for energy footprint was developed under a project founded by H2020. Two different levels of analysis are applied: Rapid Audit (RA), that is a general evaluation of the plant; and Decision Support (DS), that specifies each stage of the treatment process. Different levels of detail can be performed in the DS analysis, depending on the quantity and the quality of the available data, from Bronze to Platinum scenario. Different energetic vectors are included and energy requirement for chemical production are also included. Net energy consumption is calculated considering the energy production and recovery that may be applied in the plant. Key Performance Indicators (KPIs) are calculated, normalized into EPIs and weighted with literature values, provided by the ENERWATER database. Finally, labels are attributed for each stage of the treatment process and EPIs are aggregated into a unique indicator, the WTEI, that characterizes the energy label of the whole plant.

According to Rosso et al. [99], different models can be used to assess energy footprint of WWTPs, depending on the kind of data available as inputs, from a cumulative analysis when only energy bills and historical information are achievable, to a dynamic energy footprint modelling, where it is possible to consider unit operations separately and detailed characterizations are available. WWTP model was set up using ASM1 and ADM1 in the SIMBA[6] simulator. By applying their model to different plants, they found that activated sludge process was the most impacting unit for energy consumptions. Moreover, modelling could support the optimization of aeration system, improving effluent quality and reducing energy wastage. Optimization strategy was developed considering the F:M ratio, varying influent load and temperature.

[4] https://www.smart-plant.eu/

[5] https://enerwater-h2020.wtelecom.es/

[6] https://simba.ifak.eu/

3.2.1. Energy footprinting tools

The H2020 project ENERWATER provides a tool to perform energy footprint of a WWTP. Calculations are set into an online free application from the website. Depending on the type of WWTP selected and the different level of analysis (RA or DS), different data are required by the tool. The required data for RA assessment are: treated volume; energy and chemical consumptions along the plant; routine data from influent and effluent characterization in terms of COD, N_{tot} and P_{tot}, TS produced by the sludge line, and, in case of plants focused on water reuse, the log reduction of *E. coli*. For DS level, energy consumptions shall be measured *in situ* with specific meters installed in order to cover at least 90% of the total consumptions reported in the energy bill. Different energy vectors use, chemical dosage and energy production must be provided. For pre-treatments, treated volume is required; the primary stage needs TSS removal; biological treatments require a weighted indicator of load removal in terms of COD, N and P, called TPE; tertiary treatments are compared with *E. coli* log reduction; while sludge line is evaluated with a composite indicator, TSE. Data should be measured directly in the plant with a required frequency, in order to assess the maximum level of analysis (Platinum). Energy consumption and carbon footprint of different stages in a conventional WWTP are illustrated in Fig. 3.

3.3. Water footprint

The literature suggests two main approaches that have been developed for the calculation of the water footprint of a product [104]. The first one follows a volumetric approach and was developed by the Water Footprint Network (WFN) [105], whereas the second one is based on a LCA approach as introduced by the LCA community [106].

According to the WFN, the water footprint of a product is an important spatial and temporal indicator of freshwater use that looks into both direct and indirect water use of the product through the whole supply chain [107,108]. The WFA approach aims to identify the availability of freshwater resources identifying three types of different water-use sources, the blue water (WF_{blue}), that refers to the freshwater consumed for the production of a service or a product and not returned to the initial catchment, the green water (WF_{green}) that is defined as the precipitation water consumed instead of becoming a run-off and the grey water (WF_{gray}) that indicates the pollution of the freshwater resources. The methodology consists of four stages: (i) goal definition (ii) assessment of the water footprint (iii) evaluation of sustainability and (iv) formulation of the response scheme [105]. This approach has been focused on identifying the water-relating hotspots of a product's life cycle and proposing actions towards improved integrated water management schemes, better resource allocations and use of more water efficient processes and products [109]. Recently, the water use-related environmental, social and economic dimensions have been integrated in this approach towards a water sustainability assessment; relevant studies, though, are still limited [110].

On the contrary, the LCA-based water footprint approach of a product intends to assess water-based impacts through the whole supply chain and implement Life Cycle Inventory methods [106]. This approach has been extensively used for the assessment of different alternatives towards the minimisation of products' environmental impacts [111]. However, most of the widely accepted LCA methods disregarded water-based impacts and water use [112]. Hence, the UNEP-SETAC WULCA project [106] aims to introduce a framework towards an LCA-based water footprint assessment. In that direction, a new ISO standard on water footprint (BS ISO 14046, 2014) has been published proposing guidelines and requirements for LCA-based Water Footprint assessment.

Generally, the LCA approach targets the quantification of potential impacts on humans and climate connected with water deprivation and even water contamination and focuses on the assessment of environmental indicators such as eutrophication, ecotoxicity, human toxicity and water scarcity [113]. The methodology comprises four steps including goal and scope definition, Life Cycle Inventory (LCI) accounting and impact assessment and interpretation. Even though both methodologies aim to contribute towards water preservation, the different approaches that they follow result in non-comparable quantitative indicators. However, as Boulay and colleagues have suggested [114], the quantitative indicators of the blue water footprint can be potentially used as inventory flows in the LCA methodology.

The Water Footprint Assessment methodology was developed by the WFN and is divided in four steps: First, goals and scope are defined; then the water footprint (WF) is calculated, using the following equation:

$$WF = WF_{blue} + WF_{green} + WF_{gray} \quad (1)$$

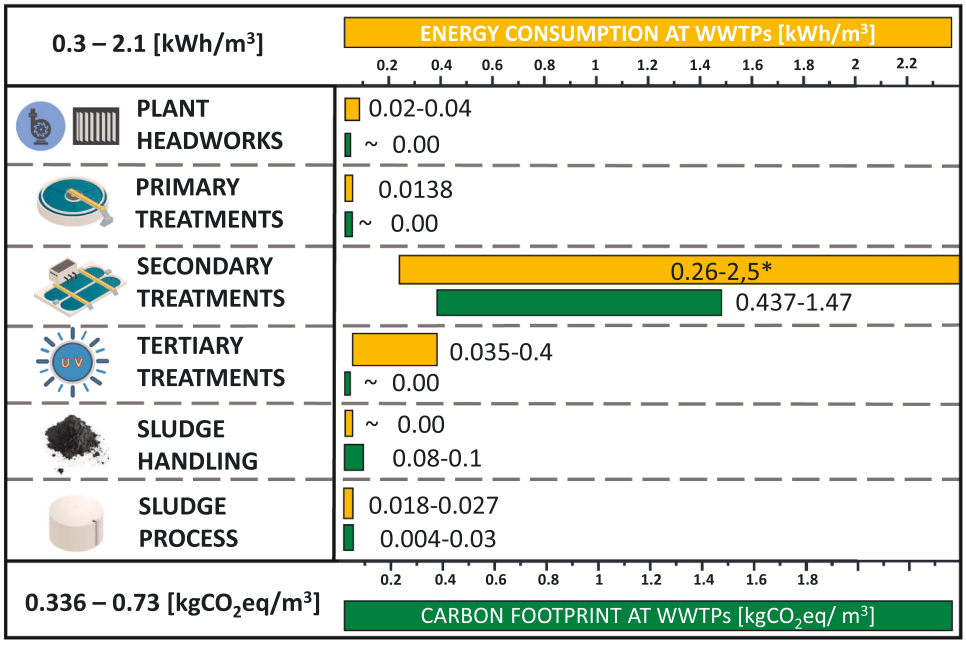


Fig. 3. Energy and carbon footprints at different stages in a WWTP (Based on the data taken from [34,100–103]).

Once WF is calculated, the sustainability is evaluated considering environmental, economic and social aspects. Finally, strategies to improve the water footprint are provided.

Regarding the evaluation of WF_{gray}, Morera and others [115] proposed a method based on a mass balance of pollutants (p) at the discharge points of WWTPs:

(2)

where Q_{eff} is the effluent flow, $C_{eff\,p}$ is the concentration of the pollutant p in the effluent water, $C_{nat\,p}$ is the concentration of the pollutant p in the water body, $WF_{gray\,p}$ is the grey water footprint calculated for each pollutant (p) and $C_{max\,p}$ is the maximum level of concentration admitted for the pollutant p. The total grey water footprint WF_{gray}represents the maximum of the diluting freshwater required for each pollutant p, as shown in the equation below.

(3)

WF_{gray} depends on local regulatory requirements for water quality; thus, this indicator cannot be used to compare WWTPs that discharge into water bodies with different quality characteristics. The indicator Water Footprint Reduction (GWF) was then proposed by Gu et al. [23] to represent the reduction in freshwater volume required to assimilate the pollutants due to WWTP, independently from water quality standards. GWF is calculated as follows:

(4)

where C_{in} and C_{out} represent pollutant concentrations in the influent and in the effluent, respectively, while $V_{treated}$ is the water volume treated in the time period under analysis.

The eGWF indicator was introduced to evaluate the nexus between energy consumptions ($E_{consumed}$) and water footprint, considering the energy consumed to reduce the WF_{gray} in WWTPs:

(5)

A greater eGWF corresponds to higher GWF for a unit energy input into the wastewater treatment process.

3.4. Integrated models and tools

WWTP management implies a series of impacts on different sectors, connected into the WEFC nexus. Catchment, sewer network, WWTP and water bodies receptors are highly interconnected. Characteristics, efficiencies or variations in each stage of the urban water cycle can affect other stages. In order to quantify the WEFC nexus, all the steps that involve wastewater production, transportation, treatment and reuse should be considered. Integrated Urban Wastewater System (IUWS) was developed to assess those linkages. Integrated models can help providing a wider panorama of all the interconnections that implies the urban wastewater cycle, from its catchment to the final destination.

Urban Wastewater System (UWS) modelling was developed to consider the interconnections amongst catchment, sewer network, WWTP and river system following a holistic approach. Benchmark Simulation Model BSM-UWS is an open-source system-wide model developed in Matlab, used to simulate, predict and evaluate different strategies in UWS. BSM-UWS is applied to provide an integrated system assessment. The toolbox uses different sub-models for each UWS stage, connected through interfaces in which mass-balances are verified. The BSM2 dynamic influent pollutant disturbance scenario generator (DIPDSG) is used to simulate the catchment model is simulated using, in order to obtain flow and concentration of pollutants in different wastewater sources: domestic, industrial, stormwater and infiltration to sewer. Sewer network sub-model considers the transport of wastewater and first flush loads from the catchment to WWTP, considering the presence of storage tanks and pumping stations. WWTP is modelled through ASM2d, while river water system is simulated using the IWA River Water Quality Model (RWQM).

Performances of each sector are assessed using evaluation indexes. For the sewer network overflow duration, frequency, volume and overflow quality index (OQI, kg pollutant units/day) are considered. WWTP efficiency is evaluated considering influent quality index (IQI, kg pollutant units/day) and effluent quality index (EQI, kg pollutant units/day). River water system is evaluated considering DO and unionized NH_3 concentrations. Three main control strategies were used by Saagi and others [116]. Sewer network system efficiency is evaluated considering the flowrate at WWTP inlet, manipulated by varying the flows from the storage tanks. Concerning the WWTP, the DO concentration in the aeration tanks is measured, modulating the oxygen transfer coefficients k_La. River water system is evaluated through the concentration of NH_3, controlled by the thresholds set up for the by-pass flows in the WWTP.

BSM-UWS is applied on different tools. WESTforIUWS, developed by Dansk Hydraulisk Institut in 2016, developed water quality-based indicators, including uncertainty and sensitivity analysis. SIMBA# water, developed by the German Institut für Automation und Kommunikation in 2016, uses hydrological and hydrodynamic models for the sewer network, biochemical and physical processes to simulate the WWTP and modules for biochemical processes in the river. CITY DRAIN [117] is an open-source Matlab based toolbox that uses hydrological models for sewers and river systems as well as a simplified model for WWTP [117]. It allows the users to create new blocks in addition to the existing model library [118]. The models and tools that are commonly used to measure carbon, energy and footprints in WWTPs are summarized in Table 1.

3.5. What is missing?

In recent years, modelling development gave a great contribution for the estimation of WWTP footprints, providing support tools for decision-making processes. WEFC nexus models can be used to evaluate designed solutions or scenarios, and support decision making. Even if more specific models are being constructed to fit WWTP conditions, still not all the footprint assessments can rely on specific tools for WWTP modelling. Different models are based on different hypothesis for process simulation or are focused on different parameters. A comprehensive tool that considers all the sectors of the WEFC nexus is still missing.

In addition, besides several nexus studies at wide scale, ranging from global to regional to national, but with very coarse resolution, local specificities must be addressed when designing effective management plans because international and regional decisions have a direct effect at the local level.

Nexus quantification depends on accurate, open access information. Decision-makers are often hindered to compare management options, make informed decisions balancing economic, social and environmental interests, and subsequently evaluate and prioritize potential sustainability solutions. To overcome this bottleneck, a mechanism is needed for data and monitoring requirements and trustable information provision.

4. The nexus approach in reclaimed water reuse for precision irrigation and smart agriculture in peri-urban areas

There is a great share of water and energy resources in agriculture for food production. In a most common sense, water and energy are required for irrigation, food production/processing and energy production, while food production can also count for biofuel feedstock [6,121,122]. Approximately 70% of the total water demand is represented by irrigation in agricultural production. In the Mediterranean region, which faces severe problems on water availability due to climate

Table 1
A summary of some of the models and tools used to measure carbon, energy and water footprints in WWTPs and to quantify the nexus.

Footprint	Model/method	Tool	Reference	Advantages	Disadvantages
Carbon footprint	EF, IPCC Guidelines	Carbon Footprint Calculation Tool (CFCT, 2014)	[88]	Easy to use, based on simple calculations; EF database available	Uncertainty of EFs; representativeness of EF to site-specific conditions
	Mass balances, ASM1, sub-models for secondary settler and AD	CF TOOL CTRL	[119]	Implemented sub-models to simulate different operations of WWTPs	CH_4 direct emissions from biologic unit not included
	Diffusive Emissions Estimation Model DEEM		[80,90]	Easy application for Life Cycle Assessment	CH_4 direct emissions from biologic unit not included
	Activated Sludge Models for Nitrogen ASMN		[80,120]	Implemented N_2O formation in nitrification and denitrification processes and CH_4 production and emissions in the sewer system	Model complexity
	BSM2G		[44,80]	Implemented indexes for strategy control	Over-parametrization, calibration required
	BSM-e		[80,93]	Included N_2O and CO_2 stripping	Overparameterization, calibration required
Energy footprint	ENERWATER	On-line tool	[1]	Estimation of WWTPs energy efficiency	Measured data required for Decision Support
Water footprint	Water Footprint Network (WFN)		[105]	Evaluation of the impact in water resource of a product or activity	Limited studies in the urban water cycle and in water and wastewater infrastructures
Integrated footprint	BSM-UWS	WESTforIUWS	[116,118]	Implemented water quality-based objectives, uncertainty and sensitivity analysis	Model complexity
		SIMBA# water	[116,118]	Controller programming with industry standard languages	Model complexity
		CITY DRAIN	[116 118]	Open-source toolbox	Simplified models

change, this demand is projected to increase up to 18% until the end of this century [123]. In fact, it is estimated that the Mediterranean region could save 35% of water by implementing more efficient irrigation and conveyance systems [124]. Therefore, the optimal use of limited water, energy, and land resources should be well considered for a sustainable agricultural system.

Although, urban and rural areas are geographically dispersed, their functioning is tightly interconnected [125]. As urbanization and industrialization have increased especially in developed countries, some developing countries have experienced pressure from the decline in both quality and quantity of farmland resources, which further witnessed remarkable transitions in rural livelihoods. Some common livelihood strategies during these transitions include rural-urban migration, off-farm employment, adjusted cropping patterns, land transfer, and changes in production inputs [126]. The complexity in the nexus approach is utmost important for areas where agricultural activities are the primary source of income, such as peri-urban areas [127].

The concept of wastewater reuse in agriculture refers to further use of treated wastewater for crop irrigation. This is essential for managing water resources, especially in arid areas that face water stress due the irregular availability of water sources throughout the hydrological year [128]. Recycle and re-utilization of nutrients and water from urban wastewater to urban agriculture can be a remarkable strategy to efficiently address the WEFC nexus. Although these cycles may still use energy and increase GHG emissions, there are advantages of water reuse and nutrient recovery [129]. Some of the environmental benefits associated with the agricultural reuse of advanced treated wastewater include reduced pressure on aquifers, successful groundwater recharge, reduction in synthetic fertilizer demand and expenses due to nutrients presence in reclaimed water, and higher crop yields for some crop types that are grown with reused water [130]. When water reuse implementations are coupled with advanced, water-saving, drip irrigation technologies, e.g., sensor-based systems (see Section 5), the impacts on water conservation and overall crop production can be remarkable. Besides these advantages, health issues should be considered and risks should be addressed when urban wastewater is used for irrigation [131]. Food safety and environmental pollution (i.e. soil and aquifers) are the main critical points in these practices. A new method called wastewater reuse effectiveness index (WREI) was developed [132] from the concept of the nexus approach by elaborating the concepts of trade-offs and thresholds, which measures the effectiveness of wastewater reuse and assesses environmental risks.

As already addressed and summarized by Fernandez García et al. [133], the concerns about optimizing WEFC nexus in irrigated areas have led to the development of different strategies aimed at reducing the use of energy and water resources. These strategies can be grouped into: (i) measures based on energy audits and benchmarking analysis, (ii) measures with respect to water distribution network management, (iii) strategies to optimize the performance of pumping station, (iv) solutions to minimize overall operational costs, (v) actions towards water distribution design, and (vi) smart irrigation. In smart irrigation, sensors are developed to adopt precision irrigation and smart agriculture and to support the decision making for applying the right amount of water at the right time and at the right place [134 136]. Sensors can estimate the volumetric water content and according to evo-transpiration crop models the right quantity of water could be determined to avoid water stress [137].

Considering the system management of water service, the issue of water reuse involves different levels of stakeholders in many national and local applications. For instance, a great part of water for agriculture is managed by irrigation and reclamation consortia in Italy, which are responsible for providing the farmers water for irrigation and for draining water from the fields. The activity of the irrigation and reclamation consortia is under the supervision of the Regional Authority, but it receives the inputs also by municipalities and farmers association. A simplified scheme for the involvement of stakeholders for a smart irrigation is depicted in Fig. 4. In this scenario, the engagement of end-users and next generation of irrigation channels/distribution network equipped with interconnected meters could make possible to implement a match-making tool to maximize the reuse of water from WWTP.

5. Digital solutions and serious games addressing the nexus

Many cities are currently initiating public-private partnerships and/or special financing to adopt the smart city model with respect to sustainable urban development. The basic idea behind the smart city concept is to increase the effectiveness of city governance by creating positive feedbacks between end users and innovative technologies, building a communication paradigm in which the objects of everyday life are equipped with digital solutions [138]. The main goal of the smart

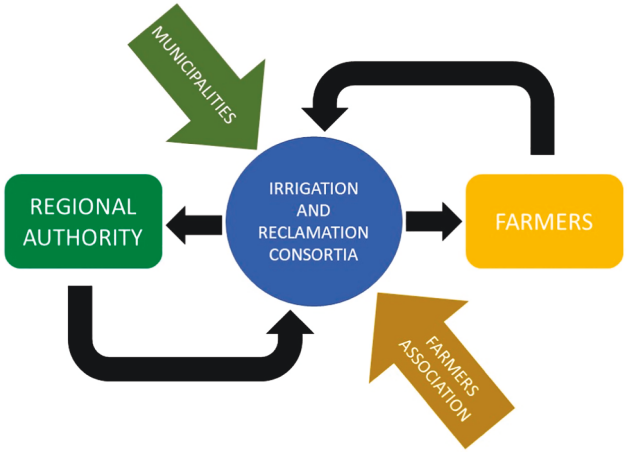


Fig. 4. Involved stakeholders for a digitalized irrigation architecture.

city model is to reduce costs, improve efficiencies, and ease the life of citizens by providing urban planners with definitive tools [139]. Hence, smart cities are believed to be a promising solution to environmental challenges of urban life in the near future where resources and processes can be managed in a closed system [140]. The integration of smart cities with digital solutions offers the chance to reorganize urban infrastructures, likewise transportation or food and water supply, in much smarter ways [141,142]. When developing a smart city with proposed possible digital solutions, the nexus approach must be placed at the heart of the plan. Recently, the digital and ecological transition which is permeating the global (e.g. The Agenda 2030 for Sustainable Development) and European policies and initiatives (e.g. New European Green Deal) has integrated the "smart" with the "circular" approach to cities and regions. This approach is being highly applied in the water management, and not only for the urban life but also should be taken into account for rural areas that was highlighted by Hosseini and colleagues [141]. Despite the afore-mentioned points of motivation, some critical concerns and gaps regarding the concept of smart city were also addressed elsewhere [138].

Nevertheless, many public and private water service providers have been reclaiming their conventional supply-oriented strategies toward a more sustainable urban water management. On the other hand, this transmission requires heavy work originated from accurate, adequate and reliable data that can be thoroughly and cost-effectively interpreted to help utilities improve their services, reduce water and energy losses and manage demand [143].

Intelligent metering (or smart metering) has high potential to evolve customer engagement and management of urban water by utilities into technologies that encourages sustainable urban water management. In general, intelligent metering is a blend of components and procedures designed for the continuous and remote monitoring and assessment of water use (also electricity and gas) to inform strategic planning processes and to aid decision making. These systems include four key processes: (i) measurement; (ii) transfer of the data; (iii) data process and analysis; and (iv) feedback. Through these steps, the determination of water consumption, in real-time or near real-time, is enabled and the possibility to read consumption both locally and remotely is provided [143]. However, one of the main challenges stands as the vast volume of continuously accumulating information is used to ensure that digital technology enhances urban water, electricity and gas management [144].

In urban water cycle, functional subsystems, e.g., drinking water treatment and distribution, wastewater and stormwater collection, wastewater treatment and discharge, are all interconnected and managed by computing and control systems through physical domain sensors [20]. In a CPS framework, monitoring and controlling processes for digital representations work in real time, with interactions between virtual and physical spaces of systems [21]. The application of CPS in the water sector can bring high added value to the IUWM. Online monitoring and warning systems, so-called (bio)sensors, has received considerable attention in recent years in the integrated assessment of water networks such as WWTPs, combined sewer overflows (CSOs) as well as receiving water bodies [145,146]. Sensors allow the physical infrastructures to be monitored and controlled, and provide high sensitivity, selectivity, reliability, simplicity, low-cost and real-time response. There is also an increasing interest in operating WWTPs with advanced digital control systems to minimize energy consumption by utilizing the energy (biogas) potential. For this purpose, digital solutions are often used to optimize performances across sectors, e.g. wet-weather control of WWTPs aimed at temporarily increasing their capacity to avoid CSO and bypass during rain [147].

Biosensor-based strategies for wastewater monitoring can be classified into three main categories for the detection of (i) organic materials (ii) heavy metals and (iii) microorganisms [146]. Currently, the ongoing (2019–2022) H2020 Innovation Action Digital.Water-City (DWC[7]) deploys digital solutions with the aid of sensors for water services at the communities in five European urban and peri-urban areas in Berlin, Milan, Copenhagen, Paris and Sofia. These solutions are expected to minimize health-related-risks in water reuse and bathing water by improved decision support and added-value from on-line data and advanced modelling. In addition, based on scientifically sound and field validated wastewater treatment and crop growth models, a web-based serious game is being developed for near real-time assessment of the WEFC nexus, engaging public to overcome social and economic barriers to water reuse, as summarized in Fig. 5.

Serious games have presented an alternative way to didactic tools from an innovative strand for raising awareness on sustainable water management in the last years [148]. Serious games are often described as the games that are not intended only for amusement and entertainment but also have a specific aim with an explicit educational purpose [149,150] and for these reason a rational design is mandatory [151]. A serious game approach brings a learning objective with a fun activity that aims to increase the potential for learning uptake, self-learning and knowledge retention [152]. These games help to (i) create a realistic learning environment (ii) elevate the potential for interactive visualization (iii) improve social learning and decision making skills and (iv) enable the involvement of stakeholders from the beginning of the planning process [150,152].

While serious games have been widely used in education and training, especially in military or flight simulators [152], in the last decade, such games combining computer simulation and role playing games have been developed also in water-related issues such as water management [153-155], wastewater infrastructures management [156], water governance and policy [157], as well as land management [148] and optimal sensor placement to localize leaks in water distribution systems [158].

As the understanding of the WEFC nexus has become a more important approach in a holistic way, few serious games have been demonstrated in recent years to address the nexus from regional to global scales. These games allow to investigate the trade-offs and synergies between sectors and enable the stakeholders to better understand nexus-related policies. For instance, Sušnik et al. [152] developed a serious game within the EU H2020 project SIM4NEXUS[8] and investigated the potential cross-nexus implications and synergies due to policy interferences for several case studies at different scales as well as at different time horizons. The authors provided a frame for complex systems that depict the interactions between the water, energy, food, land and climate sectors within the nexus that can be modelled against future

[7] http://www.digital-water.city
[8] http://www.sim4nexus.eu

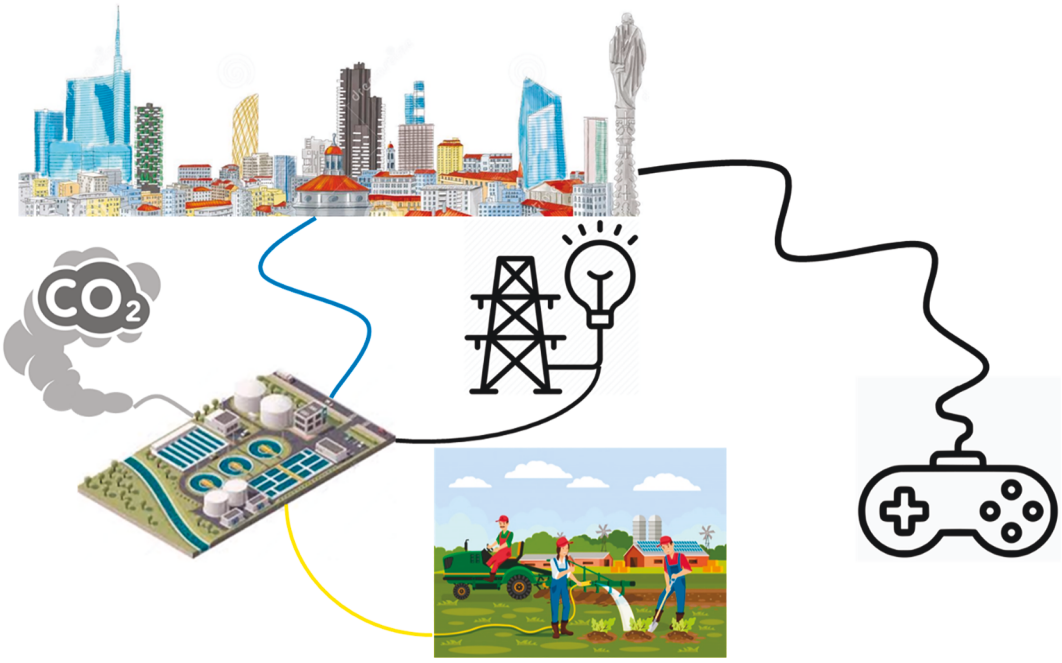


Fig. 5. Schematic of the data-driven serious game on the water reuse, carbon, energy, food and climatic nexus under development in H2020 DWC (taken from DWC website[7]).

climate scenarios.

The water industry of the future is expected to be digital, smart and resource efficient. At this point, benefits provided by digital solutions can reduce the fragmentation of knowledge across the water value chain and among stakeholders by facilitating information exchange through increased interoperability of data and digital systems.

6. Further recommendations for research and application to bring nexus perspective to operators

Water, energy and food are interlinked resources managed by separate institutions and operators to facilitate decision-making and optimization, which overlooks the interdependences and interconnectivity of the resources. In order to implement the nexus concept, development of multilevel governance systems, investment into human and social infrastructure, encouraging innovations across water-using industries, and leveraging public–private partnerships, while involving local citizens in co-creation and decision-making processes, must be supported. Solutions should come from all stages of supply chains, from development to consumption, to provide water, food, and energy protection to a growing and urbanizing global population under high climate challenges to face. The phrase "more crop per drop" is commonly used to describe increased water productivity, whereas the phrase "more biomass per decrease" is more fitting to the nexus concept [159].

It is obvious that the provision of water, energy, and food protection for citizens within a geographical area is concerned with the consumption side of the region rather than the development side. As a result, trade between regions is essential to safeguard these three sources. However, we must not forget that sustainability does not mean self-sufficiency. So far, only few projects have considered the nexus concept in practice for efficient and sustainable use of resources in the water sector. For instance, H2020 SIM4NEXUS[8] applies the nexus concept in 12 case studies at different scales to better understand interlinkages of decision making across spatial scales, and support decision making in the short-, medium- and long-term. H2020 MAGIC-NEXUS[9] created a set of relationships between identified factors to explore the complexity between water, soil, energy, and climate while integrating social challenges, stakeholders and citizens perceptions related to the nexus into the analysis.

Finally, demographic change, urbanisation, climate change, digitalisation as well as the circular economy are all tightly interconnected with water, energy, and food, offering both challenges and opportunities for Europe to lead the way towards a sustainable future. In order to fit to the digital age, we must give serious efforts to move towards a greener Europe supported by an integrated approach to the establishment of WEFE-smart communities and society, even by implementing effective strategies and policymaking with the participation of citizens to achieve sustainable development by complying with the Sustainable Development Goals (SDGs) by 2030. In order to contribute to the achievement of SDGs, even many urban wastewater utilities are willing to implement nexus-oriented management to support their city's energy transition policy in the last years. However, serious difficulties are faced by the water and wastewater utility managers/operators often originated from legislative and regulatory framework that is not considering the nexus for sustainable urban integrated wastewater management and reuse. The regulations governing how wastewater utilities should operate and how open energy markets should function are proving currently highly restrictive to the utilities' aspirations to provide flexibility for energy networks [14]. According to the conclusion achieved in the workshop "H2020 Water Innovations for Sustainable Impacts in Industries and Utilities"[10], digitalization can help to increase water sector operators' awareness concerning the nexus and longer-term impact of their day-by-day activity. Demonstrating the nexus can provide water utilities with opportunities to decrease energy consumption, to improve performance and to link better with the water tariffing system. In this nexus, the whole water supply chain needs to be considered from water acquisition up to wastewater treatment and disposal or reuse. Awareness of water

[9] http://www.magic-nexus.eu/

[10] https://op.europa.eu/en/publication-detail/-/publication/4cac17d6-49bb-11ea-8aa5-01aa75ed71a1/language-en/format-PDF/source-115839553.

utility operators and value chain actors can be raised by generally addressing and promoting the nexus in regulatory framework, initially without any obligation or target to be achieved. There is a need of widely validating integrated models and tools that are addressing and assessing the WEFC nexus, and smart urban infrastructure with peri-urban agriculture can be the best testbed where field data can be used to support knowledge-based regulatory, legislative and operational decisions. Data mining should be applied to public sources (e.g. Copernicus, EUROSTAT) and integrate social and economic drivers into data assessment. Locally, energy, water, carbon footprint need to be measured in case studies, also through citizen science, and relevant key performance indicators including social and economic drivers/factors need to be derived.

7. Final remarks and conclusion

Significant energy and resource recovery can be achieved in wastewater treatment and recycling as well as in sludge management using innovative technologies. However, the challenge here is to integrate WEFC nexus in the urban and peri-urban context considering all their key components as a whole. Although some remarkable efforts have been given to develop a large variety of models and tools to assess climate change impacts and ecosystem services and to provide relevant information to policy makers at a range of different levels, there is also a need for tools that focus on the holistic and integrated assessment of all sectors in the nexus. The nexus concept is indeed critical for achieving adequate water, energy and food securities which are naturally interconnected, but currently still regulated and governed by traditional sectorial approaches [159]. In fact, the nexus approach should be adopted to determine system trade-offs and synergies to provide a stronger evidence base for policy-makers to ensure sustainable use of natural resources [160].

Unless water and agricultural policies can guarantee revenues for reuse-based farming systems which include payment for recovered resources, public regulation, and subsidization of the energy costs of reclaimed water appear to be the only way forward, even considering the impacts on regional water and carbon footprint. This approach can help to better understand, measure, and monitor the indicators of the complex interactions between water treatment/reuse, carbon emission, energy consumption, smart agriculture and climate variability. The models and tools can be used to demonstrate decision support systems based on multi-sources data platforms, including quality and quantity monitoring, interconnected smart sensor networks, advanced predictive analytics and big data solutions. Digital solutions bring high relevance to address current and future water-related issues, namely the protection of human health, the technical, environmental and economic performance and return on investment of water infrastructures as well as the public and end-user awareness on water reuse management. Given the importance of these tools and models and digital solutions for decision makers, water utilities must upgrade their strategies based on best available technologies and practices. This way, multiple actors - water utilities, reclamation managers, farmers, policy makers and even citizens can benefit from real data-driven decision making and better coordinate for informed decisions. Social acceptance and incentives to apply nexus-oriented practices also confirm the need to integrate social dimension into integrated nexus models and tools through a participatory approach and explicit representation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Longo S, Mauricio-Iglesias M, Soares A, Campo P, Fatone F, Eusebi AL, et al. ENERWATER a standard method for assessing and improving the energy efficiency of wastewater treatment plants. Appl Energy 2019;242:897 910. https://doi.org/10.1016/j.apenergy.2019.03.130.

[2] Landa-Cansigno O, Behzadian K, Davila-Cano DI, Campos LC. Performance assessment of water reuse strategies using integrated framework of urban water metabolism and water-energy-pollution nexus. Environ Sci Pollut Res 2020;27:4582 97. https://doi.org/10.1007/s11356-019-05465-8.

[3] Veldhuis AJ, Yang A. Integrated approaches to the optimisation of regional and local food energy water systems. Curr Opin Chem Eng 2017;18:38 44. https://doi.org/10.1016/j.coche.2017.09.001.

[4] Hülsmann S, Susnik J, Rinke K, Langan S, van Wijk D, Janssen AB, et al. Integrated modelling and management of water resources: the ecosystem perspective on the nexus approach. Curr Opin Environ Sustain 2019;40:14 20. https://doi.org/10.1016/j.cosust.2019.07.003.

[5] van den Heuvel L, Blicharska M, Masia S, Susnik J, Teutschbein C. Ecosystem services in the Swedish water-energy-food-land-climate nexus: anthropogenic pressures and physical interactions. Ecosyst Serv 2020;44. https://doi.org/10.1016/j.ecoser.2020.101141.

[6] Li M, Fu Q, Singh VP, Ji Y, Liu D, Zhang C, et al. An optimal modelling approach for managing agricultural water-Tenergy-food nexus under uncertainty. Sci Total Environ 2019;651:1416 34. https://doi.org/10.1016/j.scitotenv.2018.09.291.

[7] Capodaglio AG. Integrated, decentralized wastewater management for resource recovery in rural and peri-urban areas. Resources 2017;6. https://doi.org/10.3390/resources6020022.

[8] Wu S, Austin D, Liu L, Dong R. Performance of integrated household constructed wetland for domestic wastewater treatment in rural areas. Ecol Eng 2011;37:948 54. https://doi.org/10.1016/j.ecoleng.2011.02.002.

[9] Mabhaudhi T, Nhamo L, Mpandeli S, Nhemachena C, Senzanje A, Sobratee N, et al. The water energy food nexus as a tool to transform rural livelihoods and well-being in Southern Africa. Int J Environ Res Public Health 2019;16. https://doi.org/10.3390/ijerph16162970.

[10] Massoud MA, Tarhini A, Nasr JA. Decentralized approaches to wastewater treatment and management: applicability in developing countries. J Environ Manage 2009;90:652 9. https://doi.org/10.1016/j.jenvman.2008.07.001.

[11] Priya AK, Pachaiappan R, Kumar PS, Jalil AA, Vo D-VN, Rajendran S. The war using microbes: a sustainable approach for wastewater management. Environ Pollut 2021;275:116598. https://doi.org/10.1016/j.envpol.2021.116598.

[12] Cipolletta G, Ozbayram EG, Eusebi AL, Akyol Ç, Malamis S, Mino E, et al. Policy and legislative barriers to close water-related loops in innovative small water and wastewater systems in Europe: a critical analysis. J Clean Prod 2021;288. https://doi.org/10.1016/j.jclepro.2020.125604.

[13] Novotny V. Water-energy nexus: Retrofitting urban areas to achieve zero pollution. Build Res Inf 2013;41:589 604. https://doi.org/10.1080/09613218.2013.804764.

[14] Moss T, Hüesker F. Politicised nexus thinking in practice: integrating urban wastewater utilities into regional energy markets. Urban Stud 2019;56:2225 41. https://doi.org/10.1177/0042098017735229.

[15] Bach PM, Rauch W, Mikkelsen PS, McCarthy DT, Deletic A. A critical review of integrated urban water modelling - urban drainage and beyond. Environ Model Softw 2014;54:88 107. https://doi.org/10.1016/j.envsoft.2013.12.018.

[16] Lee M, Keller AA, Chiang PC, Den W, Wang H, Hou CH, et al. Water-energy nexus for urban water systems: a comparative review on energy intensity and environmental impacts in relation to global water risks. Appl Energy 2017;205:589 601. https://doi.org/10.1016/j.apenergy.2017.08.002.

[17] Vasilaki V, Massara TM, Stanchev P, Fatone F, Katsou E. A decade of nitrous oxide (N2O) monitoring in full-scale wastewater treatment processes: a critical review. Water Res 2019;161:392 412. https://doi.org/10.1016/j.watres.2019.04.022.

[18] Venkatesh G, Chan A, Bratteb H. Understanding the water-energy-carbon nexus in urban water utilities: Comparison of four city case studies and the relevant influencing factors. Energy 2014;75:153 66. https://doi.org/10.1016/j.energy.2014.06.111.

[19] Ganora D, Hospido A, Husemann J, Krampe J, Loderer C, Longo S, et al. Opportunities to improve energy use in urban wastewater treatment: a European-scale analysis. Environ Res Lett 2019;14:44028. https://doi.org/10.1088/1748-9326/ab0b54.

[20] Sun C, Puig V, Cembrano G. Real-time control of urban water cycle under cyber-physical systems framework. Water (Switzerland) 2020;12. https://doi.org/10.3390/w12020406.

[21] Wang Z, Song H, Watkins DW, Ong KG, Xue P, Yang Q, et al. Cyber-physical systems for water sustainability: challenges and opportunities. IEEE Commun Mag 2015;53:216 22. https://doi.org/10.1109/MCOM.2015.7105668.

[22] Wang Y, Vuran MC, Goddard S. Cyber-physical systems in industrial process control. Univ Nebraska-Lincoln Lincoln, NE, USA 2008. https://doi.org/10.1145/1366283.1366295.

[23] Gu Y, Dong YN, Wang H, Keller A, Xu J, Chiramba T, et al. Quantification of the water, energy and carbon footprints of wastewater treatment plants in China considering a water-energy nexus perspective. Ecol Indic 2016;60:402 9. https://doi.org/10.1016/j.ecolind.2015.07.012.

[24] Zhang P, Zhang L, Chang Y, Xu M, Hao Y, Liang S, et al. Food-energy-water (FEW) nexus for urban sustainability: a comprehensive review. Resour Conserv Recycl 2019;142:215 24. https://doi.org/10.1016/j.resconrec.2018.11.018.

[25] Heard BR, Miller SA, Liang S, Xu M. Emerging challenges and opportunities for the food energy water nexus in urban systems. Curr Opin Chem Eng 2017;17: 48 53. https://doi.org/10.1016/j.coche.2017.06.006.

[26] Wang XC, Klemes JJ, Wang Y, Dong X, Wei H, Xu Z, et al. Water-Energy-Carbon Emissions nexus analysis of China: an environmental input-output model-based approach. Appl Energy 2020;261. https://doi.org/10.1016/j.apenergy.2019.114431.

[27] Foglia A, Andreola C, Cipolletta G, Radini S, Akyol Ç, Eusebi AL, et al. Comparative life cycle environmental and economic assessment of anaerobic membrane bioreactor and disinfection for reclaimed water reuse in agricultural irrigation: a case study in Italy. J Clean Prod 2021;293:126201. https://doi.org/10.1016/j.jclepro.2021.126201.

[28] Foglia A, Akyol Ç, Frison N, Katsou E, Eusebi AL, Fatone F. Long-term operation of a pilot-scale anaerobic membrane bioreactor (AnMBR) treating high salinity low loaded municipal wastewater in real environment. Sep Purif Technol 2020; 236:116279. https://doi.org/10.1016/j.seppur.2019.116279.

[29] Reddy VR, Cunha DGF, Kurian M. A water-energy-food nexus perspective on the challenge of eutrophication. Water (Switzerland) 2018;10:1 13. https://doi.org/10.3390/w10020101.

[30] Gondhalekar D, Ramsauer T. Nexus City: operationalizing the urban Water-Energy-Food Nexus for climate change adaptation in Munich, Germany. Urban Clim 2017;19:28 40. https://doi.org/10.1016/j.uclim.2016.11.004.

[31] Nair S, George B, Malano HM, Arora M, Nawarathna B. Water-energy-greenhouse gas nexus of urban water systems: review of concepts, state-of-art and methods. Resour Conserv Recycl 2014;89:1 10. https://doi.org/10.1016/j.resconrec.2014.05.007.

[32] Panepinto D, Fiore S, Zappone M, Genon G, Meucci L. Evaluation of the energy efficiency of a large wastewater treatment plant in Italy. Appl Energy 2016;161: 404 11. https://doi.org/10.1016/j.apenergy.2015.10.027.

[33] Gu Y, Li Y, Li X, Luo P, Wang H, Robinson ZP, et al. The feasibility and challenges of energy self-sufficient wastewater treatment plants. Appl Energy 2017;204: 1463 75. https://doi.org/10.1016/J.APENERGY.2017.02.069.

[34] Longo S, d Antoni BM, Bongards M, Chaparro A, Cronrath A, Fatone F, et al. Monitoring and diagnosis of energy consumption in wastewater treatment plants. A state of the art and proposals for improvement. Appl Energy 2016;179: 1251 68. https://doi.org/10.1016/j.apenergy.2016.07.043.

[35] FundacionOPTI. Estudio de Prospectiva Consumo Energetico en el sector del agua; 2012.

[36] Foladori P, Vaccari M, Vitali F. Energy audit in small wastewater treatment plants: Methodology, energy consumption indicators, and lessons learned. Water Sci Technol 2015;72:1007 15. https://doi.org/10.2166/wst.2015.306.

[37] Gandiglio M, Lanzini A, Soto A, Leone P, Santarelli M. Enhancing the energy efficiency of wastewater treatment plants through co-digestion and fuel cell systems. Front Environ Sci 2017;5:1 21. https://doi.org/10.3389/fenvs.2017.00070.

[38] Bodík I, Kubaska M. Energy and sustainability of operation of a wastewater treatment plant. Environ Prot Eng 2013;39:15 24. https://doi.org/10.5277/EPE130202.

[39] Mamais D, Noutsopoulos C, Dimopoulou A, Stasinakis A, Lekkas TD. Wastewater treatment process impact on energy savings and greenhouse gas emissions. Water Sci Technol 2015;71:303 8. https://doi.org/10.2166/wst.2014.521.

[40] Arnold ME, Merta ES. Towards energy self-sufficiency in wastewater treatment by optimized sludge treatment. Water Pract Technol 2011;6. https://doi.org/10.2166/wpt.2011.069.

[41] Mizuta K, Shimada M. Benchmarking energy consumption in municipal wastewater treatment plants in Japan. Water Sci Technol 2010;62:2256 62. https://doi.org/10.2166/wst.2010.510.

[42] Mininni G, Laera G, Bertanza G, Canato M, Sbrilli A. Mass and energy balances of sludge processing in reference and upgraded wastewater treatment plants. Environ Sci Pollut Res 2015;22:7203 15. https://doi.org/10.1007/s11356-014-4013-2.

[43] Gurung K, Tang WZ, Sillanpaa M. Correction to: Unit Energy Consumption as Benchmark to Select Energy Positive Retrofitting Strategies for Finnish Wastewater Treatment Plants (WWTPs): a Case Study of Mikkeli WWTP (Environmental Processes, (2018), 5, 3, (667-681), 10.1007/s40710-018-0310-. Environ Process 2018;5:931. https://doi.org/10.1007/s40710-018-0340-5.

[44] Flores-Alsina X, Arnell M, Amerlinck Y, Corominas L, Gernaey K V, Guo LS. A dynamic modelling approach to evaluate GHG emissions from wastewater treatment plants. Proc. World Congr. Water, Clim. Energy, Dublin, Ireland: 2012, p. 1 8.

[45] Maktabifard M, Zaborowska E, Makinia J. Achieving energy neutrality in wastewater treatment plants through energy savings and enhancing renewable energy production. vol. 17. Springer Netherlands; 2018. https://doi.org/10.1007/s11157-018-9478-x.

[46] EnvironmentAgency. Evidence: Transforming wastewater treatment to reduce carbon emissions. Bristol, UK; 2009.

[47] SevernTrentPlc. Annual Report and Accounts 2012; 2012. https://doi.org/10.1007/s00355-016-0977-9.

[48] Puchongkawarin C, Gomez-Mont C, Stuckey DC, Chachuat B. Optimization-based methodology for the development of wastewater facilities for energy and nutrient recovery. Chemosphere 2015;140:150 8. https://doi.org/10.1016/j.chemosphere.2014.08.061.

[49] Batstone DJ, Hülsen T, Mehta CM, Keller J. Platforms for energy and nutrient recovery from domestic wastewater: a review. Chemosphere 2015;140:2 11. https://doi.org/10.1016/j.chemosphere.2014.10.021.

[50] Frison N, Katsou E, Malamis S, Oehmen A, Fatone F. Nutrient removal via nitrite from reject water and polyhydroxyalkanoate (PHA) storage during nitrifying conditions. J Chem Technol Biotechnol 2015;90:1802 10. https://doi.org/10.1002/jctb.4487.

[51] Akyol Ç, Foglia A, Ozbayram EG, Frison N, Katsou E, Eusebi AL, et al. Validated innovative approaches for energy-efficient resource recovery and re-use from municipal wastewater: From anaerobic treatment systems to a biorefinery concept. Crit Rev Environ Sci Technol 2020;50:869 902. https://doi.org/10.1080/10643389.2019.1634456.

[52] Puyol D, Batstone DJ, Hülsen T, Astals S, Peces M, Kromer JO. Resource recovery from wastewater by biological technologies: opportunities, challenges, and prospects. Front Microbiol 2017;7:1 23. https://doi.org/10.3389/fmicb.2016.02106.

[53] Mo W, Zhang Q. Can municipal wastewater treatment systems be carbon neutral? J Environ Manage 2012;112:360 7. https://doi.org/10.1016/j.jenvman.2012.08.014.

[54] Capodaglio AG, Olsson G. Energy issues in sustainable urban wastewater management: use, demand reduction and recovery in the urban water cycle. Sustain 2020;12. https://doi.org/10.3390/su12010266.

[55] Ashrafi O, Yerushalmi L, Haghighat F. Greenhouse gas emission and energy consumption in wastewater treatment plants: Impact of operating parameters. Clean - Soil, Air, Water 2014;42:207 20. https://doi.org/10.1002/clen.201200158.

[56] Bani Shahabadi M, Yerushalmi L, Haghighat F. Estimation of greenhouse gas generation in wastewater treatment plants - model development and application. Chemosphere 2010;78:1085 92. https://doi.org/10.1016/j.chemosphere.2009.12.044.

[57] IPCC. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia,. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press; 2013.

[58] Caniani D, Caivano M, Pascale R, Bianco G, Mancini IM, Masi S, et al. CO2 and N2O from water resource recovery facilities: evaluation of emissions from biological treatment, settling, disinfection, and receiving water body. Sci Total Environ 2019;648:1130 40. https://doi.org/10.1016/j.scitotenv.2018.08.150.

[59] Daniel JS, Fleming EL, Portmann RW, Velders GJM, Jackman CH, Ravishankara AR. Options to accelerate ozone recovery: ozone and climate benefits. Atmos Chem Phys 2010;10:7697 707. https://doi.org/10.5194/acp-10-7697-2010.

[60] Daelman MRJ, Van Voorthuizen EM, Van Dongen LGJM, Volcke EIP, Van Loosdrecht MCM. Methane and nitrous oxide emissions from municipal wastewater treatment - results from a long-term study. Water Sci Technol 2013; 67:2350 5. https://doi.org/10.2166/wst.2013.109.

[61] Lackner S, Gilbert EM, Vlaeminck SE, Joss A, Horn H, van Loosdrecht MCM. Full-scale partial nitritation/anammox experiences - an application survey. Water Res 2014;55:292 303. https://doi.org/10.1016/j.watres.2014.02.032.

[62] Schaubroeck T, De Clippeleir H, Weissenbacher N, Dewulf J, Boeckx P, Vlaeminck SE, et al. Environmental sustainability of an energy self-sufficient sewage treatment plant: Improvements through DEMON and co-digestion. Water Res 2015;74:166 79. https://doi.org/10.1016/j.watres.2015.02.013.

[63] Desloover J, Vlaeminck SE, Clauwaert P, Verstraete W, Boon N. Strategies to mitigate N 2O emissions from biological nitrogen removal systems. Curr Opin Biotechnol 2012;23:474 82. https://doi.org/10.1016/j.copbio.2011.12.030.

[64] Law Y, Ye L, Pan Y, Yuan Z. Nitrous oxide emissions from wastewater treatment processes. Philos Trans R Soc B Biol Sci 2012;367:1265 77. https://doi.org/10.1098/rstb.2011.0317.

[65] Massara TM, Malamis S, Guisasola A, Baeza JA, Noutsopoulos C, Katsou E. A review on nitrous oxide (N2O) emissions during biological nutrient removal from municipal wastewater and sludge reject water. Sci Total Environ 2017; 596 597:106 23. https://doi.org/10.1016/j.scitotenv.2017.03.191.

[66] Kosonen H, Heinonen M, Mikola A, Haimi H, Mulas M, Corona F, et al. Nitrous oxide production at a fully covered wastewater treatment plant: results of a long-term online monitoring Campaign. Environ Sci Technol 2016;50:5547 54. https://doi.org/10.1021/acs.est.5b04466.

[67] Daelman MRJ, van Voorthuizen EM, van Dongen UGJM, Volcke EIP, van Loosdrecht MCM. Seasonal and diurnal variability of N2O emissions from a full-scale municipal wastewater treatment plant. Sci Total Environ 2015;536:1 11. https://doi.org/10.1016/j.scitotenv.2015.06.122.

[68] Stillwell AS, Hoppock DC, Webber ME. Energy recovery from wastewater treatment plants in the United States: a case study of the energy-water nexus. Sustainability 2010;2:945 62. https://doi.org/10.3390/su2040945.

[69] Hao X, Li J, van Loosdrecht MCM, Jiang H, Liu R. Energy recovery from wastewater: Heat over organics. Water Res 2019;161:74 7. https://doi.org/10.1016/j.watres.2019.05.106.

[70] Jenicek P, Kutil J, Benes O, Todt V, Zabranska J, Dohanyos M. Energy self-sufficient sewage wastewater treatment plants: Is optimized anaerobic sludge digestion the key? Water Sci Technol 2013;68:1739 43. https://doi.org/10.2166/wst.2013.423.

[71] Haimi H, Mulas M, Corona F, Vahala R. Data-derived soft-sensors for biological wastewater treatment plants: an overview. Environ Model Softw 2013;47:88 107. https://doi.org/10.1016/j.envsoft.2013.05.009.

[72] Corominas L, Garrido-Baserba M, Villez K, Olsson G, Cortes U, Poch M. Transforming data into knowledge for improved wastewater treatment operation: A critical review of techniques. Environ Model Softw 2018;106:89 103. https://doi.org/10.1016/j.envsoft.2017.11.023.

[73] Zhang R, Xie WM, Yu HQ, Li WW. Optimizing municipal wastewater treatment plants using an improved multi-objective optimization method. Bioresour Technol 2014;157:161 5. https://doi.org/10.1016/j.biortech.2014.01.103.

[74] Qiao J, Zhang W. Dynamic multi-objective optimization control for wastewater treatment process. Neural Comput Appl 2018;29:1261 71. https://doi.org/10.1007/s00521-016-2642-8.

[75] Arnell M, Rahmberg M, Oliveira F, Jeppsson U. Multi-objective performance assessment of wastewater treatment plants combining plant-wide process models and life cycle assessment. J Water Clim Chang 2017;8:715 29. https://doi.org/10.2166/wcc.2017.179.

[76] Flores-Alsina X, Gallego A, Feijoo G, Rodriguez-Roda I. Multiple-objective evaluation of wastewater treatment plant control alternatives. J Environ Manage 2010;91:1193 201. https://doi.org/10.1016/j.jenvman.2010.01.009.

[77] Endo A, Yamada M, Miyashita Y, Sugimoto R, Ishii A, Nishijima J, et al. Dynamics of water energy food nexus methodology, methods, and tools. Curr Opin Environ Sci Heal 2020;13:46 60. https://doi.org/10.1016/j.coesh.2019.10.004.

[78] Massara TM, Solís B, Guisasola A, Katsou E, Baeza JA. Development of an ASM2d-N2O model to describe nitrous oxide emissions in municipal WWTPs under dynamic conditions. Chem Eng J 2018;335:185 96. https://doi.org/10.1016/j.cej.2017.10.119.

[79] Henze M, Gujer W, Mino T, van Loosdrecht M. Activated Sludge Models ASM1, ASM2, ASM2d and ASM3. London, UK: IWA Publishing; 2000. https://doi.org/https://doi.org/10.2166/9781780402369.

[80] Mannina G, Ekama G, Caniani D, Cosenza A, Esposito G, Gori R, et al. Greenhouse gases from wastewater treatment - a review of modelling tools. Sci Total Environ 2016;551 552:254 70. https://doi.org/10.1016/j.scitotenv.2016.01.163.

[81] Alex J, Benedetti L, Copp J, Gernaey K V, Jeppsson U, Nopens I, et al. Benchmark Simulation Model no . 1 (BSM1). Control 2008;1.

[82] IWA. BSM1. IWA Model Integr Assess; 2020.

[83] Jeppsson U, Pons M-N, Nopens I, Alex J, Copp JB, Gernaey KV, et al. Benchmark Simulation Model no . 2 (BSM2). Water Sci Technol 2018;1:99. https://doi.org/10.1016/0005-7967(76)90069-3.

[84] Mannina G, Rebouças TF, Cosenza A, Chandran K. A plant-wide wastewater treatment plant model for carbon and energy footprint: Model application and scenario analysis. J Clean Prod 2019;217:244 56. https://doi.org/10.1016/j.jclepro.2019.01.255.

[85] ISPRA. Italian Greenhouse Gas Inventory 1990-2018. Roma; 2020.

[86] Federal Office for the Environment FOEN. Switzerland s Greenhouse Gas Inventory 1990-2018. Bern; 2020.

[87] IPCC. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories; 2019.

[88] Gustavsson DJI, Tumlin S. Carbon footprints of Scandinavian wastewater treatment plants. Water Sci Technol 2013;68:887 93. https://doi.org/10.2166/wst.2013.318.

[89] Behling Ian, Bonifazi E, Hinton S. Workbook For Estimating Operational GHG Emissions, Version 10. UK Water Industry Research Limited; 2016.

[90] Rodriguez-Garcia G, Hospido A, Bagley DM, Moreira MT, Feijoo G. A methodology to estimate greenhouse gases emissions in Life Cycle Inventories of wastewater treatment plants. Environ Impact Assess Rev 2012;37:37 46. https://doi.org/10.1016/j.eiar.2012.06.010.

[91] Guo L, Porro J, Sharma KR, Amerlinck Y, Benedetti L, Nopens I, et al. Towards a benchmarking tool for minimizing wastewater utility greenhouse gas footprints. Water Sci Technol 2012;66:2483 95. https://doi.org/10.2166/wst.2012.495.

[92] Guisasola A, Sharma KR, Keller J, Yuan Z. Development of a model for assessing methane formation in rising main sewers. Water Res 2009;43:2874 84. https://doi.org/10.1016/j.watres.2009.03.040.

[93] Sweetapple C, Fu G, Butler D. Identifying key sources of uncertainty in the modelling of greenhouse gas emissions from wastewater treatment. Water Res 2013;47:4652 65. https://doi.org/10.1016/j.watres.2013.05.021.

[94] Larsen TA. CO2-neutral wastewater treatment plants or robust, climate-friendly wastewater management? A systems perspective. Water Res 2015;87:513 21. https://doi.org/10.1016/j.watres.2015.06.006.

[95] Gao H, Scherson YD, Wells GF. Towards energy neutral wastewater treatment: methodology and state of the art. Environ Sci Process Impacts 2014;16:1223 46. https://doi.org/10.1039/c4em00069b.

[96] Kim D, Bowen JD, Ozelkan EC. Optimization of wastewater treatment plant operation for greenhouse gas mitigation. J Environ Manage 2015;163:39 48. https://doi.org/10.1016/j.jenvman.2015.07.005.

[97] Chou MS, Cheng WH. Gaseous emissions and control in wastewater treatment plants. Environ Eng Sci 2005;22:591 600. https://doi.org/10.1089/ees.2005.22.591.

[98] C-FOOT-CTRL. Developing on line tools to monitor, control and mitigate GHG emissions in WWTPs - Deliverable 3.1 Development of the dynamic software tool; 2016.

[99] Rosso D, Jiang LM, Sobhani R, Wett B. Energy Footprint Modelling: A tool for process optimisation in Large Wastewater Treatment Plants. Water Pract Technol 2012;7. https://doi.org/10.2166/wpt.2012.018.

[100] Haas D De, Foley J, Lant P. Energy and Greenhouse Footprints of Wastewater Treatment Plants in South-East Queensland. Adv Water Manag Centre, Univ Queensland, St Lucia, Brisbane, QLD 4072 2008.

[101] Masuda S, Sano I, Hojo T, Li YY, Nishimura O. The comparison of greenhouse gas emissions in sewage treatment plants with different treatment processes. Chemosphere 2018;193:581 90. https://doi.org/10.1016/j.chemosphere.2017.11.018.

[102] Khan MA, Ngo HH, Guo W, Liu Y, Nghiem LD, Chang SW, et al. Optimization of hydraulic retention time and organic loading rate for volatile fatty acid production from low strength wastewater in an anaerobic membrane bioreactor. Bioresour Technol 2019;271:100 8. https://doi.org/10.1016/j.biortech.2018.09.075.

[103] Lazarova V, Choo K-H, Cornel P. Water-Energy Interactions in Water Reuse. vol. 11. London, UK: IWA Publishing; 2012. https://doi.org/10.2166/9781780400662.

[104] McGlade J, Werner B, Young M, Matlock M, Jefferies D, Sonnemann G, et al. Measuring Water Use in a Green Economy; 2012.

[105] Hoekstra AY, Chapagain AK, Aldaya MM, Mekonnen MM. The Water Footprint Assessment Manual: Setting the Global Standard. London, UK: Earthscan; 2011. https://doi.org/10.1080/0969160x.2011.593864.

[106] Kounina A, Margni M, Bayart JB, Boulay AM, Berger M, Bulle C, et al. Review of methods addressing freshwater use in life cycle inventory and impact assessment. Int J Life Cycle Assess 2013;18:707 21. https://doi.org/10.1007/s11367-012-0519-3.

[107] Hoekstra AY. Water footprint assessment: evolvement of a new research field. Water Resour Manag 2017;31:3061 81. https://doi.org/10.1007/s11269-017-1618-5.

[108] Paterson W, Rushforth R, Ruddell BL, Konar M, Ahams IC, Gironas J, et al. Water footprint of cities: a review and suggestions for future research. Sustain 2015;7:8461 90. https://doi.org/10.3390/su7078461.

[109] Morillo JG, Martín M, Camacho E, Díaz JAR, Montesinos P. Toward precision irrigation for intensive strawberry cultivation. Agric Water Manag 2015;151:43 51. https://doi.org/10.1016/j.agwat.2014.09.021.

[110] Jefferies D, Munoz I, Hodges J, King VJ, Aldaya M, Ercin AE, et al. Water footprint and life cycle assessment as approaches to assess potential impacts of products on water consumption. Key learning points from pilot studies on tea and margarine. J Clean Prod 2012;33:155 66. https://doi.org/10.1016/j.jclepro.2012.04.015.

[111] Feng K, Hubacek K, Pfister S, Yu Y, Sun L. Virtual scarce water in China. Environ Sci Technol 2014;48:7704 13. https://doi.org/10.1021/es500502q.

[112] Mila I, Canals L, Chenoweth J, Chapagain A, Orr S, Anton A, et al. Assessing freshwater use impacts in LCA: Part I - inventory modelling and characterisation factors for the main impact pathways. Int J Life Cycle Assess 2009;14:28 42. https://doi.org/10.1007/s11367-008-0030-z.

[113] Kounina A, Margni M, Shaked S, Bulle C, Jolliet O. Spatial analysis of toxic emissions in LCA: a sub-continental nested USEtox model with freshwater archetypes. Environ Int 2014;69:67 89. https://doi.org/10.1016/j.envint.2014.04.004.

[114] Boulay AM, Hoekstra AY, Vionnet S. Complementarities of water-focused life cycle assessment and water footprint assessment. Environ Sci Technol 2013;47:11926 7. https://doi.org/10.1021/es403928f.

[115] Morera S, Corominas L, Poch M, Aldaya MM, Comas J. Water footprint assessment in wastewater treatment plants. J Clean Prod 2016;112:4741 8. https://doi.org/10.1016/j.jclepro.2015.05.102.

[116] Saagi R, Flores-Alsina X, Kroll S, Gernaey KV, Jeppsson U. A model library for simulation and benchmarking of integrated urban wastewater systems. Environ Model Softw 2017;93:282 95. https://doi.org/10.1016/j.envsoft.2017.03.026.

[117] Achleitner S, Moderl M, Rauch W. CITY DRAIN - an open source approach for simulation of integrated urban drainage systems. Environ Model Softw 2007;22:1184 95. https://doi.org/10.1016/j.envsoft.2006.06.013.

[118] Saagi R. Benchmark Simulation Model for Integrated Urban Wastewater Systems : Model Development and Control Strategy Evaluation; 2017.

[119] Baeza J, Gabriel D, Guisasola A, Lafuente J, Katsou E, Massara T, et al. On-line monitoring, control and mitigation of greenhouse gases emissions in WWTPs. In: 7th Int. Conf. Biotech. Air Pollut. Control Bioenergy, La Coruna, Spain; 2017.

[120] Porro J, Guo L, Sharma KR, Amerlinck Y, Benedetti L, Shaw A, et al. Towards a benchmarking tool for minimizing wastewater utility greenhouse gas footprints. 8th IWA Symp. Syst. Anal. Integr. Assess., vol. 66, 2012, p. 2483 95. https://doi.org/10.2166/wst.2012.495.

[121] Tian H, Lu C, Pan S, Yang J, Miao R, Ren W, et al. Optimizing resource use efficiencies in the food energy water nexus for sustainable agriculture: from conceptual model to decision support system. Curr Opin Environ Sustain 2018;33:104 13. https://doi.org/10.1016/j.cosust.2018.04.003.

[122] Daccache A, Ciurana JS, Rodriguez Diaz JA, Knox JW. Water and energy footprint of irrigated agriculture in the Mediterranean region. Environ Res Lett 2014;9. https://doi.org/10.1088/1748-9326/9/12/124014.

[123] UNEP/MAP Plan Bleu. State of the environment and development in the Mediterranean. Technical Report. Athens; 2019.

[124] Fader M, Shi S, Von Bloh W, Bondeau A, Cramer W. Mediterranean irrigation under climate change: More efficient irrigation needed to compensate for increases in irrigation water requirements. Hydrol Earth Syst Sci 2016;20:953 73. https://doi.org/10.5194/hess-20-953-2016.

[125] Sukhwani V, Shaw R, Mitra BK, Yan W. Optimizing Food-Energy-Water (FEW) nexus to foster collective resilience in urban-rural systems. Prog Disaster Sci 2019;1:100005. https://doi.org/10.1016/j.pdisas.2019.100005.

[126] Qi X, Li J, Yuan W, Wang RY. Coordinating the food-energy-water nexus in grain production in the context of rural livelihood transitions and farmland resource

constraints. Resour Conserv Recycl 2021;164:105148. https://doi.org/10.1016/j.resconrec.2020.105148.
[127] Medina-Santana AA, Flores-Tlacuahuac A, Cardenas-Barron LE, Fuentes-Cortes LF. Optimal design of the water-energy-food nexus for rural communities. Comput Chem Eng 2020;143:107120. https://doi.org/10.1016/j.compchemeng.2020.107120.
[128] Jaramillo MF, Restrepo I. Wastewater reuse in agriculture: a review about its limitations and benefits. Sustain 2017;9. https://doi.org/10.3390/su9101734.
[129] Miller-Robbie L, Ramaswami A, Amerasinghe P. Wastewater treatment and reuse in urban agriculture: exploring the food, energy, water, and health nexus in Hyderabad, India. Environ Res Lett 2017;12. https://doi.org/10.1088/1748-9326/aa6bfe.
[130] Sapkota AR. Water reuse, food production and public health: Adopting transdisciplinary, systems-based approaches to achieve water and food security in a changing climate. Environ Res 2019;171:576 80. https://doi.org/10.1016/j.envres.2018.11.003.
[131] Weckenbrock P, Alabaster G. Designing Sustainable Wastewater Reuse Systems: Towards an Agroecology of Wastewater Irrigation. Gov. Nexus Water, Soil Waste Resour. Considering Glob. Chang., Switzerland: Springer International Publishing; 2015, p. 1 230. https://doi.org/10.1007/978-3-319-05747-7_8.
[132] Kurian M. The water-energy-food nexus: Trade-offs, thresholds and transdisciplinary approaches to sustainable development. Environ Sci Policy 2017;68:97 106. https://doi.org/10.1016/j.envsci.2016.11.006.
[133] Fernandez García I, Merida García A, Rodríguez Díaz JA, Barrios PM, Poyato EC. Water-energy nexus in irrigated areas. Lessons from real case studies. Water Scarcity Sustain. Agric. Semiarid Environ. Tools, Strateg. Challenges Woody Crop., Elsevier; 2018, p. 41 59. https://doi.org/10.1016/B978-0-12-813164-0.00002-8.
[134] Smith R, Baillie J, McCarthy A, Raine SR, Baillie CP. Review of precision irrigation technologies and their application. Natl Cent 2010.
[135] Kamienski C, Soininen JP, Taumberger M, Dantas R, Toscano A, Cinotti TS, et al. Smart water management platform: IoT-based precision irrigation for agriculture. Sensors (Switzerland) 2019;19. https://doi.org/10.3390/s19020276.
[136] Abioye EA, Abidin MSZ, Mahmud MSA, Buyamin S, Ishak MHI, Rahman MKIA, et al. A review on monitoring and advanced control strategies for precision irrigation. Comput Electron Agric 2020;173:105441. https://doi.org/10.1016/j.compag.2020.105441.
[137] Allen RG, Pereira LS, Raes D, Smith M. FAO Irrigation and Drainage Paper No.56. Food Agric Organ United Nations 1998;56:e156. https://doi.org/10.1016/S0141-1187(05)80058-6.
[138] Colding J, Barthel S. An urban ecology critique on the Smart City model. J Clean Prod 2017;164:95 101. https://doi.org/10.1016/j.jclepro.2017.06.191.
[139] Naphade M, Banavar G, Harrison C, Paraszczak J, Morris R. Smarter cities and their innovation challenges. Computer (Long Beach Calif) 2011;44:32 9.
[140] Klein B, Koenig R, Schmitt G. Managing Urban Resilience: Stream Processing Platform for Responsive Cities. Informatik-Spektrum 2017;40:35 45. https://doi.org/10.1007/s00287-016-1005-2.
[141] Hosseini S, Frank L, Fridgen G, Heger S. Do not forget about smart towns: how to bring customized digital innovation to rural areas. Bus Inf Syst Eng 2018;60:243 57. https://doi.org/10.1007/s12599-018-0536-2.
[142] Ramaswami A, Russell AG, Culligan PJ, Rahul Sharma K, Kumar E. Meta-principles for developing smart, sustainable, and healthy cities. Science (80-) 2016;352:940 3. https://doi.org/10.1126/science.aaf7160.
[143] Boyle T, Giurco D, Mukheibir P, Liu A, Moy C, White S, et al. Intelligent metering for urban water: a review. Water (Switzerland) 2013;5:1052 81. https://doi.org/10.3390/w5031052.
[144] Stewart RA, Nguyen K, Beal C, Zhang H, Sahin O, Bertone E, et al. Integrated intelligent water-energy metering systems and informatics: visioning a digital multi-utility service provider. Environ Model Softw 2018;105:94 117. https://doi.org/10.1016/j.envsoft.2018.03.006.
[145] Sitzenfrei R, Rauch W. From Water Networks to a Digital City : a Shift of Paradigm in Assessment of Urban Water Systems. 12th Int Conf Urban Drain 2011:9.
[146] Ejeian F, Etedali P, Mansouri-Tehrani HA, Soozanipour A, Low ZX, Asadnia M, et al. Biosensors for wastewater monitoring: a review. Biosens Bioelectron 2018;118:66 79. https://doi.org/10.1016/j.bios.2018.07.019.
[147] Mikkelsen PS. Water solutions for smart cities Smart water systems make cities more liveable, resilient and sustainable. In: Smart Cities - creating liveable, sustainable and prosperous societies, State of Green; 2018, p. 14 14.
[148] Schulze J, Martin R, Finger A, Henzen C, Lindner M, Pietzsch K, et al. Design, implementation and test of a serious online game for exploring complex relationships of sustainable land management and human well-being. Environ Model Softw 2015;65:58 66. https://doi.org/10.1016/j.envsoft.2014.11.029.
[149] Mendler de Suarez J, Bachofen C, Fortugno N, Goentzel J, Gonçalves P, Grist N, et al. Games for a new climate : Experiencing the complexity of future risks. In: Report, P.C.T.F. (Ed.), The Frederick S.Pardee Center for the Study of the Longer-range Future. Boston: 2012.
[150] Aubert AH, Bauer R, Lienert J. A review of water-related serious games to specify use in environmental Multi-Criteria Decision Analysis. Environ Model Softw 2018;105:64 78. https://doi.org/10.1016/j.envsoft.2018.03.023.
[151] Mildner P, 'Floyd Mueller F. Design of Serious Games. In: Dorner R, Gobel S, Effelsberg W, Wiemeyer J, editors. Serious Games Found. Concepts Pract., Cham: Springer International Publishing; 2016, p. 57 82. https://doi.org/10.1007/978-3-319-40612-1_3.
[152] Susnik J, Chew C, Domingo X, Mereu S, Trabucco A, Evans B, et al. Multi-stakeholder development of a serious game to explore the water-energy-food-land-climate nexus: The SIM4NEXUS approach. Water (Switzerland) 2018;10. https://doi.org/10.3390/w10020139.
[153] Medema W, Furber A, Adamowski J, Zhou Q, Mayer I. Exploring the potential impact of serious games on social learning and stakeholder collaborations for transboundary watershed management of the St. Lawrence river basin. Water (Switzerland) 2016;8. https://doi.org/10.3390/w8050175.
[154] Savic DA, Morley MS, Khoury M. Serious gaming for water systems planning and management. Water (Switzerland) 2016;8:1 17. https://doi.org/10.3390/w8100456.
[155] Van der Wal MM, de Kraker J, Kroeze C, Kirschner PA, Valkering P. Can computer models be used for social learning? A serious game in water management. Environ Model Softw 2016;75:119 32. https://doi.org/10.1016/j.envsoft.2015.10.008.
[156] Prat P, Aulinas M, Turon C, Comas J, Poch M. Role playing games: a methodology to acquire knowledge for integrated wastewater infrastructures management in a river basin scale. Water Sci Technol 2009;59:1809 16. https://doi.org/10.2166/wst.2009.212.
[157] Douven W, Mul ML, Son L, Bakker N, Radosevich G, Hendriks A. Games to Create Awareness and Design Policies for Transboundary Cooperation in River Basins: Lessons from the Shariva Game of the Mekong River Commission. Water Resour Manag 2014;28:1431 47. https://doi.org/10.1007/s11269-014-0562-x.
[158] Arbesser-Rastburg G, Fuchs-Hanusch D. Serious sensor placement-optimal sensor placement as a serious game. Water (Switzerland) 2020;12. https://doi.org/10.3390/w12010068.
[159] Bidoglio G, Vanham D, Bouraoui F, Barchiesi S. The water-energy-food-ecosystems (WEFE) nexus. Encycl Ecol 2018;4:459 66. https://doi.org/10.1016/B978-0-12-409548-9.11036-X.
[160] Biggs EM, Bruce E, Boruff B, Duncan JMA, Horsley J, Pauli N, et al. Sustainable development and the water-energy-food nexus: a perspective on livelihoods. Environ Sci Policy 2015;54:389 97. https://doi.org/10.1016/j.envsci.2015.08.002.

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Water-Energy-Food-Climate Nexus in an Integrated Peri-Urban Wastewater Treatment and Reuse System: From Theory to Practice

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Abstract: This paper develops a framework for the identification, assessment and analysis of the water reuse-carbon-energy-food-climatic (WEFC) nexus in an integrated peri-urban wastewater treatment and reuse system. This methodology was applied to the municipal wastewater treatment plant (WWTP) of Peschiera Borromeo (Milan, Italy) and its peri-urban district to define the most possible affirmations and conflicts following the EU regulations 741/2020. Results of this work showed that transferring the WEFC nexus from theory to practice can realize sustainable resource management in the operating environment by providing a reduction in greenhouse gas (GHG) emissions, overall energy savings, reduction in water stress and optimization of agricultural practices. Particularly, it was found that if the plant configuration is upgraded to reach water quality class C for water reuse, instead of wastewater discharge, energy savings are estimated to reach up to 7.1% and carbon emissions are supposed to be reduced up to 2.7%. In addition, enhancing water quality from class C to class A resulted in increments in energy and carbon footprint of 5.7% and 1.7%, respectively. Nevertheless, higher quality crops can be cultivated with reclaimed water in class A, with bigger economic revenues and high recovery of nutrients (e.g., recovery of 154,450 kg N/y for tomato cultivation).

Keywords: carbon footprint; energy assessment; greenhouse gas emission; nutrients recovery; wastewater disinfection; agricultural reuse

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1. Introduction

Water is rooted and highly interlinked to natural resources and their productivity [1]. Water management is indeed one of the pillars for the efficiency of the balance and resilience of many productivity sectors such as energy production and transmission [2]. Fragmented management of interrelated sectors, as well as their separated analysis without considering the integrated system, hide a potential risk of not achieving the desired outcomes [3]. This is caused by underestimated or even unexplored synergies, antagonisms, and ripple effects. The shift of integrated management (so-called nexus approach) requires a shift towards integrated approaches for systems analysis and assessment as well [4]. Hence, the aim should be at using water-related nexus, targeting the holistic systems analysis, and revealing the multiple and complex interactions and feedback loops between technical and biological cycles [5]. A multi-sectoral system analysis framework symbiotically managing

different resources (e.g., water, energy) is needed for modelling both socio-economic (e.g., food) and non-economic (e.g., climate) sectors to investigate the complex interactions and interdependencies between the sectors in order to promote synergies and reduce antagonisms between them [6,7]. The water-energy-food-climate (WEFC) nexus is a systematic approach to scientific investigation and design of coherent policy goals and instruments that focuses on synergies, conflicts and related trade-offs emerging in the interactions between water, energy, food and climate at bio-physical, cyber-physical, socio-economic, and governance level [8].

Urban water management considers water supply, wastewater treatment and disposal as an integrated system. The increasing demand for good quality water brings a higher necessity for treatment that enables water reuse, which in turn demands more energy use and associated greenhouse gas (GHG) emissions [9]. The energy-intensive nature of wastewater treatment creates an important part in sustaining urban water systems [10]. The modern thinking about urban water systems not only regards augmenting the existing water supply with new and alternative water sources but also ensuring that adaptive measures are applied for climate change mitigation by reducing energy use and GHG emissions [5,11].

Some authors discussed the nexus addressing issues through water and energy use in agriculture [7,12], while others focused on WWTP boundaries [13] or on water supply and treatment lines [10]. In most cases, the Water-Energy-Food (WEF) nexus is applied, and climate is often neglected in the nexus approach [14]. However, climate change has been considered one of the most threatening factors that put the sustainability of the WEF nexus at risk [15], especially considering water availability [16].

In the wastewater reuse-based agricultural sector, energy-carbon and water footprints are highly interconnected and exhibit a great example of the Water-Energy-Food-Climate (WEFC) nexus [17]. WWTPs are great energy carriers [18,19] and responsible for up to 26% of the GHG emissions of the whole water supply chain [20]. From an environmental and economic point of view related to climate change, any reduction in energy consumption and associated with GHG emissions is valuable [21]. Furthermore, the nutrients and reuse potential of reclaimed wastewater are valuable and can be reutilized in urban agriculture as a potential strategy to support local communities. Between 50% and 90% of the total water demand is represented by agriculture, and the reuse for agricultural irrigation is by far the most established end-use for reclaimed water and is seen as a solution to overcome global water stress [22]. Hence, integrated wastewater treatment and reuse systems are a typical case of WEFC interactions both at city and farm levels, bridging the gap between food and climate sectors. For instance, Gondhalekar and Ramsauer [11] applied the urban WEF nexus for climate change adaptation in a neighborhood of Munich, Germany. The authors provided 26% saving of current freshwater supply by wastewater recycling and reuse coupled with rainwater harvesting as well as 66% of local demand for fruit and 246% of local demand for vegetables by intensive urban agriculture.

In recent years, EU regulations such as 2020/741 "Regulation on minimum requirements for water reuse" [23] and strategies such as "Farm to Fork" have enabled the valorization of wastewater. In the meantime, many wastewater utility managers are seeking to implement nexus-oriented projects to support their city's energy transition policy [24]. However, the nexus has remained conceptual so far and eventually many operators and/or reclamation managers do not know how this approach can bring added value to the operating environment. Currently, innovative ways such as serious games, participatory processes, and bottom-up approaches are adopted to make the nexus more tangible and usable by water utilities, policymakers and citizens [25,26]. The nexus analysis in urban development requires a common matrix to measure the carbon, energy and water efficiency of proposed scenarios and technical options. In many cases, relative carbon, energy and water footprints are calculated and evaluated to quantify environmental impacts [27]. According to the European Environmental Agency (EEA), in 2007, at least 11% of Europe's population and 17% of the territory were estimated to be affected by water scarcity, with a

related cost of droughts over the past thirty years at EUR 100 billion. In the Mediterranean region, 20% of the population lives under constant water stress, which increases up to over 50% in summer. Moreover, water losses are important issues to be addressed for a more efficient distribution system. Non-conventional water resources, e.g., reclaimed wastewater reuse for irrigation, are innovating solutions to be implemented by a smart water management system. It can guarantee safe and good quality of reclaimed water for irrigation, and it will help to satisfy the high-water demand of the agriculture sector. In this paper, we therefore wanted to showcase a practical application of the WEFC nexus in an integrated peri-urban system for wastewater treatment and reuse system in Milan, Italy, to highlight the relationships between different sectors including wastewater treatment, irrigation infrastructure and agricultural district. In this regard, the nexus approach was adopted to define connections and interactions between water reuse, carbon and energy footprint, as well as food production in various water reuse scenarios and conditions.

2. Materials and Methods

2.1. Description of the WWTP and Peri-Urban Area

The peri-urban district of Peschiera Borromeo WWTP is geographically located in the Lombardy Padana Plain. The treatment capacity of the plant is 566,000 population equivalent (PE) with a total average inflow rate of 216,000 m^3/d treated in two different wastewater lines. Line 1 consists of a conventional activated sludge process followed by biological filtration to remove inorganic nitrogen and a final chemical disinfection using peracetic acid. Line 2 is designed and will be used for reuse purposes that include a two-stage upflow biological filtration (Biofor®) and two parallel lines of ultra-violet (UV) disinfection. The water demand of the peri-urban areas in the south of Milan (near Parco Agricolo Sud Milano) is mainly required for irrigation purposes. The surrounding agricultural land has an area of approximately 2500 ha and its main crop is corn, and its water demand can be widely covered by the treated effluent of Line 2 of the WWTP. Agriculture is a crucial sector for the economy of the region. The most common irrigation techniques are still traditional and rely on border irrigation, which implies a water demand of about 1.5–2 L/s/ha of over the agricultural season. The current water sources come from rivers, and it is distributed by gravity through a network of open unlined canals. The irrigation network of canals and the related water delivery systems that supply water to farmers is managed by the consortium Est Ticino Villoresi, which is one of the most important irrigation consortia in Italy. Current water distribution and irrigation systems are still characterized by poor efficiency and are affected by significant losses. In addition, a decrease in freshwater availability is observed in the territory, which makes water reuse a valid option.

2.2. Nexus Approach

For the evaluation of the WEFC nexus, single sector footprints and single subsystem' stages were initially considered, to define the significative elements that characterized each impact. Footprints were first calculated individually for each sector, from energy to carbon and water, and were determined separated for each stage of the integrated system, from the WWTP to the field. Where available, standardized methods were used.

2.3. Energy Footprint

The recently standardized ENERWATER methodology [19] was adopted to assess the energy audit of the WWTP. All the electromechanical equipment serving the treatment units was listed, defining for each the working hours, the absorbed power, and the functioning characteristics. Measured data from the energy meters installed along the treatment units were collected and elaborated, to evaluate the monitored consumptions and assign to each unit the respective energy demand. Moreover, energy bills were collected to verify effective consumption. Each electromechanical equipment was assigned to one of the 5 stages in which the plant layout was divided into. In this way, consumption could

be normalized in respect to the parameter that most affected the single stage, i.e., the entering flowrates (in m^3) for the preliminary treatments (stage 1), the amount of total solids removed (kg TSS removed) for primary treatments (stage 2), the amounts of organics and nutrients removed for the biologic unit (stage 3) expressed in terms of total pollutant equivalent (kg TPE removed), the pathogen log reduction for m^3 of treated water for the disinfection unit (stage 4), and the solids load processed expressed as total solid equivalent (kg TSE processed) for sludge treatment (stage 5). In this way, key performance indicators (KPIs) were calculated according to the standard method [19]. Calculations were performed considering one year operation of the plant. Finally, a global indicator of plant performances, the "water treatment energy index" (WTEI), was calculated and a label describing the global efficiency of the plant was assigned as reported in Table 1.

Table 1. Labels for the general representation of a WWTP energy efficiency according to the WTEI (adapted by Longo et al. [19]).

ENERGY EFFICIENCY	WTEI
A	X < 0.110
B	0.110 X < 0.220
C	0.220 X < 0.330
D	0.330 X < 0.440
E	0.440 X < 0.550
F	0.550 X < 0.775
G	X 0.775

2.4. Carbon Footprint Assessment

The Italian Regulatory Authority for Energy, Networks and Environment (ARERA) introduced the additional indicator G5.3 "Carbon Footprint of the water treatment service" according to the standard ISO 14064-1, 2019. This guideline provides general information for the assessment of the carbon footprint of activity, but it does not include specifications for single sectors, such as water or wastewater service. International guidelines, such as IPCC [28], can support providing Emission Factors (EFs) groped in sectorial activities, but site-specific conditions can differ significantly from the ones assumed.

In this work, emissions were calculated according to Equation (1) in agreement with IPCC Guidelines [28].

$$Emission \ \frac{tonCO_2e}{y} \quad Activity\ data \ \frac{quantity}{y} \quad EF \ \frac{tonGHGs}{quantity} \quad GWP \qquad (1)$$

where, for each greenhouse gas (*GHG*), emissions were calculated multiplying an Activity Data (*AD*), which is the representative quantity of an activity or a service that cause the emission, by an Emission Factor (*EF*), which represent the specific contribution, and a Global Warming Potential (*GWP*) to convert each GHG into CO_2 equivalents.

In general, AD represents a quantity generated or used, of energy, mass, or volume, representing the key parameter for each emission category. As concerning WWTP, the main activities are related to the amount of wastewater and pollutants treated (m^3/y, tonCOD/y, tonN/y, ...), chemicals (ton/y) and energy consumed (MWh/y), as explained by Gustavsson and Tumlin [29]. Annual operational data were collected from the plant and elaborated to obtain the needed activity data to describe GHG emissions from a WWTP operation.

Applied EFs were collected from literature, guidelines, and national and international databases, such as IPCC guidelines [28,30–32] and ISPRA [33–35].

Emissions were grouped into categories, distinguishing between direct and indirect ones. Regarding direct emissions, the main sources were biological processes of organic oxidation, nitrification and denitrification [36]. Indirect emissions were related to energy consumption, chemical dosing and waste disposal and were considered too, even if their sources were located outside the physical boundaries of WWTPs, since they were caused

by specific management strategies under the responsibility of the water utility. The main sources of direct emissions included biogas combustion, WWTP processes and fugitive emissions, while emissions for dissolved gases on the water body, energy and chemical consumption, transports and sludge disposal were considered as indirect.

Utilized GWPs were obtained by the IPCC Fifth Assessment Report, AR5 (IPCC, 2019) [37].

2.5. Water Footprint

The water footprint was addressed by distinguishing four different cases of water quality, depending on the classes foreseen in EU Regulation 741/2020. The current discharge limits satisfy the requirements of class D, which was considered as the non-reuse option. The plant has to satisfy Italian Regulation 2006/52 for effluent quality, with stricter restrictions for nitrogen and phosphorus concentrations, which must be under 10 mg/L and 1 mg/L, respectively. Moreover, *E.coli* concentrations at the effluent must be lower than 5000 CFU/100 mL.

For the irrigation of crops where the edible part is not in direct contact with reclaimed water, class C could be used in case drip irrigation is applied to furtherly reduce risks; otherwise, a minimum of class B is necessary. Class A is required for food crops consumed raw where the edible part is in direct contact with reclaimed water. In this way, the quality class reached determined the possible applications available.

2.6. Water Reuse

Single footprints were interconnected to each other. For instance, energy consumption impacted both on energy footprint and on carbon footprint, since the emissions related to energy production must be included in the carbon footprint assessment. In the same way, the use of chemicals impacted both the GHG emitted and the energy requirements for their production. Moreover, to provide a certain quantity of water of a defined quality for irrigation, WWTP efficiency must be improved, increasing the energy demand to enhance treatment performances, especially in the disinfection unit, and increasing the related GHG emissions accordingly. Footprints at WWTP level were then extended, to include the agricultural sector. For water reuse applications, the cultivated crops to be irrigated with the reclaimed water were selected according to the requirements provided by the EU 741/2020 for each class and considering the most diffused species of the integrated peri-urban system in the surroundings of Peschiera Borromeo WWTP. Data on culture productivity, nutrient demand and carbon sequestration were collected from literature and national and international guidelines. Table 2 shows the demands of different crop types selected in this study. Water quality class influence the type of crops that could be irrigated, with varying water and nutrient demand, as well as different GHG emissions and carbon sequestration. Finally, it is important to highlight that the water demand for the cultivation of the three selected crops in the surrounding agricultural lands (2500 ha) of the peri-urban of Milan can be widely covered by the treated effluent of Line 2 of the WWTP (25,929,332 m^3/y).

Table 2. Characteristics and requirements for crops cultivated in the peri-urban area of Peschiera Borromeo.

Parameter	Unit	Corn	Carrot	Tomato
Crop productivity	ton/ha	10	50	100
Nutrient demand—N	kg N/ha	135–235	150	250
Nutrient demand—P	kg P/ha	58–80	70	65
Emissions/Carbon sequestration	tonCO_2e/ha	3.52	2.27	2.11
Crop water demand	m^3/ha	5000	5200	5400
Expected revenue	/kg	0.26	0.48	0.78

Different irrigation methods were taken into consideration to address the water demand of selected crops, varying from traditional to more technologic techniques, such

as drip irrigation. Each irrigation method is characterized by a certain level of efficiency, considered as the ratio between the quantity of water that effectively reaches the crop in respect to the amount of water provided. In these terms, surface irrigation is characterized by the lowest efficiency level, equal to 0.5; sprinkler irrigation has an efficiency of 0.7; drip irrigation, which is the most efficient method, has an efficiency of 0.9.

It is evident that drip irrigation is by far the technology able to provide the highest saving of water during irrigation. Hence, drip irrigation was selected as irrigation technology to calculate water needs for crops and energy consumption during irrigation. Energy requirements for irrigation and the consequent carbon footprint were calculated, to evaluate the requirements connected to the production of each crop and define the specific relationships between irrigation method, water demand, energy and carbon footprint. Once calculated single-subsystem footprints, different combinations of WWTP treatment efficiency level and irrigated crop were considered, and their integrated cross-sectorial footprints were defined. The information was collected to provide a reliable database for the implementation of a serious game, based on the real data of the demo-case of the peri-urban area of Peschiera Borromeo WWTP.

2.7. Assessment of Water Reuse Scenarios

For each scenario considered, single sector and single stage footprints were calculated and combined, linking energy requirements needed to achieve a defined water quality class, the water demand required to the cultivated crop depending also on the irrigation method used, and the related carbon footprint. Water quality impacted on energy consumptions and carbon footprint of the WWTP, but also limited the kind of crops that could be irrigated, as well as the irrigation method. On the other side, crop quality and irrigation techniques influenced the amount of water needed, as well as the energy and fuel consumption, GHG emissions and carbon sequestration. A summary of requirements needed for each scenario is provided in Table 3, where standard limits are related to the EU regulations 741/2020.

Table 3. Water reuse requirements for different scenarios according to EU Regulation 741/2020.

Parameter	Unit	Non Reuse	Reuse Class C	Reuse Class B	Reuse Class A
E.coli	CFU/100 mL	5000	1000	100	10
Biochemical Oxygen Demand (BOD_5)	mg/L	25	25	25	10
Total Suspended Solids (TSS)	mg/L	35	35	35	10
Turbidity	NTU	-	-	-	5
Irrigated crop	-	-	Corn	Corn	Corn-Tomato-Carrot
Irrigation method	-	-	Drip irrigation	All irrigation methods	All irrigation methods

2.7.1. Non-Reuse Scenario

The baseline scenario was the non-reuse case (Figure 1), where the WWTP treats wastewater to meet the minimum regulatory limits for discharge. Since in this case, the discharged wastewater is not in direct contact with cultures, no particular attention needs to be paid to toxicity issues for chemical overdosing. Considering that the conventional treatment line of Peschiera Borromeo WWTP was not designed for water reuse and was characterized by a chemical disinfection unit with peracetic acid, the same chemical was considered to simulate the non-reuse scenario. The destination for sludge disposal considered was the production of defecation gypsum, as it is one of the most common pathways in the area.

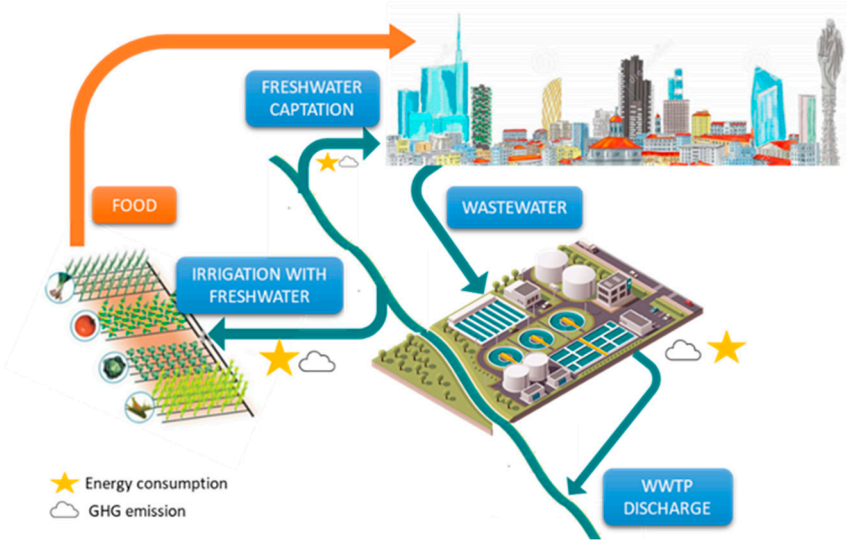


Figure 1. Scheme of the non-reuse scenario.

2.7. Reuse Scenarios

Different reuse scenarios were analyzed combining water quality class, irrigation method used and cultivated crop (Figure 2). On WWTP level, the treatment configuration included UV disinfection instead of chemical dosing.

Treatment Line 2 of Peschiera-Borromeo WWTP used biological filtration as secondary treatment, and it was already able to respect standards for BOD_5 and TSS related to Reuse class A (Table 3). In any case, standard limits for BOD_5 and TSS could be easily accomplished by adding a sand-filtration unit after the biological stage. UV doses were selected to respect limits for *E. coli* provided by the EU regulations 741/2020 for the different water quality classes [32,33].

In the peri-urban district of Milan, water reuse was practiced according to crop demand, for five months between April and August.

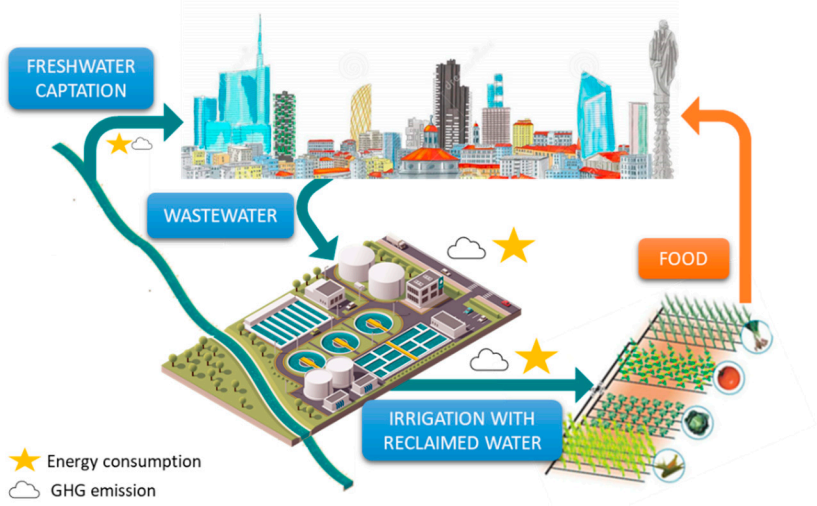


Figure 2. Scheme of the reuse scenarios.

Reuse class C

Class C was characterized by the lowest quality requirements for food crop irrigation but implied additional measures to guarantee an acceptable level of risk. Edible parts of crops must have no direct contact with reclaimed water and must be irrigated using more precise techniques, such as the drip method. Even if nutrient content in reclaimed water is usually not sufficient to compensate crop demand, part of the synthetic fertilizer commonly used for crop growth could be saved anyway. It is specified that in the application in the exam, nutrients concentration in wastewater were assumed to be in compliance with discharge limits of current Italian regulation 152/2006, since in case of non-acceptability for reuse, water quality is needed in any case to satisfy requirements for discharge.

usually not sufficient to compensate crop demand, part of the synthetic fertilizer commonly used for crop growth could be saved anyway. It is specified that in the application in the exam, nutrients concentration in wastewater were assumed to be in compliance with discharge limits of current Italian regulation 152/2006, since in case of non-acceptability for reuse, water quality is needed in any case to satisfy requirements for discharge. Further improvements could consider fertigation practices or sludge application on the cultivated fields, to optimize resources recovery and further decrease impacts due to nutrient removal from the water line or sludge disposal. However, all analyzed reuse scenarios were focused on the water line, while sludge application in the cultivated fields was not considered, since further considerations on the type of post-treatment and final product quality were needed.

Reuse class B

If reclaimed water was provided with class B standards, any irrigation method could be applied for food crops without edible parts in direct contact with water. In this way, the reclaimed water provided by the WWTP could be used for a wider range of potential users, that did not need to satisfy specific requirements of irrigation techniques applied. On the other side, the energy and carbon footprint of WWTP would increase, since more efforts were required to provide a higher quality reclaimed water, especially as concern tertiary treatments for pathogen reduction.

Reuse class A

Reclaimed water in class A had the strictest quality standards. All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw could be irrigated and all irrigation methods could be used. WWTPs must increase the efficiencies of their tertiary and disinfection treatments, to guarantee concentration of *E. coli* as low as 10 CFU/100 mL. Where UV disinfection units were applied, higher energy consumptions for lamps and consequently higher carbon footprint were foreseen; meanwhile, for chemical units, the higher dosage of reagents was necessary, which implied an increase in energy and carbon footprint related to their production. Moreover, overdosage must not impact health and environmental risk for water reuse [38].

3. Results

Benefits and Applications from Reuse Scenarios

Considering only at the WWTP level, delivering a lower quality effluent seems to imply fewer impacts, since the plant consumes less energy and thus produces fewer carbon emissions. However, it is also characterized by less efficient configuration. In the same way, on the agriculture side, when the treated wastewater is discharged, high-quality fresh water is consumed for irrigation purposes and a greater quantity of synthetic fertilizers is required for crop growth.

Footprint assessments showed the interconnections between water, energy and carbon in an application of water reuse for agricultural irrigation. Carbon emissions and energy consumptions varied depending on the water quality required to be delivered (Table 4). For example, upgrading plant performances to reach higher quality classes of treated wastewater implied an increase in the energy and in the carbon footprint of the plant, but higher quality crops could be cultivated with the reclaimed wastewater. In Table 4 calculations have also taken into account the energy consumption (1160 MWh/y) and GHGs emission (515 tonCO_2eq/y) for drip irrigation of the investigated district.

If the WWTP is upgraded to reach water quality class C, instead of wastewater discharge, energy savings were estimated to reach 7.1%, in agreement with results of previous studies [39,40], and carbon emissions were supposed to be reduced up to 2.7%. When the water quality was aimed to reach class B, then 5.8% energy footprint reduction was achieved, while the carbon footprint was reduced by 2.0%. However, enhancing water quality from class C to class A implied the additional WWTP increments on energy and carbon footprint of 5.7% and 1.7%, respectively. On a more global and complex view, however, lower quality effluent wastewater means more contamination and bigger impacts on health and the environment. Moreover, higher quality crops can be cultivated

with reclaimed water in class A, with bigger economic revenues for farmers. To provide the readers with additional information on carbon footprint calculation, details of GHG emissions grouped for categories and related to the operation of Peschiera-Borromeo WWTP are reported in Table 5.

Table 4. Footprints for reuse and non-reuse scenarios.

Category	Unit	Non-Reuse	Reuse Class C	Reuse Class B	Reuse Class A
Energy footprint	MWh/y	16,374	15,196	15,427	16,121
Carbon footprint	tonCO_2eq/y	24,505	23,849	23,952	24,260
Treated wastewater	millions of m^3/y	25.9			
Nutrients in the wastewater effluent	mgN/L	7.8			
	mgP/L	0.6			

Table 5. GHGs emissions at Peschiera-Borromeo WWTP grouped by categories.

GHG Emission Category	Unit	Value			
Biogenic emission due to WWTP operation	tonCO2eq/y	7278			
Transport	tonCO2eq/y	66			
Use of chemicals	tonCO2e/y	2787			
Dissolved gases in the effluent	tonCO2eq/y	6937			
Waste management (production of defecation gypsum from sludge)	tonCO2eq/y	753			
Energy consumption for WWTP operation	tonCO2eq/y	4718			
Disinfection	tonCO2eq/y	Chemical 1451	UV-Class C 795	UV-Class B 898	UV-Class A 1206

In a similar way, information about energy consumptions of the different units of Peschiera-Borromeo WWTP are reported in Table 6 in terms of KPI and WTEI in agreement with the standardized ENERWATER methodology [19]. Particularly, it is interesting to observe that the upgrade of the disinfection units did not determine changes in the classification of the energy efficiency of the plant, which remained in class F.

Finally, in Table 7 are resumed data on saved energy, reduced GHG emission, recovered water and nutrients for the irrigation of corn, carrot and tomato with reclaimed water compared to the non-reuse scenario. During drip irrigation, the edible part of corn is not in direct contact with reclaimed water. Hence, this crop can be irrigated by class C water. On the contrary, tomato and carrot need to be irrigated by class A water. Particularly, the lowest energy consumption and GHG emission were obtained during corn irrigation, due to the smallest energy need for UV disinfection (Table 4). On the contrary, during irrigation of carrots and tomatoes, which have a higher water demand (Table 2), it was possible to have a higher recovery of nutrients and water. In addition, these two crops can have a higher economic revenue (Table 1).

Table 6. KPIs for energy consumptions at Peschiera-Borromeo WWTP grouped by treatment stages and WTEI related to one year of operation.

Stage	Treatment Unit	Unit	Value			
Stage 1	Pre-treatments	kWh/m^3	0.101			
Stage 2	Primary treatment	kWh/kg TSS rem	0.646			
Stage 3	Secondary treatment	kWh/kg TPE	0.535			
Stage 4	Disinfection	kWh/(m^3 logred)	Chemical 0.025	UV-Class C 0.011	UV-Class B 0.012	UV-Class A 0.013
Stage 5	Sludge treatment	kWh/kg TSE	0.223			
WWTP	WTEI	-	0.719	0.686	0.686	0.691
	Global efficiency	-	F	F	F	F

Table 7. Saved energy, reduced GHG emission, and recovered water and nutrients during water reuse.

Crop	Water Quality Class	Saved Energy (MWh/y)	Reduced GHG Emission (ton-CO_{2eq}/y)	Recovered Water (Millions of m^3/y)	Recovered Nitrogen (kg N/y)	Recovered Phosphorus (kg P/y)
Corn	C	1178	656	17.8	139,140	10,703
Carrot	A	253	245	19.1	149,026	11,464
Tomato	A	253	245	19.8	154,450	11,881

4. Discussion

Stricter environmental regulations, the requirements for wastewater and for higher efficiency in the removal of pollutants at a lower cost, as well as the generation of energy from wastewater treatment clarify the need for attention to the new methods and technologies [41]. The description and the quantification of the water reuse-based WEFC nexus in an integrated peri-urban wastewater treatment and reuse system includes cross-sectorial analysis, linking energy, carbon and water footprints, as well as cross-stage assessments of all the sub-systems involved. More energy and carbon emissions must be considered to improve wastewater quality to satisfy reuse requirements. Different crops and different irrigation methods can be combined, always keeping regulatory requirements and technological level achievable in mind. Once defined all the interlinkages that construct the nexus, those relationships can be used to develop applications to show and evaluate all the inter-related outcomes, explaining and communicating the efforts and the benefits for the sustainable development of a peri-urban system.

Based on our assessment of the agricultural water reuse scenarios for Peschiera Borromeo WWTP, remarkable reduction in carbon and energy footprints were obtained. Within the reuse scenarios, energy footprint increased as the water quality (reuse class) increased due to required high standards and associated treatment technologies/methods. On the other hand, carbon footprint remained almost the same in all reuse scenarios. Similarly, Lahlou and co-authors [42] used the WEF nexus approach to identify the optimal allocation of 13 sources of treated wastewater to be used in cultivating alfalfa, achieving reductions in greywater footprint, energy and carbon footprints in Qatar that faces severe food insecurity due to its limited water resources. The authors found greywater footprint, energy for transportation, and carbon footprint associated with the growth of 1 ton of alfalfa as 917 m^3, 70 kWh and 34 kg-CO_2eq for Erakhiya farm, and 1770 m^3, 68 kWh and 18 kg-CO_2eq for Wadi Al Araig farm.

Considering the major resource savings (including water and energy) and reduction in GHG emissions, farmers, policymakers, reclamation managers and agronomists recognize the necessity for WEFC nexus in integrated wastewater and reuse systems, and propose and implement innovative solutions based on this concept especially in the local dimension [43]. This approach can help to measure and monitor the indicators of the complex interactions between water treatment/reuse, carbon emission, energy consumption, smart agriculture and climate variability [17]. Furthermore, digitalization can improve the collection of the data required for the footprint assessments, providing a common space for their storage and elaboration. Standardized offline data from periodic lab analyses or other traditional management practices can be supported and improved with real-time signals from the monitoring network of sensors in the WWTP and in the field, improving the frequency and the level of control. Moreover, coupling inputs from the field and from WWTP can optimize water and resources management.

5. Conclusions

The evaluation of complex and multi-sectorial practices, such as water reuse for agricultural irrigation, must be performed considering the WEFC nexus of the integrated system. Single audits or partial sub-stage considerations may not be representative of the whole system, since they may not include significative aspects or counterbalances. In this paper, the nexus approach was used to evaluate water reuse practices in a peri-urban integrated system, analyzing different sectorial footprints, from energy to water and carbon, for different sub-stages that constituted the integrated system, from WWTP to the irrigated field. Only by using a nexus approach and considering all the integrated systems can a comprehensive assessment be performed, and the benefits of water reuse (i.e., energy and carbon footprints) can be clearly highlighted. The nexus approach is fundamental for a better understanding of all the connections that interlink water, energy, carbon, food and climate sectors and allows evaluating all the impacts and consequences of different strategies on water reuse practices. The nexus approach can be used to increase awareness of all the efforts required for the application of sustainable practices and the benefits that can be apported, improving the acceptability and willingness to pay for more sustainable water and resource management actions.

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References

1. Miralles-Wilhelm, F. Development and application of integrative modeling tools in support of food-energy-water nexus planning—A research agenda. *J. Environ. Stud. Sci.* **2016**, *6*, 3–10. [CrossRef]
2. Klein, B.; Koenig, R.; Schmitt, G. Managing Urban Resilience: Stream Processing Platform for Responsive Cities. *Inform.-Spektrum* **2017**, *40*, 35–45. [CrossRef]

3. Brouwer, F.; Avgerinopoulos, G.; Fazekas, D.; Laspidou, C.; Mercure, J.F.; Pollitt, H.; Ramos, E.P.; Howells, M. Energy modelling and the Nexus concept. *Energy Strateg. Rev.* **2018**, *19*, 1–6. [CrossRef]
4. Dargin, J.; Daher, B.; Mohtar, R.H. Complexity versus simplicity in water energy food nexus (WEF) assessment tools. *Sci. Total Environ.* **2019**, *650*, 1566–1575. [CrossRef] [PubMed]
5. Nair, S.; George, B.; Malano, H.M.; Arora, M.; Nawarathna, B. Water-energy-greenhouse gas nexus of urban water systems: Review of concepts, state-of-art and methods. *Resour. Conserv. Recycl.* **2014**, *89*, 1–10. [CrossRef]
6. Hülsmann, S.; Sušnik, J.; Rinke, K.; Langan, S.; van Wijk, D.; Janssen, A.B.; Mooij, W.M. Integrated modelling and management of water resources: The ecosystem perspective on the nexus approach. *Curr. Opin. Environ. Sustain.* **2019**, *40*, 14–20. [CrossRef]
7. Li, M.; Fu, Q.; Singh, V.P.; Ji, Y.; Liu, D.; Zhang, C.; Li, T. An optimal modelling approach for managing agricultural water-Tenergy-food nexus under uncertainty. *Sci. Total Environ.* **2019**, *651*, 1416–1434. [CrossRef]
8. Van den Heuvel, L.; Blicharska, M.; Masia, S.; Sušnik, J.; Teutschbein, C. Ecosystem services in the Swedish water-energy-food-land-climate nexus: Anthropogenic pressures and physical interactions. *Ecosyst. Serv.* **2020**, *44*, 101141. [CrossRef]
9. Bassano, C.; Deiana, P.; Vilardi, G.; Verdone, N. Modeling and economic evaluation of carbon capture and storage technologies integrated into synthetic natural gas and power-to-gas plants. *Appl. Energy* **2020**, *263*, 114590. [CrossRef]
10. Meng, F.; Liu, G.; Liang, S.; Su, M.; Yang, Z. Critical review of the energy-water-carbon nexus in cities. *Energy* **2019**, *171*, 1017–1032. [CrossRef]
11. Gondhalekar, D.; Ramsauer, T. Nexus City: Operationalizing the urban Water-Energy-Food Nexus for climate change adaptation in Munich, Germany. *Urban Clim.* **2017**, *19*, 28–40. [CrossRef]
12. Tian, H.; Lu, C.; Pan, S.; Yang, J.; Miao, R.; Ren, W.; Yu, Q.; Fu, B.; Jin, F.F.; Lu, Y.; et al. Optimizing resource use efficiencies in the food–energy–water nexus for sustainable agriculture: From conceptual model to decision support system. *Curr. Opin. Environ. Sustain.* **2018**, *33*, 104–113. [CrossRef]
13. Gu, Y.; Dong, Y.N.; Wang, H.; Keller, A.; Xu, J.; Chiramba, T.; Li, F. Quantification of the water, energy and carbon footprints of wastewater treatment plants in China considering a water-energy nexus perspective. *Ecol. Indic.* **2016**, *60*, 402–409. [CrossRef]
14. González-Bravo, R.; Sauceda-Valenzuela, M.; Mahlknecht, J.; Rubio-Castro, E.; Ponce-Ortega, J.M. Optimization of Water Grid at Macroscopic Level Analyzing Water-Energy-Food Nexus. *ACS Sustain. Chem. Eng.* **2018**, *6*, 12140–12152. [CrossRef]
15. Zeng, X.T.; Zhang, J.L.; Yu, L.; Zhu, J.X.; Li, Z.; Tang, L. A sustainable water-food-energy plan to confront climatic and socioeconomic changes using simulation-optimization approach. *Appl. Energy* **2019**, *236*, 743–759. [CrossRef]
16. Cansino-Loeza, B.; Ponce-Ortega, J.M. Sustainable assessment of Water-Energy-Food Nexus at regional level through a multi-stakeholder optimization approach. *J. Clean. Prod.* **2021**, *290*, 125194. [CrossRef]
17. Radini, S.; Marinelli, E.; Akyol, Ç.; Eusebi, A.L.; Vasilaki, V.; Mancini, A.; Frontoni, E.; Bischetti, G.B.; Gandolfi, C.; Katsou, E.; et al. Urban water-energy-food-climate nexus in integrated wastewater and reuse systems: Cyber-physical framework and innovations. *Appl. Energy* **2021**, *298*, 117268. [CrossRef]
18. Capodaglio, A.G.; Olsson, G. Energy issues in sustainable urban wastewater management: Use, demand reduction and recovery in the urban water cycle. *Sustainability* **2020**, *12*, 266. [CrossRef]
19. Longo, S.; Mauricio-Iglesias, M.; Soares, A.; Campo, P.; Fatone, F.; Eusebi, A.L.; Akkersdijk, E.; Stefani, L.; Hospido, A. ENERWATER–A standard method for assessing and improving the energy efficiency of wastewater treatment plants. *Appl. Energy* **2019**, *242*, 897–910. [CrossRef]
20. Vasilaki, V.; Massara, T.M.; Stanchev, P.; Fatone, F.; Katsou, E. A decade of nitrous oxide (N2O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Res.* **2019**, *161*, 392–412. [CrossRef]
21. Venkatesh, G.; Chan, A.; Brattebø, H. Understanding the water-energy-carbon nexus in urban water utilities: Comparison of four city case studies and the relevant influencing factors. *Energy* **2014**, *75*, 153–166. [CrossRef]
22. Foglia, A.; Andreola, C.; Cipolletta, G.; Radini, S.; Akyol, Ç.; Eusebi, A.L.; Stanchev, P.; Katsou, E.; Fatone, F. Comparative life cycle environmental and economic assessment of anaerobic membrane bioreactor and disinfection for reclaimed water reuse in agricultural irrigation: A case study in Italy. *J. Clean. Prod.* **2021**, *293*, 126201. [CrossRef]
23. EU Regulation. *REGULATION (EU) 2020/741 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 May 2020 on Minimum Requirements for Water Reuse*; EU: Luxembourg, 2020; Volume 2019.
24. Moss, T.; Hüesker, F. Politicised nexus thinking in practice: Integrating urban wastewater utilities into regional energy markets. *Urban Stud.* **2019**, *56*, 2225–2241. [CrossRef]
25. Laspidou, C.S.; Mellios, N.; Kofinas, D. Towards ranking the water-energy-food-land use-climate nexus interlinkages for building a nexus conceptual model with a Heuristic Algorithm. *Water* **2019**, *11*, 306. (In Switzerland) [CrossRef]
26. Sušnik, J.; Chew, C.; Domingo, X.; Mereu, S.; Trabucco, A.; Evans, B.; Vamvakeridou-Lyroudia, L.; Savić, D.A.; Laspidou, C.; Brouwer, F. Multi-stakeholder development of a serious game to explore the water-energy-food-land-climate nexus: The SIM4NEXUS approach. *Water* **2018**, *10*, 139. (In Switzerland) [CrossRef]
27. Yang, Y.J.; Goodrich, J.A. Toward quantitative analysis of water-energy-urban-climate nexus for urban adaptation planning. *Curr. Opin. Chem. Eng.* **2014**, *5*, 22–28. [CrossRef]
28. Bartram, D.; Short, M.D.; Ebie, Y.; Farkas, J.; Gueguen, C.; Peters, G.M.; Zanzottera, N.M.; Karthik, M. Guidelines for National Greenhouse Gas Inventories-Wastewater Treatment and Discharge. 2019. Available online: https://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/5_Volume5/V5_6_Ch6_Wastewater.pdf (accessed on 30 September 2021).

29. Gustavsson, D.J.I.; Tumlin, S. Carbon footprints of Scandinavian wastewater treatment plants. *Water Sci. Technol.* **2013**, *68*, 887–893. [CrossRef]
30. Guendehou, G.H.S.; Koch, M.; Hockstad, L.; Pipatti, R.; Yamada, M. Incineration and Open Burning of Waste. *2006 IPCC Guidel. Natl. Greenh. Gas Invent. Waste* **2006**, *5*, 1–26.
31. IPCC. Chapter 5: Incineration and Open Burning. IPCC, 2019. Available online: https://www.ipcc-nggip.iges.or.jp/public/2019rf/pdf/5_Volume5/19R_V5_5_Ch05_IOB.pdf (accessed on 30 September 2021).
32. IPCC. Chapter 3: Solid Waste Disposal. IPCC, 2019. Available online: https://www.ipcc-nggip.iges.or.jp/public/2019rf/pdf/5_Volume5/19R_V5_3_Ch03_SWDS.pdf (accessed on 30 September 2021).
33. SINANET. Fattori di Emissione del Trasporto su Strada, Rete del Sistema Informativo Nazionale Ambientale. ISPRA, 2018. Available online: http://www.sinanet.isprambiente.it/it/sia-ispra/fetransp/fattori-emissione-trasporto-stradale/view (accessed on 30 September 2021).
34. ISPRA. Fattori di Emissione Atmosferica di CO_2 e Altri Gas a Effetto Serra Nel Settore Elettrico. ISPRA, 2017; ISBN 9788844808129. Available online: https://www.isprambiente.gov.it/it/pubblicazioni/rapporti/fattori-di-emissione-atmosferica-di-co2-e-altri-gas-a-effetto-serra-nel-settore-elettrico (accessed on 30 September 2021).
35. Romano, D.; Arcarese, C.; Bernetti, A.; Caputo, A.; Contaldi, M.; Cordella, M.; De Lauretis, R.; Di Cristofaro, E.; Gagna, A.; Gonella, B.; et al. Italian Greenhouse Gas Inventori 1990–2018. *National Inventory Report*. 2020. Available online: http://www.sinanet.isprambiente.it/it/sia-ispra/serie-storiche-emissioni/national-inventory-report/view (accessed on 30 September 2021).
36. Zhan, X.; Hu, Z.; Wu, G. *Greenhouse Gas Emission and Mitigation in Municipal Wastewater Treatment Plants*; IWA Publishing: London, UK, 2017; Volume 16, ISBN 9781780406312.
37. Meyer, R.K.; Pachauri, L.A. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. 2014. Available online: https://epic.awi.de/id/eprint/37530/ (accessed on 30 September 2021).
38. Hochstrat, R.; Peter, M.; Wintgens, T.; Kraus, F.; Seis, W.; Miehe, U.; Frijns, J. Water Reuse Safety Plans-a manual for practitioners. *Deliv. D3.4 Demoware* **2017**, *1*, 43.
39. Tak, S.; Kumar, A. Chlorination disinfection by-products and comparative cost analysis of chlorination and UV disinfection in sewage treatment plants: Indian scenario. *Environ. Sci. Pollut. Res.* **2017**, *24*, 26269–26278. [CrossRef]
40. Cotton, C.A.; Owen, D.M.; Ćline, G.C.; Brodeur, T.P. UV disinfection costs for inactivating cryptosporidium. *J. Am. Water Work. Assoc.* **2001**, *93*, 82–94. [CrossRef]
41. Fetanat, A.; Tayebi, M.; Mofid, H. Water-energy-food security nexus based selection of energy recovery from wastewater treatment technologies: An extended decision making framework under intuitionistic fuzzy environment. *Sustain. Energy Technol. Assess.* **2021**, *43*, 100937. [CrossRef]
42. Lahlou, F.Z.; Mackey, H.R.; McKay, G.; Onwusogh, U.; Al-Ansari, T. Water planning framework for alfalfa fields using treated wastewater fertigation in Qatar: An energy-water-food nexus approach. *Comput. Chem. Eng.* **2020**, *141*, 106999. [CrossRef]
43. Rodias, E.; Aivazidou, E.; Achillas, C.; Aidonis, D.; Bochtis, D. Water-energy-nutrients synergies in the agrifood sector: A circular economy framework. *Energies* **2021**, *14*, 159. [CrossRef]

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Comparative life cycle environmental and economic assessment of anaerobic membrane bioreactor and disinfection for reclaimed water reuse in agricultural irrigation: A case study in Italy

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ABSTRACT

Reuse of treated wastewater for irrigation purposes is a measure to reduce water stress and over-exploitation of freshwater resources. This study aims to investigate the environmental and economic impacts of a current conventional wastewater treatment plant (WWTP) in Peschiera Borromeo (Milan, Italy), and compare possible scenarios to enable reclaimed water reuse for agriculture. Accordingly, we propose alternative disinfection methods (i.e. enhanced UV, peracetic acid) and replace conventional activated sludge (CAS) with upflow anaerobic sludge blanket (UASB) for biological treatment and use anaerobic membrane bioreactor (AnMBR) as the tertiary treatment. Life cycle assessment (LCA) and life cycle costing (LCC) were implemented on the existing full-scale wastewater treatment line and the hypothetical scenarios. In most cases, the impact categories are primarily influenced by fertilizer application and direct emissions to water (i.e. nutrients and heavy metals). The baseline scenario appears to have the largest environmental impact, except for freshwater eutrophication, human ecotoxicity and terrestrial ecotoxicity. As expected, water depletion is the most apparent impact category between the baseline and proposed scenarios. The UASB + AnMBR scenario gives relatively higher environmental benefits than the other proposed scenarios in climate change (−28%), fossil fuel depletion (−31%), mineral resource depletion (−52%), and terrestrial ecotoxicity compared to the baseline. On the other hand, the highest impact on freshwater eutrophication is also obtained by this scenario since the effluent from the anaerobic processes is rich in nutrients. Moreover, investment and operational costs vary remarkably between the scenarios, and the highest overall costs are obtained for the UASB + AnMBR line mostly due to the replacement of membrane modules (24% of the total cost). The results highlighted the importance of the life cycle approach to support decision making when considering possible upgrading scenarios in WWTPs for water reuse.

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1. Introduction

Mediterranean region has been facing increasing pressure from water scarcity and droughts where freshwater availability is likely to decrease substantially by 2%-15% for 2 °C increase of global temperature due to climate change alone (MedECC Network, 2019). Between 50% and 90% of the total water demand in the Mediterranean basin is dedicated to irrigation, and this demand is projected to rise by 18% until the end of the century (UNEP/MAP Plan Bleu, 2019). Meanwhile, seawater intrusion is another critical problem along the Mediterranean coasts as a consequence of over-exploitation of groundwater (Giannoccaro et al., 2019). All of these issues together with population and economic growth continuously stress freshwater supplies, which consequently increase the demand for non-conventional water resources (Lee et al., 2018).

Reclaimed wastewater reuse is seen as a solution to help to

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address above-mentioned challenges, but its potential remains largely untapped from a technical and legislative point of view (Rizzo et al., 2018). Treated wastewater can be used either for non-potable purposes, such as aquifers recharge, irrigation/fertigation, and industrial use, or as a source for drinking water supply after additional treatments. This can help to protect the environment and to enhance water security by managing water resources of the hydrological cycle in a more circular way (Diaz-Elsayed et al., 2019; Giannoccaro et al., 2019). The reuse for agricultural irrigation is by far the most established end-use for reclaimed water (Rizzo et al., 2020). However, the use of reclaimed water relies on many types of advances, not only related to technological approaches but also health, socioeconomic and legal aspects (Salgot and Folch, 2018). In most cases, water reuse strategies are often intended to address the problem of water scarcity without aggravating other environmental problems, thus reflecting the need for their environmental assessment (Meneses et al., 2010). Moreover, water reuse practices can be expensive since a high degree of treatment is required and a separate piping system is needed for the reuse systems to distribute the water.

Currently, approximately 1 billion cubic meters of treated urban wastewater is reused in the EU annually, which accounts for about 2.4% of the treated urban wastewater effluents and less than 0.5% of annual EU freshwater withdrawals. Water-scarce EU countries such as Italy, Spain, and Greece only reuse between 5% and 12% of their effluents (EC, 2020a). This is mainly due to the existing constraints for reclaimed water reuse at the national level. For example, in Italy, the agricultural use of reclaimed water is strongly restricted by law D.Lgs 185/2003 (Ventura et al., 2019). Indeed, the treated wastewater must comply with a range of water directives at the EU and national levels to protect the environment, but the reuse of reclaimed water has to comply with additional directives/regulations depending on the purpose (Vojtěchovská Šrámková et al., 2018). Recently, the European Commission has developed the Regulation 2020/741 on "*Minimum Requirements for Water Reuse*" (EC, 2020b), where specific indications are provided for the assessment of reclaimed water reuse.

Tertiary treatment (including filtration and/or disinfection) is commonly required to meet the quality standards of reused treated wastewater (Carré et al., 2017). Conventionally, chemical or physical disinfection is applied during wastewater treatment, complying with the stringent microbial safety required for water reuse (Angelakis and Snyder, 2015). Alternatively, well designed and operated membrane bioreactors (MBRs) can also provide efficient removals of solids and pathogens (Foglia et al., 2020). Hai et al. (2014) provided an in-depth overview of the mechanisms and influencing factors of pathogens removal by MBRs and highlighted the practical issues, such as reduced chemical disinfectant dosages and associated economic and environmental benefits. Anaerobic MBR (AnMBR) is a very attractive technology in terms of energy efficiency with energy recovered from sewage and without aeration requirements. In fact, AnMBR has been reported to be net energy positive, leading in cost savings up to €0.023 per m^3 of treated water (Pretel et al., 2016). At the same time, the combined used of anaerobically treated effluent for fertigation can further reduce CO_2 emissions (Jiménez-Benítez et al., 2020).

In most cases, decisions about wastewater treatment are primarily influenced by direct capital and operating costs as long as the design meets the required standards, while life-cycle cost (LCC) and life-cycle environmental impacts are rarely considered (Awad et al., 2019). The consideration of a life cycle perspective can help to achieve sustainable wastewater treatment. The Life Cycle Thinking approach is widely applied to assess the environmental sustainability of treatment processes and reveal trade-offs across various environmental impact categories. Besides, life cycle assessment (LCA) provides quantitative information that can support decision making in water reuse practices when considering possible operational scenarios during a strategic planning of reclaimed water reuse (Corominas et al., 2020). For instance, Meneses et al. (2010) investigated tertiary treatment alternatives (i.e. chlorination plus UV treatment; ozonation; and ozonation plus hydrogen peroxide) to enable urban wastewater reuse for non-potable uses (both agricultural and urban uses). Although the assessed disinfection methods had similar environmental impacts, most of the indicators were about 50% higher than the UV disinfection except for the acidification (100% higher) and photochemical oxidation (less than 5%), while chlorination plus UV treatment disinfection was found to have the lowest impact. Up to date, there have been few studies that investigated the LCA of tertiary disinfection methods for reclaimed water reuse (Carré et al., 2017; Muñoz et al., 2009; Pan et al., 2019; Pasqualino et al., 2011) (see the **e-Supplementary file**). Although LCA and market prospects for AnMBR technology are discussed in the review work of Krzeminski et al. (2017b), there are still limited studies on the LCA of AnMBRs for urban wastewater treatment and water reuse mainly due to the lack of full-scale data (Krzeminski et al., 2017a).

In this study, advanced tertiary treatment processes were assessed within the frameworks of life cycle approach to analyze water reuse options in the municipal WWTP of Peschiera Borromeo in Northern Italy. LCA and LCC were carried out to compare the impacts of treated wastewater discharge and using conventional sources to supply the water and nutrient demand of the surrounding agricultural area (Baseline scenario) with proposed alternative reuse strategies. Fertigation coupled with different disinfection methods, such as peracetic acid (PAA) and UV-disinfection, was evaluated as the alternative scenarios. Furthermore, a third scenario was suggested to replace the conventional activated sludge (CAS) process with an anaerobic biological process (i.e. upflow anaerobic sludge blanket (UASB)) and to use AnMBR as tertiary treatment and finally to reuse the effluent in fertigation practice. The main aim was to identify: i) potential environmental and economic benefits and ii) undesired impacts of integrated wastewater treatment and water reuse system. We believe that the outcomes of this work can help to guide reclamation managers for possible upgrading opportunities in WWTPs considering the sustainability aspects.

2. Materials and methods

2.1. Description of the study area

2.1.1. Peschiera Borromeo WWTP

The target WWTP is located in the municipality of Peschiera Borromeo (Lombardy, Italy) and serves a large urban territory (Milan and neighboring municipalities) with a total catchment area of 2230 ha. Currently, the final effluent is discharged into the Lambro River. The plant has a real treatment capacity of 322,376 population equivalent (PE) with a total average inflow rate of 126,322 m^3/d in 2019 treated in two different wastewater lines as shown in Fig. 1. Line 1 (Fig. 1a) collects and treats the wastewater from the municipalities of Brugherio (MB), Carugate, Cassina de' Pecchi, Cernusco sul Naviglio, Cologno Monzese, Peschiera Borromeo, Pioltello, Segrate, and Vimodrone. Line 2 treats the wastewater from the eastern district of Milan. After pre- and primary treatments, Line 1 consists of a CAS process followed by biological filtration to remove inorganic nitrogen and a final chemical disinfection using PAA. Line 2 (Fig. 1b) includes a two-stage upflow biological filtration (Biofor ®) and two parallel lines of UV disinfection operating at a UV dose of 50 mW/cm^2. Although Line 2 is designed for the purpose of reclaimed water reuse, the effluent is

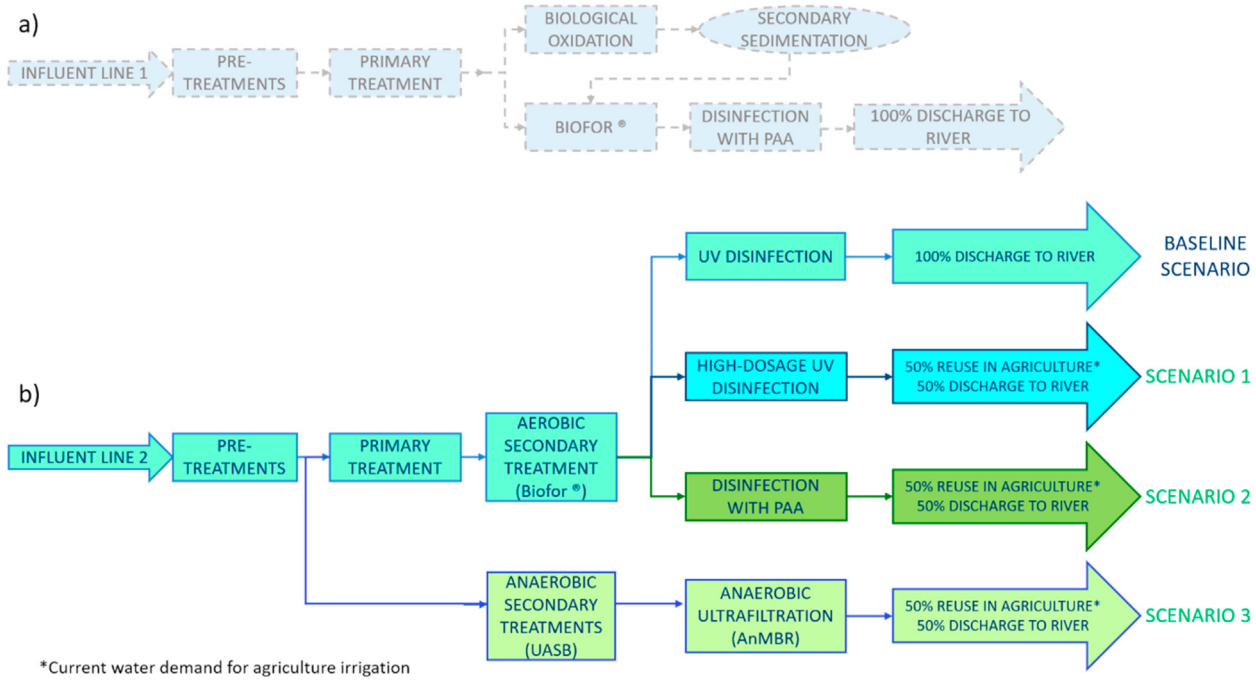


Fig. 1. Flow scheme of the Peschiera Borromeo WWTP: a) Line 1 and b) baseline and proposed scenarios applied to Line 2.

discharged into the Lambro River in both cases. The sludge line consists of the following processes: gravity and dynamic pre-thickening, two-stage anaerobic digestion, gravity post-thickening, and dewatering via centrifuges. The dewatered sludge is transformed in defecation lime and then applied as soil improver. The produced biogas is valorized in two combined heat and power (CHP) units recovering electricity for internal usage and thermal energy to heat the digesters. The biogas is stored in two gasometers where the unused fraction is burned by two torches.

2.1.2. Surrounding irrigation area

Peri-urban areas in the south of Milan (near Parco Agricolo Sud Milano) suffer from water scarcity. Its water demand (12.03 hm^3/y) is mainly required for irrigation. This request can be widely covered by the outflow of Line 2. The surrounding agricultural land has an area of approximately 1500 ha and its main crop is tomato. The nutrient needs (N and P) of tomato in drip irrigation systems are 160 kg N/ha/y and 20 kg P/ha/y (Jiménez-Benítez et al., 2020).

2.2. Treatment scenarios

In order to enable the reuse of the final effluent for agricultural purposes, the following proposed scenarios focused only on the Line 2 of Peschiera Borromeo WWTP. The environmental impacts of the current no reuse configuration was compared to alternative reclamation solutions permitting water reuse. Table 1 presents the effluent characteristics of the plant and the wastewater reuse limits set out by the current Italian legislation as well as those established by the new European Regulation 2020/741 on minimum requirements for water reuse (EC, 2020b).

The initial (baseline) scenario refers to the current treatment chain of Line 2 where the final effluent is discharged on surface water and the irrigation and nutrient demand are supplied by freshwater and spreading of mineral fertilizers, respectively.

To comply with the water reuse regulation, the proposed reuse scenarios (Fig. 1) involve upgrading or process modifications of Line 2 as follows:

- UV disinfection at higher UV dose (Scenario 1),
- Chemical disinfection using peracetic acid PAA (Scenario 2)
- Biological treatment with UASB followed by AnMBR (Scenario 3).

In Scenario 1, the existing UV disinfection operates at a dose of 80 mW/cm^2 to ensure a 3.5 log reduction (DEMOWARE, 2016) required to achieve a quality effluent of Class A. In Scenario 2, the UV disinfection is substituted by chemical disinfection unit of 2200 m^3, with a contact time of 49 min and a dosage of 5 mgPAA/L to guarantee the same log reduction (Antonelli et al., 2013) of Scenario 1. Finally, in Scenario 3, an UASB reactor is installed replacing the aerobic secondary treatment. The UASB reactor works at ambient temperature and has a volume of 24,106 m^3, with a hydraulic retention time (HRT) of 9 h. Then, the UASB is coupled with an anaerobic hollow-fiber ultrafiltration membrane as the tertiary treatment. The membrane (267,842 m^2) has a nominal pore size of 0.03 μm and operates at the specific flux of 10 $L/m^2/h$. The ultrafiltration technology in Scenario 3 provides pathogen-free effluent. Therefore, all the alternative scenarios are modeled to reach reclaimed water of class A quality (*E. coli* < 10 CFU/100 ml).

The described configurations are assumed to treat the entire inflow rate of Line 2 (64,282 m^3/d). On the other hand, the effective request of water for irrigating the surrounding area is accounted for the half of the WWTP flow. Therefore, 32,959 m^3/d are reused in agriculture and 31,323 m^3/d are discharged in the Lambro river. At the same time, the nutrient demand of crops is first covered by the N- and P-content of the reclaimed water and then by a supplementary amount of mineral fertilizer if needed.

2.3. Life cycle assessment methodology

The above-described scenarios were compared to determine the sustainability of the different water reclamation and reuse practices in terms of environmental and economic impacts. The study was carried out following four phases: goal and scope definition, inventory analysis, impact assessment and interpretation. This

Table 1
Effluent concentrations and wastewater reuse limits.

Parameters	Unit	Effluent Line 1	Effluent Line 2	DM183/2005[a]	2020/741 Class A	2020/741 Class B	2020/741 Class C
E. coli	CFU/100 ml	284	847	<10	<10	<100	<1000
COD	mg/l	19.3	17.9	<100	-	-	-
BOD_5	mg/l	6.7	6	<20	<10	<25	<25
TN	mg/l	10.3	8.4	<15	[b]	[b]	[b]
NH_4	mg/l	3.9	1.1	<2	[b]	[b]	[b]
TP	mg/l	0.5	0.7	<2	[b]	[b]	[b]
TSS	mg/l	7.2	6.5	<10	<10	<35	<35
Al	mg/l	0.19	0.12	<1	[b]	[b]	[b]
Fe	mg/l	0.19	0.31	<2	[b]	[b]	[b]

[a] Italian Ministerial Decree on Water Reuse.
[b] defined by a site-specific risk assessment to be carried out.

approach was followed within the framework and principles universally valid to plan and conduct an LCA as established by ISO14044 (ISO, 2006).

The analysis considered the environmental impact directly related to the treatment system (foreground system), as well as the background impact from the supplementary supply chains delivering energy, chemicals, or auxiliaries (background system) using the Ecoinvent v.3.6 databases published and maintained by the Ecoinvent Centre in Switzerland, since it is the most renowned database for life cycle inventory (LCI) datasets. It contains approximately 4500-5000 harmonized, reviewed and validated datasets for use in LCA that are all fully documented. The Life Cycle Impact Assessment (LCIA) phase was largely automated thanks to the use of LCA software Umberto LCA + v10.0 in this research. It uses graphic modeling of the product life cycle and allows analyzing, assessing and visualizing the environmental impacts in different impact categories.

2.3.1. System boundaries and functional unit

The physical system boundaries (Fig. 2) were defined according to the goal and scope of the study, i.e. the comparison of different tertiary treatment schemes. It included not only the water line processes (L2) but also the water and nutrient demand of the surrounding irrigation area (1500 ha). To model foreground and background processes, the following data were considered: the volume and the quality of all water streams, direct GHG emissions from processes, energy consumption, production and transportation of chemicals, wastes disposal, surface water withdrawal and production and spreading of fertilizer. To compare the environmental performance of the different scenarios, 1 m^3 of treated wastewater was selected as the functional unit.

2.3.2. Life cycle inventory

A summary of the LCI of the scenarios is given in Table 2. The data refer to the main units investigated in this study. The principal parameters of the foreground processes (primary data) were provided by the water utility of the Peschiera Borromeo WWTP. Water quality, consumption of energy and chemicals, amount of waste produced, and related distance to disposal sites refer to the information gathered in 2019. For alternative scenarios, relevant literature values were mainly considered. In Scenario 1, to apply a UV dose of 80 mJ/cm^2 (DEMOWARE, 2016), the disinfection unit utilizes 5472 kWh/d of electricity. Irrigating with the treated wastewater, 275 kgN/d and 34 kgP/d are provided to crops. Therefore, a supplementary consumption of mineral fertilizer (383 kgN/d and 48 kgP/d) were considered to ensure required plant growth (Jiménez-Benítez et al., 2020). In Scenario 2, the chemical disinfection consumes 2009 kg/d of 16% PAA and 43 kWh/d of electricity. The need for supplementary mineral fertilizer (N and P) was assumed to be equal to Scenario 1. In Scenario 3, based on the data taken from the study of Pretel et al. (2013), the electricity consumption of the UASB was accepted to be 900 kWh/d, while the electricity and thermal energy productions were taken as 1350 kWh/d and 4236 MJ/d, respectively. Furthermore, the electricity consumption of the AnMBR was calculated as 12,381 kWh/d according to Pretel et al. (2013). Considering the membrane cleaning, the amount of NaOCl at 15% for the ordinary cleaning and citric acid at 100% for the recovery cleaning were estimated as 618 kg/d and 93 kg/d, respectively. The N-content in the AnMBR effluent exceeds the N-demand for crops growth, thus only 18 kg/d of supplementary P-fertilizer was considered to be applied to cover the crop requirements.

Regarding the background processes, the following assumptions were considered: the PAA production was modeled by the production processes of acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2) assuming that the production of 1 kg of PAA requires 0.45 kg of CH_3COOH, 0.79 kg of H_2O_2 and 0.28 kg of water (Buonocore et al., 2018). The lifetime of a UV lamp is equal to 10,000 h as indicated by the WWTP manager. The residues from screening (disposed of in municipal incineration) were assumed to be composed of 50% of "waste packaging paper" and 50% of "plastic mixture" (Buonocore et al., 2018; Doka, 2003). The final disposal in landfill of the residues from gritting was simulated with "disposal, inert waste, to inert material landfill" (Buonocore et al., 2018; Lorenzo-Toja et al., 2016). The electricity was modeled based on the "Market for electricity, low voltage [IT]".

As conducted in other studies (Yoshida et al., 2018), "calcium ammonium nitrate production [RER]" and "triple superphosphate production [RER]" were considered for the N and P fertilizer production, respectively. The mineral fertilizer application was, instead, modeled by the Ecoinvent process "fertilising, by broadcaster [CH]".

The impact of transport derives from "Freight, lorry 3.5-7.5 metric ton, EURO 4" for chemicals and "Freight, lorry 16-32 metric, EURO 4" for wastes and sludge disposal. Furthermore, direct GHGs emissions like non-fossil carbon dioxide, fossil methane, and dinitrogen monoxide were also considered in the model.

2.3.3. Impact assessment

The life cycle impact assessment was carried out by applying the "ReCiPe 2008 Midpoint (H) V1.13 no LT" (results without long-term emissions) method for the following impact categories: climate change (CC), fossil fuel depletion (FD), freshwater eutrophication (FE), mineral resource depletion (MRD), water depletion (WD), freshwater ecotoxicity (T-FET), human toxicity (T-HT) and terrestrial ecotoxicity (T-TET).

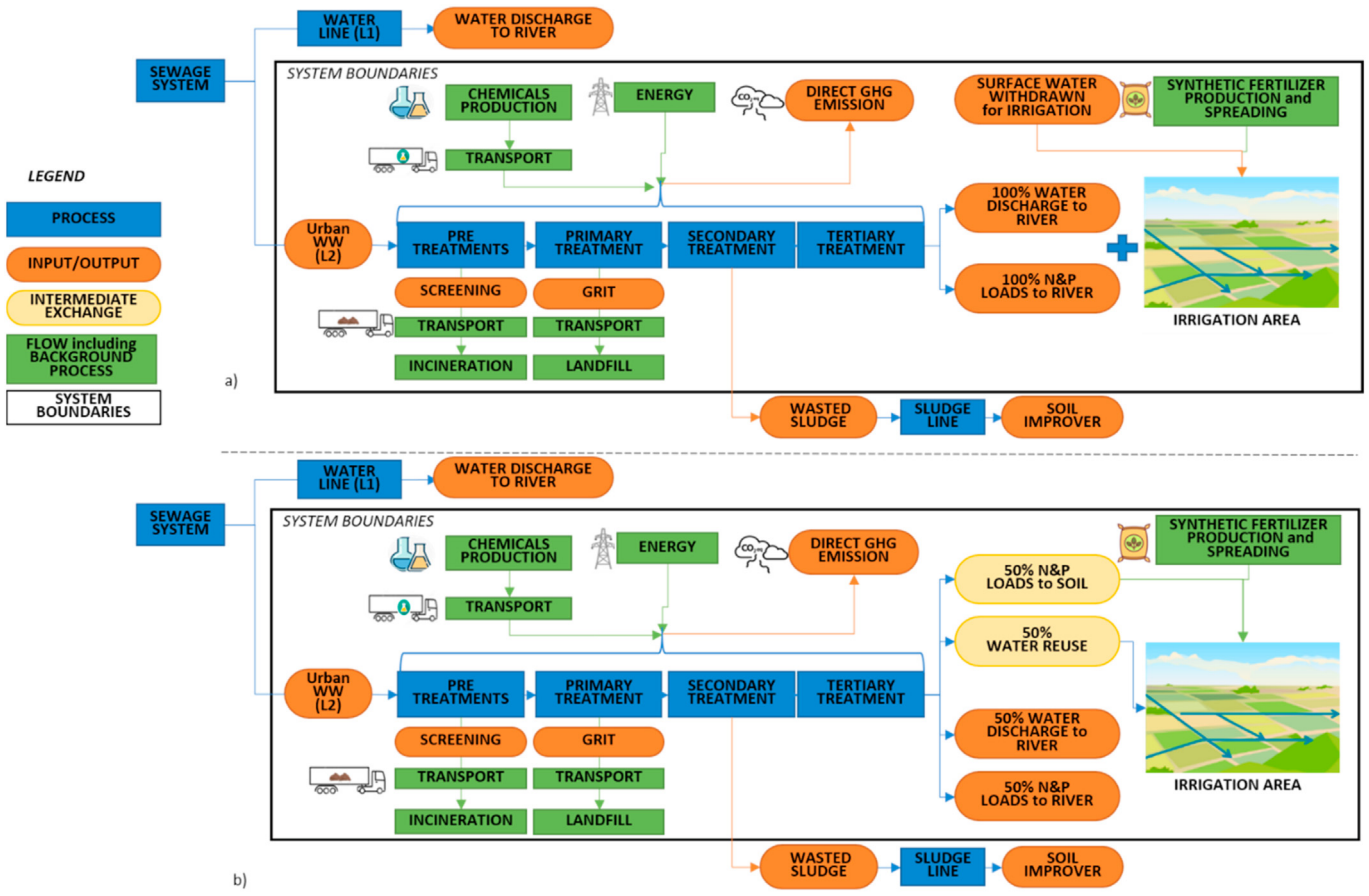


Fig. 2. System boundaries for the life cycle assessment: a) baseline configuration; b) alternative scenarios.

Table 2
Life cycle inventory of the operation stage in the scenarios.

		Baseline scenario	Scenario 1	Scenario 2	Scenario 3
		No reuse	Reuse of class A reclaimed water		
Parameters	Units	UV	High dosage UV	PAA	AnMBR
Q treated (L2)	m^3/d	64,282	64,282	64,282	64,282
Q discharged to river	m^3/d	64,282	31,323	31,323	31,323
Q required by crop	m^3/d	32,959	32,959	32,959	32,959
Q surface water withdrawn	m^3/d	32,959	0	0	0
Q water reused for irrigation	m^3/d	0	32,959	32,959	32,959
TN effluent concentration	g/m^3	8	8	8	24
TN required by crop	kg/d	658	658	658	658
TN added by water	kg/d	0	275	275	791
TN added by mineral fertilizers	kg/d	658	383	383	-
Excess TN to soil	kg/d	-	-	-	133
TN discharged to surface water	kg/d	536.35	261.35	261.35	751.73
TP effluent concentration	g/m^3	1.04	1.04	1.04	1.94
TP required by crop	kg/d	82	82	82	82
TP added by water	kg/d	-	34	34	64
TP added by mineral fertilizers	kg/d	82	48	48	18
Excess TP to soil	kg/d	0	0	0	0
TP discharged to surface water	kg/d	67.12	32.71	32.71	60.88
Consumed electricity (secondary treatments)	kWh/d	9792	9792	9792	900
Consumed electricity (tertiary treatments)	kWh/d	2517	5472	43	12,381
Consumed electricity (whole plant)	kWh/d	20,318	23,273	17,844	21,290
Produced electricity	kWh/d	0	0	0	1350
Self-produced heat	MJ/d	0	0	0	4236
PAA at 16% w/w	kg/d	0	0	2009	0
Citric acid at 100% w/w (membrane cleaning)	kg/d	0	0	0	93
NaOCl at 15% w/w (membrane maintenance)	kg/d	0	0	0	618

2.4. Life cycle cost assessment methodology

Direct capital costs include the cost of infrastructures, mechanical equipment and installation, and electrical and automation systems (Harclerode et al., 2020). For the conventional treatment facilities, the capital expenditures (CAPEX) was developed based on the scaling of costs from comparable projects implemented by the authors, while costs for less common processes like AnMBR were estimated using equipment market pricing and estimated quantities for materials, such as concrete, tank covers, and pre-engineered buildings. The effects of price development (e.g. rising energy prices) and inflation (i.e. the loss of value for money) were not considered in the calculation. The investment cost for a conventional aerobic secondary treatment was taken as 0.04 €/m^3 considering a lifetime of 25 years (Harclerode et al., 2020). Similarly, the CAPEX for the disinfection units were assumed to be 0.0008 and 0.0002 €/m^3 for the UV (Scenario 1) and the PAA disinfection (Scenario 2), respectively (Collivignarelli et al., 2000; Luukkonen et al., 2015). For Scenario 3, a specific total CAPEX of 0.096 €/m^3 was assumed for both secondary and tertiary treatments (Harclerode et al., 2020). For operating expenses (OPEX), most of the information was provided by the water utility, otherwise the Ecoinvent database was considered.

The economic lifetime was set to 25 years to be conservative since the investment cost includes both constructions and buildings with a typical lifespan higher than 30 years and machinery to be replaced every 20 years or less. This choice is stated in the "EVALUATION of the Council Directive 91/271/EEC of May 21, 1991" concerning urban waste-water treatment that suggests a lifetime of 25 years for WWTPs. Table 3 provides a summary of the main CAPEX and specific OPEX values.

The total cost in the results is reported as the annual costs, corresponding to the annual OPEX with the CAPEX per annum:

Annual costs = OPEX (€/y) + CAPEX (€/y).

CAPEX (€/y) = ($\sum$ [[investment costs (€))/(economic lifetime (y))

3. Results

3.1. Baseline scenario assessment

Fig. 3a illustrates the allocation between foreground and background environmental impacts. The foreground impact is dominating among all impact categories mainly as a result of the agricultural activities (fertilizer spreading) and the direct emissions to air, water, and soil that are related to the treatment process and to the final effluent discharge. More than 96% of the impact on freshwater eutrophication (FE), water depletion (WD), and human toxicity (T-HT) are caused by direct emissions. Fig. 3b shows the breakdown of the environmental footprint among the different stages of the water treatment supply chain, namely: pre- and primary treatments, biological process, disinfection and final use. The latter includes water withdrawal and fertilizer application in agriculture. As expected, the most significant environmental impact is related to the final use, followed by primary treatment where phosphorous is chemically removed by dosing poly-aluminium chloride (PAC). Specifically, the final use causes about 75% of the impact on climate change and fossil fuel depletion, and more than 98% on freshwater eutrophication and water depletion. The relative impact of primary treatments (>7%), as well as biological processes (>6%), are more evident on climate change, fossil fuel depletion, mineral resource depletion and terrestrial ecotoxicity categories. As an energy-intensive process, the disinfection affects mainly the fossil fuel depletion and climate change categories; however, it is still significantly lower than the other stages (<2%). Fig. 3c shows the contribution analysis of each impact category based on the origin of the impact and related resources (i.e. energy, chemicals, direct emissions, etc.). Fertilizer spreading has a significant contribution to climate change, fossil fuel depletion, mineral resource depletion and terrestrial ecotoxicity since it is strongly related to fossil fuel combustion. The direct emissions to water refer to the nutrients and heavy metals content of the discharged effluent to the surface water body. They affect mainly the freshwater eutrophication, the freshwater ecotoxicity, and human ecotoxicity with relative contributions of about 77%, 65%, and 86%, respectively. Approximately 20% of the environmental burden in the freshwater eutrophication category is due to the P-content in the irrigation water (direct emission to soil) and 80% is due to the P-content in the discharged water (direct emission to water). The water depletion is influenced almost entirely by the direct withdrawal of water from the environment while climate change, fossil fuel depletion, and terrestrial ecotoxicity are mainly affected by electricity consumption and transportation. The chemicals mostly have an impact on the categories of terrestrial ecotoxicity (20%), fossil fuel depletion (8%), freshwater ecotoxicity (7%), and climate change (7%).

Table 3
CAPEX and OPEX considered in the scenarios.

CAPEX costs (Peschiera WWTP)	U.M	Values	Reference
Preliminary and primary treatment	k€	8836	Harclerode et al. (2020)
Conventional activated sludge secondary treatment	k€	22,396	Harclerode et al. (2020)
Disinfection UV	k€	470	Collivignarelli et al. (2000)
Disinfection PAA	k€	147	Luukkonen et al. (2015)
Anaerobic treatment (UASB + AnMBR)	k€	36,450	Harclerode et al. (2020)
Biogas conditioning and CHP	k€	11,772	Harclerode et al. (2020)
Specific OPEX costs (Peschiera WWTP)	**U.M**	**Values**	**Reference**
Electricity	€/kWh	0.14	Company information
PAA 16%	€/kg	0.74	Company information
NaOCl 100%	€/kg	0.34	Ecoinvent EURO2005
Citric acid 100%	€/kg	0.78	Ecoinvent EURO2005
MBR replacement frequency	years	10	Harclerode et al. (2020)
MBR replacement cost for WW treated	€/m^3/d	190	Harclerode et al. (2020)
UV replacement frequency	hours	10,000	Trojan UV technical factsheet
UV lamp cost	€/lamp	343	Trojan UV technical factsheet
N fertilizer	€/kg N	0.47	Ecoinvent EURO2005
P fertilizer	€/kg P_2O_5	0.24	Ecoinvent EURO2005
Number of labors	N°	6	Company information
Labor salary	€/h	25	Company information
Irrigation water withdrawn from the channel	€/m^3	0.016	ISPRA (2012)
Reclaimed water market price	€/m^3	0.016	ISPRA (2012)

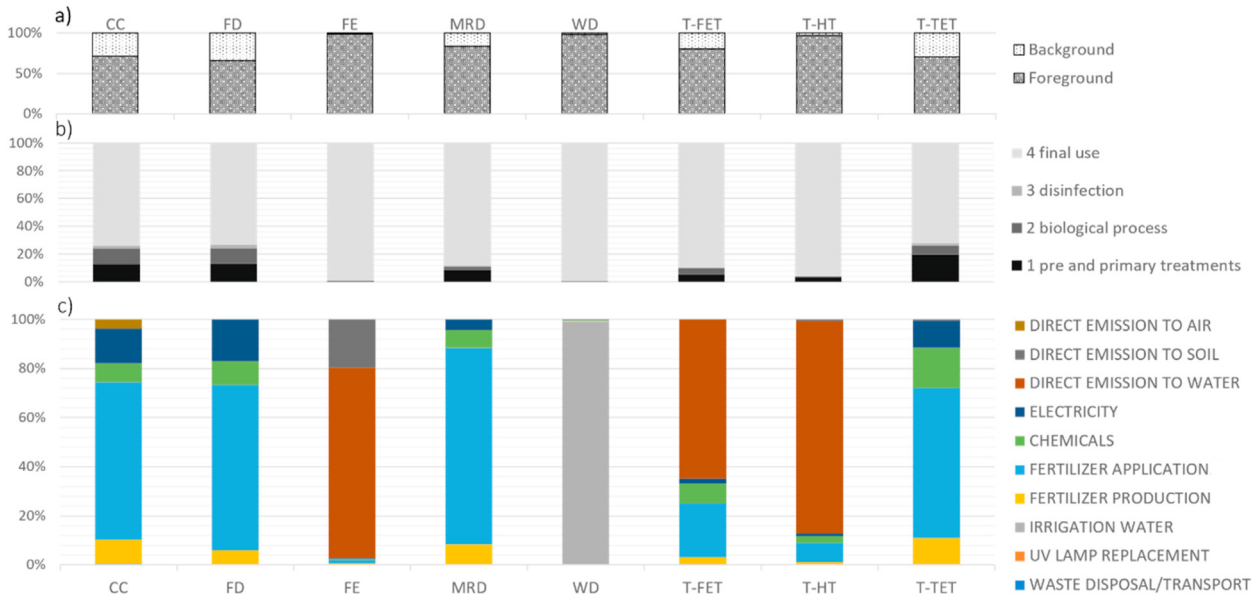


Fig. 3. Environmental profile of the existing treatment configuration (baseline scenario) in the Peschiera Borromeo WWTP **a)** foreground and background environmental impacts **b)** impact of each treatment stage **c)** contributions on each impact category.

3.2. Scenario analysis

An overall comparison of the relative impacts of each scenario is presented in Fig. 4. In most impact categories, the proposed water reuse scenarios show significant environmental benefits. The baseline scenario represents the largest environmental impact in all categories, except for freshwater eutrophication, human toxicity, and terrestrial ecotoxicity. As expected, the largest benefit is observed in water depletion category since the abstraction of freshwater is replaced with reclaimed water reuse. Scenario 1 and 2 show a significant reduction in freshwater eutrophication due to the lower amount of P directly discharged into the river. On the other hand, Scenario 3 rises the impact on freshwater eutrophication since the UASB + AnMBR effluent is highly rich in nutrients that leads to higher rate of P-release even if the same low quantity of water is discharged. However, due to the savings of producing and spreading mineral fertilizer, Scenario 3 has a relatively lower impact on fossil fuel depletion (68%). A slight reduction of 3% and 6% in fossil fuel depletion impact is observed in Scenarios 1 and 2 compared to baseline scenario, respectively, since they are strongly related to fossil fuel combustion required in energy production and in the transport of the disinfection agents. Looking at the toxicity-related categories, the toxicity in the water environment is higher in the Baseline since traces of heavy metals present in the effluent are fully discharged into the river. However, increased toxicity levels for terrestrial and human categories are observed in the alternative scenarios where the toxic compounds are partially sent to the soil.

3.2.1. Scenario 1 - enhanced UV disinfection

Scenario 1 is the upgraded version of the baseline scenario with an enhanced UV application to reach an effluent quality of class A (*E. coli* <10) to be reused in agriculture. The current plant configuration performs poor nutrient removal resulting in a final effluent of N = 8 mg/l and P = 1 mg/l. Fig. 5 shows the environmental performance of Scenario 1 relative to the baseline scenario. Although there is higher electricity consumption in Scenario 1, the climate change impact shows a 7% reduction. This is because the avoided emissions of the displaced fertilizer production and application that are much higher than the ton of CO_2 equivalent

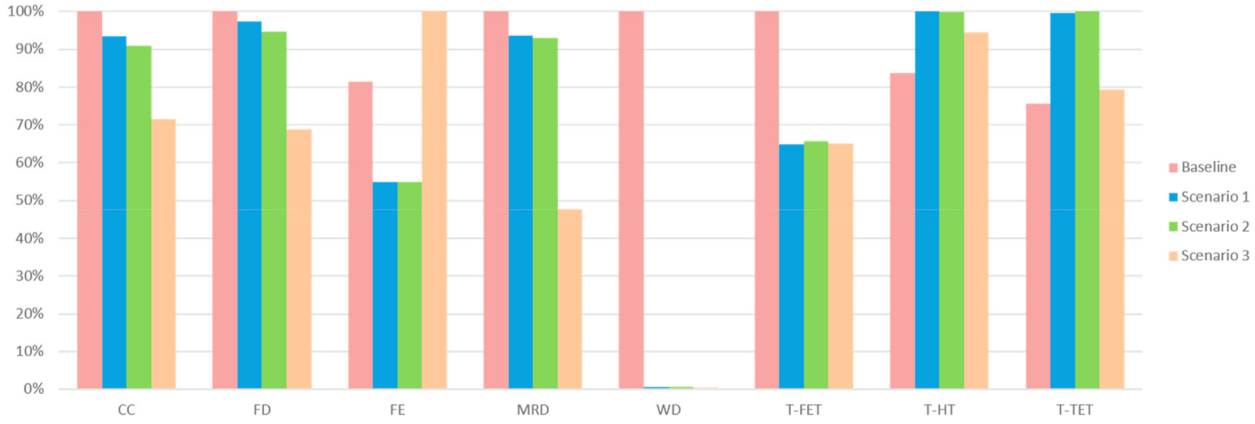


Fig. 4. Comparison of the relative environmental impacts of each scenario.

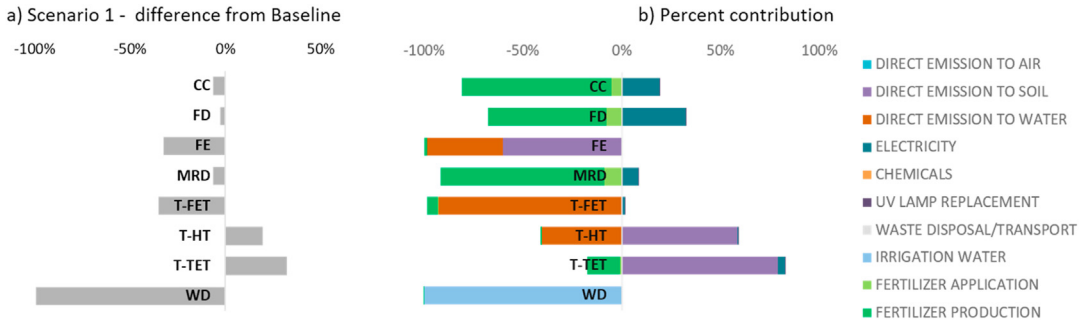


Fig. 5. Environmental performance of enhanced UV disinfection relative to baseline scenario a) as overall relative differences in each category; b) percentage contribution analysis based on the individual processes and sources of impact.

related to the intensified energy demand. Since the irrigation water comes from the reuse of reclaimed water, the largest benefit is observed in the water depletion category. Freshwater eutrophication shows a significant reduction (32%) due to the avoided direct emissions to water produced by the effluent discharge. For the same reason, a large change (−35%) is seen in the freshwater ecotoxicity category compared to the baseline scenario. However, a significant negative impact is observed on human toxicity (+19%), and terrestrial ecotoxicity (+32%) due to the presence of traces of heavy metals in the reclaimed water. The shift of direct emissions from water to the soil results in a trade-off between freshwater ecotoxicity and human toxicity and terrestrial ecotoxicity. The reduction of the mineral resource depletion (6%) is also affected by the displaced N and P fertilizer.

3.2.2. Scenario 2 - chemical disinfection using peracetic acid

Scenario 2 is the alternative version of the baseline scenario where the UV disinfection is replaced by PAA disinfection. Fig. 6 shows the environmental performance of Scenario 2 relative to the baseline scenario.

The overall environmental performance of the chemical disinfection scenario is similar to that of Scenario 1. The use of chemicals for disinfection leads to an additional impact on climate change (+9%), fossil fuel depletion (+18%) and mineral resource depletion (+6%) compared to baseline case. However, the avoided emissions from energy savings and displaced fertilizer outweigh them significantly and result in an overall reduction in most of the impact categories. The chemical disinfection of Scenario 2 shows a slightly higher reduction (2%) compared to the energy-intensive UV disinfection of Scenario 1, both in climate change and fossil fuel depletion. Similar to Scenario 1, there is a significant reduction in freshwater ecotoxicity while the end-use of water on land plays a large role in human toxicity and terrestrial ecotoxicity. Finally, the impact reduction on freshwater eutrophication is again determined by the avoided direct emissions to water.

3.2.3. Scenario 3 - biological treatment with UASB followed by AnMBR as tertiary treatment

Scenario 3 includes the UASB as biological treatment followed by the AnMBR as the tertiary treatment and thus eliminates the need for a disinfection unit. The final effluent is richer in N and P contents compared to the other scenarios as 24 mg/l and 2 mg/l, respectively. Fig. 7 shows the environmental performance of Scenario 3 relative to the baseline scenario. Besides water depletion, Scenario 3 shows much higher relative benefits than Scenario 1 and 2 in climate change (−28%), fossil fuel depletion (−31%), mineral resource depletion (−52%) and freshwater ecotoxicity (−35%) compared to the baseline scenario (Fig. 7 a). As can be seen from the contribution analysis in (Fig. 7 b) the latter is attributed to the avoided fertilizer spreading, where relative reductions of 45% in climate change, 68% in fossil fuel depletion, 74% in mineral resource depletion and 18% in freshwater ecotoxicity are obtained. The direct emissions to soil (heavy metals and nutrients) are the main contributors to human toxicity (+55%) and terrestrial ecotoxicity (+45%). It is assumed that the dissolved methane in the permeate is not recovered through advanced treatment and thus raising the global warming potential by 28%. However, this is balanced by the avoided direct emissions from aerobic processes and the reduced amount of chemicals required for P-removal via chemical precipitation. Moreover, the greater nutrient content of the effluent provides the highest fertilizer substitution rate. It produces the most positive effect on impact reduction. However, Scenario 3 shows a significantly larger impact on eutrophication (68%) due to the increased amount of direct emissions to water (+23% compared to the baseline) mainly related to the fraction of nutrient-rich water which is not reused but directly discharged into a water body. The

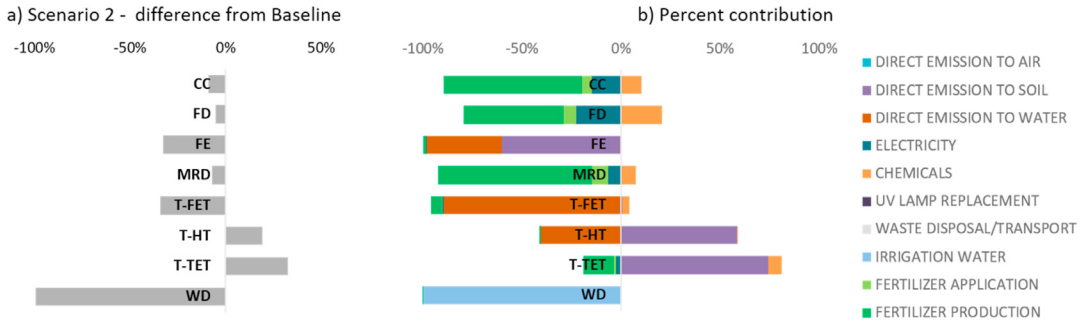


Fig. 6. Environmental performance of scenario with chemical disinfection using peracetic acid relative to baseline scenario: a) as overall relative differences in each category; b) percentage contribution analysis based on the individual processes and sources of impact.

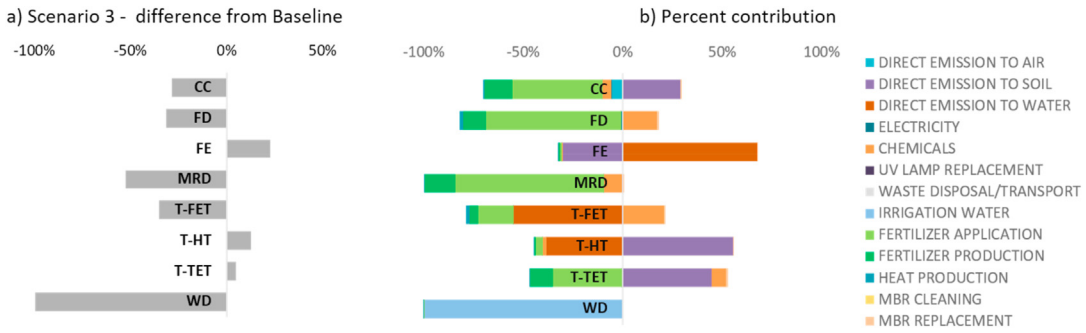


Fig. 7. Environmental performance of UASB followed by AnMBR relative to baseline scenario: a) as overall relative differences in each category; b) percentage contribution analysis based on the individual processes and sources of impact.

additional impact from membrane replacement, maintenance and cleaning results in a negligible additional impact (<1%) compared to other factors. Finally, differently from Scenarios 1 and 2, the trade-off between the toxicity categories is relatively smaller.

3.3. Economic performance assessment

The economic performance of the considered scenarios is shown in Fig. 8. In terms of biological treatment, Scenario 3 does not have a significant increase in CAPEX compared to the other scenarios. CAPEX comprises 40-44% of the total cost during the lifetime of the plant. In all scenarios, the greatest OPEX belongs to energy consumption except for Scenario 3 where the membrane replacement plays a major role followed by the energy demand. The differences between the UV disinfection (Scenario 1), PAA disinfection (Scenario 2) and the baseline are negligible in terms of total costs. On the other hand, the relative contributions are different. Specifically, in Scenario 1, the UV replacement has a relative impact of 8%, while in Scenario 2, the chemical consumption has a relative impact of 19%. The larger cost for PAA supply is balanced by lower energy consumption and the avoided periodic replacement of expensive equipment like a UV lamp. Although the Scenario 3 has the best environmental performance considering almost all the indicators, it has the highest overall costs due to the membrane investment and replacement. The substitution every ten years of the membrane modules contributes to 24% of the total cost. From a wastewater treatment point of view, the environmental benefits of Scenario 3 should encompass the highest investment and operational cost of the membrane reactor.

4. Discussion

This work demonstrated that the combination of anaerobic secondary treatment (i.e. UASB) with an ultrafiltration chamber (i.e. AnMBR) can strongly reduce the environmental impact of final discharges compared to the CAS line followed by disinfection processes (Baseline, Scenario 1 and Scenario 2) when the reclaimed water is intended to be reused in agriculture. Furthermore, it showed the necessity to recognize WWTPs more like water resource recovery facilities (WWRFs) where not only water but also

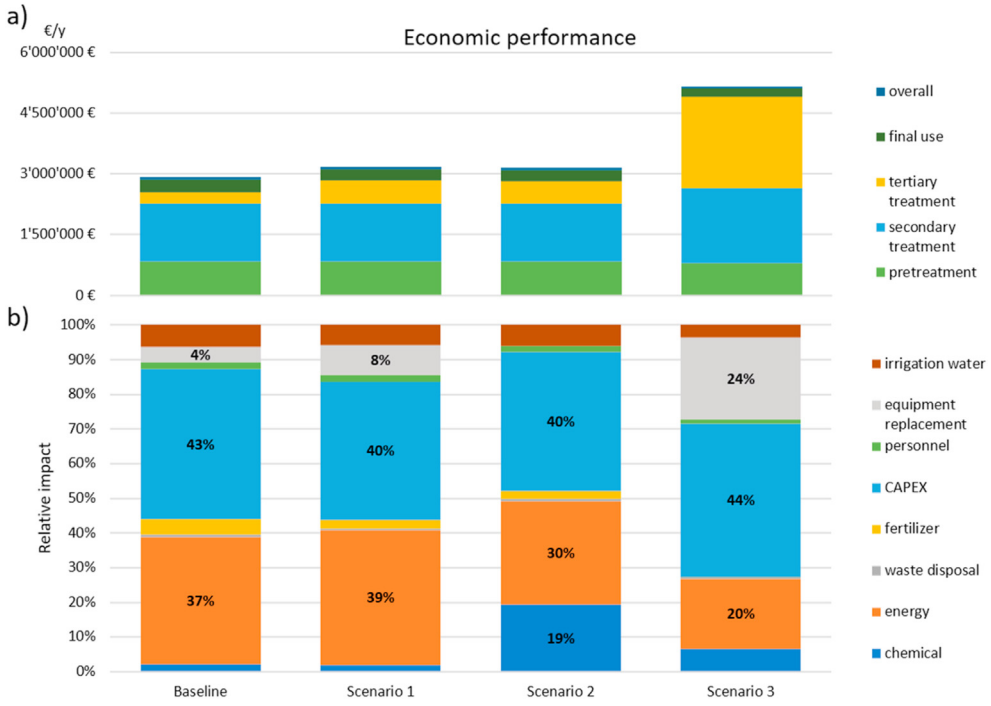


Fig. 8. Economic evaluation in each scenario: a) phase contribution b) relative impact.

value-added materials, nutrients and energy are recovered (Akyol et al., 2020), while economic cost and carbon footprint are minimized. At the same time, this could provide an economic benefit for farmers since they can reduce mineral fertilizer acquisition, resulting in an economic and environmental win-win situation. In alternative scenarios, high environmental impacts are associated with eco- and human toxicity categories as a result of using reclaimed water in agriculture. The impacts on eco- and human toxicity are primarily related to heavy metals contamination of soil. Tangsubkul et al. (2005) noted that the increased impacts on the terrestrial environments might be inevitable when selecting a technology that optimizes the recycling of wastewater nutrients, due to the potentially higher metals loading associated with the higher nutrient recovery and reuse (Fang et al., 2016). Turan et al. (2018) evaluated the effects of chitosan (CH) and biochar (BC) on growth and nutritional quality of brinjal plant together with in situ immobilization of heavy metals in a soil polluted with heavy metals due to irrigation with wastewater. This is a critical point that the reclamation managers and farmers should pay attention to the possible ways to neglect heavy metal contamination via reclaimed wastewater reuse. Strong exposure of plants to heavy metals in the soil modifies the majority of metabolic and cellular processes in plant cells, which in return pose serious ecological risks and human health hazards (Turan, 2019).

In a recent study, environmental and human health impacts of water reclamation for crop irrigation was comparatively evaluated by the combination of scenario modeling, life-cycle impact analyses and Monte Carlo simulations (Pan et al., 2019). Similar to our findings, the authors indicated that adverse environmental and human health impacts were dependent on energy and chemical inputs (such as iron chloride for enhanced phosphorus removal). In fact, the direct benefits of water reclamation could be offset by other adverse environmental and human health impacts, (e.g. mineral depletion, global warming, ozone depletion, ecotoxicity) which are associated with increased usage of energy and chemicals for rigorous removal of contaminants, that can further affect decision-making. LCA may provide some surprising results, too, such as the case of Carré et al. (2017). Five different tertiary treatments were compared where the combination of a sand filter with UV disinfection or the use of UF alone was found to be equivalent in terms of environmental impact for most of the midpoint indicators chosen although the processes vary from each other.

Specifically, in our study, the system boundaries involved the water and nutrient demand of crops, besides the different technical solutions for water reclamation. Hence, our inventory includes the off-set of mineral fertilizer production and freshwater withdrawn as conducted by previous works (Cornejo et al., 2016; Pan et al., 2019). Further, the LCI also considers the spreading of fertilizer via tractors, as well as nutrients excess due to reclaimed water. The spreading plays a major role in such impact categories related to fossil fuel combustion, while nutrients do so in the eutrophication. When fertigation is implemented, N and P are directly supplied through irrigation system avoiding the use of tractors and broadcasters. Therefore, it strongly influences and reduces the environmental impact. This also stresses the higher benefits obtained by the anaerobic processes (Scenario 3) in almost all categories, except for the freshwater eutrophication. To overcome the eutrophication issue that occurs when P-rich effluents are discharged into water bodies, using both aerobic and anaerobic treatment is recommended. This will make the modulation of the quality of the treated wastewater possible: the UASB-AnMBR effluent will provide the crops with nutrients and water while the effluent from aerobic CAS system will be used for nutrient dilution or irrigation. Temporal variability of the nutrients and water demands of crops will determine the flow rate partition between the two treatment lines.

5. Conclusion

All three proposed configurations aim to obtain an effluent quality of class A (*E. coli* < 10 CFU/100 ml) according to the Regulation (EU) 2020/741 on minimum requirements for water reuse in agriculture. The LCA clearly demonstrated that the reuse of reclaimed water provides more environmental benefits than the discharge of treated water. No significant differences were obtained between the disinfection by PAA or UV. The environmental performance of the PAA disinfection scenario is mainly affected by chemical transportation, while the UV disinfection is influenced by energy consumption. The impact related to energy consumption is expected to be less significant in the future with the increase amount of renewable energy. In almost every impact category, higher benefits were obtained by applying the anaerobic configuration (UASB + AnMBR), except for the freshwater eutrophication. Furthermore, the highest overall costs belong to the AnMBR line, but its environmental benefits can encompass the high investment and operational cost. For future research, actual removal of heavy metals, as well as contaminants of emerging concern, can be considered in the proposed scenarios, especially stressing the differences between CAS and AnMBR systems.

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CRediT authorship contribution statement

Alessia Foglia: Conceptualization, Investigation, Data curation, Methodology, Software, Formal analysis. **Corinne Andreola:** Investigation, Data curation, Methodology, Formal analysis, Software, Visualization, Writing - original draft. **Giulia Cipolletta:** Investigation, Methodology, Software, Formal analysis. **Serena Radini:** Investigation, Methodology, Software, Visualization. **Çağrı Akyol:** Conceptualization, Writing - original draft. **Anna Laura Eusebi:** Conceptualization, Supervision, Writing - review & editing. **Peyo Stanchev:** Methodology, Software, Formal analysis, Validation, Visualization, Writing - original draft. **Evina Katsou:** Conceptualization, Supervision, Writing - review & editing. **Francesco Fatone:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2021.126201.

References

Akyol, Ç., Foglia, A., Ozbayram, E.G., Frison, N., Katsou, E., Eusebi, A.L., Fatone, F., 2020. Validated innovative approaches for energy-efficient resource recovery and re-use from municipal wastewater: from anaerobic treatment systems to a biorefinery concept. Crit. Rev. Environ. Sci. Technol. 50, 869–902. https://doi.org/10.1080/10643389.2019.1634456.

Angelakis, A.N., Snyder, S.A., 2015. Wastewater treatment and reuse: past, present, and future. Water (Switzerland) 7, 4887–4895. https://doi.org/10.3390/w7094887.

Antonelli, M., Turolla, A., Mezzanotte, V., Nurizzo, C., 2013. Peracetic acid for secondary effluent disinfection: a comprehensive performance assessment. Water Sci. Technol. 68, 2638–2644. https://doi.org/10.2166/wst.2013.542.

Awad, H., Gar Alalm, M., El-Etriby, H.K., 2019. Environmental and cost life cycle assessment of different alternatives for improvement of wastewater treatment plants in developing countries. Sci. Total Environ. 660, 57–68. https://doi.org/10.1016/j.scitotenv.2018.12.386.

Buonocore, E., Mellino, S., De Angelis, G., Liu, G., Ulgiati, S., 2018. Life cycle assessment indicators of urban wastewater and sewage sludge treatment. Ecol. Indicat. 94, 13–23. https://doi.org/10.1016/j.ecolind.2016.04.047.

Carré, E., Beigbeder, J., Jauzein, V., Junqua, G., Lopez-Ferber, M., 2017. Life cycle assessment case study: tertiary treatment process options for wastewater reuse. Integrated Environ. Assess. Manag. 13, 1113–1121. https://doi.org/10.1002/ieam.1956.

Collivignarelli, C., Bertanza, G., Pedrazzani, R., 2000. A comparison among different wastewater disinfection systems: experimental results. Environ. Technol. 21, 1–16. https://doi.org/10.1080/09593332108618137.

Cornejo, P.K., Zhang, Q., Mihelcic, J.R., 2016. How does scale of implementation impact the environmental sustainability of wastewater treatment integrated with resource recovery? Environ. Sci. Technol. 50, 6680–6689. https://doi.org/10.1021/acs.est.5b05055.

Corominas, L., Byrne, D.M., Guest, J.S., Hospido, A., Roux, P., Shaw, A., Short, M.D., 2020. The application of life cycle assessment (LCA) to wastewater treatment: a best practice guide and critical review. Water Res. 184 https://doi.org/10.1016/j.watres.2020.116058.

DEMOWARE, 2016. Partial Disinfection Technologies for Water Reuse: Case Studies and Design Guidelines.

Diaz-Elsayed, N., Rezaei, N., Guo, T., Mohebbi, S., Zhang, Q., 2019. Wastewater-based resource recovery technologies across scale: a review. Resour. Conserv. Recycl. 145, 94–112. https://doi.org/10.1016/j.resconrec.2018.12.035.

Doka, G., 2003. Life cycle inventory of wastewater treatment. Swiss Cent. Life Cycle Invent. Tech. Rep. Ecoinvent Rep.

Ec, 2020a. Water reuse report. https://ec.europa.eu/environment/water/reuse.htm. (Accessed 14 September 2020).

Ec, 2020b. Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on Minimum Requirements for Water Reuse.

Fang, L.L., Valverde-Pérez, B., Damgaard, A., Plósz, B.G., Rygaard, M., 2016. Life cycle assessment as development and decision support tool for wastewater resource recovery technology. Water Res. 88, 538–549. https://doi.org/10.1016/j.watres.2015.10.016.

Foglia, A., Akyol, Ç., Frison, N., Katsou, E., Laura, A., Fatone, F., 2020. Long-term operation of a pilot-scale anaerobic membrane bioreactor (AnMBR) treating high salinity low loaded municipal wastewater in real environment. Separ. Purif. Technol. 236, 116279 https://doi.org/10.1016/j.seppur.2019.116279.

Giannoccaro, G., Arborea, S., de Gennaro, B.C., Iacobellis, V., Piccinni, A.F., 2019. Assessing reclaimed urban wastewater for reuse in agriculture: technical and economic concerns for Mediterranean regions. Water 11, 1511. https://doi.org/10.3390/w11071511.

Hai, F.I., Riley, T., Shawkat, S., Magram, S.F., Yamamoto, K., 2014. Removal of pathogens by membrane bioreactors: a review of the mechanisms, influencing factors and reduction in chemical disinfectant dosing. Water (Switzerland) 6, 3603–3630. https://doi.org/10.3390/w6123603.

Harclerode, M., Doody, A., Brower, A., Vila, P., Ho, J., Evans, P.J., 2020. Life cycle assessment and economic analysis of anaerobic membrane bioreactor whole-plant configurations for resource recovery from domestic wastewater. J. Environ. Manag. 269, 110720 https://doi.org/10.1016/j.jenvman.2020.110720.

ISPRA, 2012. Modello di indagine per la valutazione della fattibilità del riuso delle acque reflue depurate. Manuali e linee guida80/2012. ISBN: 978-88-448-0547-0.

ISO, 2006. Environmental Management—Life Cycle Assessment—Requirements and Guidelines (ISO 14044). Geneva.

Jiménez-Benítez, A., Ferrer, F.J., Greses, S., Ruiz-Martínez, A., Fatone, F., Eusebi, A.L., Mondéjar, N., Ferrer, J., Seco, A., 2020. AnMBR, reclaimed water and fertigation: two case studies in Italy and Spain to assess economic and technological feasibility and CO2 emissions within the EU Innovation Deal initiative. J. Clean. Prod. 270 https://doi.org/10.1016/j.jclepro.2020.122398.

Krzeminski, P., Katsou, E., Dosoretz, C.G., Esteban-García, A.L., Leverette, L., Malamis, S., Nenov, V., Robles, Á., Seco, A., Syron, E., 2017a. Membranes in wastewater treatment. In: Lema, J.M., Suarez, S. (Eds.), Innovative Wastewater Treatment & Resource Recovery Technologies: Impacts on Energy, Economy and Environment. IWA Publishing. https://doi.org/10.2166/9781780407876_0129, 0.

Krzeminski, P., Leverette, L., Malamis, S., Katsou, E., 2017b. Membrane bioreactors - a review on recent developments in energy reduction, fouling control, novel configurations, LCA and market prospects. J. Membr. Sci. 527, 207–227. https://doi.org/10.1016/j.memsci.2016.12.010.

Lee, E.J., Criddle, C.S., Geza, M., Cath, T.Y., Freyberg, D.L., 2018. Decision support toolkit for integrated analysis and design of reclaimed water infrastructure. Water Res. 134, 234–252. https://doi.org/10.1016/j.watres.2018.01.037.

Lorenzo-Toja, Y., Alfonsín, C., Amores, M.J., Aldea, X., Marin, D., Moreira, M.T., Feijoo, G., 2016. Beyond the conventional life cycle inventory in wastewater treatment plants. Sci. Total Environ. 553, 71–82. https://doi.org/10.1016/j.scitotenv.2016.02.073.

Luukkonen, T., Heyninck, T., Rämö, J., Lassi, U., 2015. Comparison of organic peracids in wastewater treatment: disinfection, oxidation and corrosion. Water Res. 85, 275–285. https://doi.org/10.1016/j.watres.2015.08.037.

MedECC Network, 2019. Risks Associated to Climate and Environmental Changes in the Mediterranean Region, A Preliminary Assessment by the Mediterranean Experts on Climate and Environmental Change (MedECC) Network Science-Policy Interface.

Meneses, M., Pasqualino, J.C., Castells, F., 2010. Environmental assessment of urban wastewater reuse: treatment alternatives and applications. Chemosphere 81, 266–272. https://doi.org/10.1016/j.chemosphere.2010.05.053.

Muñoz, I., Rodríguez, A., Rosal, R., Fernández-Alba, A.R., 2009. Life Cycle Assessment of urban wastewater reuse with ozonation as tertiary treatment. A focus on toxicity-related impacts. Sci. Total Environ. 407, 1245–1256. https://doi.org/10.1016/j.scitotenv.2008.09.029.

Pan, Y.R., Wang, X., Ren, Z.J., Hu, C., Liu, J., Butler, D., 2019. Characterization of implementation limits and identification of optimization strategies for sustainable water resource recovery through life cycle impact analysis. Environ. Int. 133, 105266. https://doi.org/10.1016/j.envint.2019.105266.

Pasqualino, J.C., Meneses, M., Castells, F., 2011. Life cycle assessment of urban wastewater reclamation and reuse alternatives. J. Ind. Ecol. 15, 49–63. https://doi.org/10.1111/j.1530-9290.2010.00293.x.

Pretel, R., Moñino, P., Robles, A., Ruano, M.V., Seco, A., Ferrer, J., 2016. Economic and environmental sustainability of an AnMBR treating urban wastewater and organic fraction of municipal solid waste. J. Environ. Manag. 179, 83–92. https://doi.org/10.1016/j.jenvman.2016.04.057.

Pretel, R., Robles, A., Ruano, M.V., Seco, A., Ferrer, J., 2013. Environmental impact of submerged anaerobic MBR (SAnMBR) technology used to treat urban wastewater at different temperatures. Bioresour. Technol. 149, 532–540. https://doi.org/10.1016/j.biortech.2013.09.060.

Rizzo, L., Gernjak, W., Krzeminski, P., Malato, S., McArdell, C.S., Perez, J.A.S., Schaar, H., Fatta-Kassinos, D., 2020. Best available technologies and treatment trains to address current challenges in urban wastewater reuse for irrigation of crops in EU countries. Sci. Total Environ. 710 https://doi.org/10.1016/j.scitotenv.2019.136312.

Rizzo, L., Krätke, R., Linders, J., Scott, M., Vighi, M., de Voogt, P., 2018. Proposed EU minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge: SCHEER scientific advice. Curr. Opin. Environ. Sci. Heal. 2, 7–11. https://doi.org/10.1016/j.coesh.2017.12.004.

Salgot, M., Folch, M., 2018. Wastewater treatment and water reuse. Curr. Opin. Environ. Sci. Heal. 2, 64–74. https://doi.org/10.1016/j.coesh.2018.03.005.

Tangsubkul, N., Beavis, P., Moore, S.J., Lundie, S., Waite, T.D., 2005. Life cycle assessment of water recycling technology. Water Resour. Manag. 19, 521–537. https://doi.org/10.1007/s11269-005-5602-0.

Turan, V., 2019. Confident performance of chitosan and pistachio shell biochar on reducing Ni bioavailability in soil and plant plus improved the soil enzymatic activities, antioxidant defense system and nutritional quality of lettuce. Ecotoxicol. Environ. Saf. 183, 109594. https://doi.org/10.1016/j.ecoenv.2019.109594.

Turan, V., Khan, S.A., Mahmood-ur-Rahman, Iqbal, M., Ramzani, P.M.A., Fatima, M., 2018. Promoting the productivity and quality of brinjal aligned with heavy metals immobilization in a wastewater irrigated heavy metal polluted soil with biochar and chitosan. Ecotoxicol. Environ. Saf. 161, 409–419. https://doi.org/10.1016/j.ecoenv.2018.05.082.

UNEP/MAP Plan Bleu, 2019. State of the Environment and Development in the Mediterranean. Technical Report. Athens.

Ventura, D., Consoli, S., Barbagallo, S., Marzo, A., Vanella, D., Licciardello, F., Cirelli, G.L., 2019. How to overcome barriers for wastewater agricultural reuse in Sicily (Italy)? Water (Switzerland) 11, 1–12. https://doi.org/10.3390/w11020335.

Vojtěchovská Šrámková, M., Diaz-Sosa, V., Wanner, J., 2018. Experimental verification of tertiary treatment process in achieving effluent quality required by wastewater reuse standards. J. Water Process Eng. 22, 41–45. https://doi.org/10.1016/j.jwpe.2018.01.003.

Yoshida, H., ten Hoeve, M., Christensen, T.H., Bruun, S., Jensen, L.S., Scheutz, C., 2018. Life cycle assessment of sewage sludge management options including long-term impacts after land application. J. Clean. Prod. 174, 538–547.

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Validation of an evidence-based methodology to support regional carbon footprint assessment and decarbonisation of wastewater treatment service in Italy

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ABSTRACT

In this paper, a new regional methodological approach for determining direct and indirect emissions from wastewater treatment plants (WWTPs) is proposed. Additionally, an entire territorial wastewater treatment service located in the northern Italy and serving 411,484 PE was assessed. The most accurate emission factor identification is presented using appropriate on-site measurements, monitoring different aerated operational units and sampling several streams in 12 relevant WWTPs of different treatment capacities, ranging from 3000 to 73,000 PE. Dissolved greenhouse gas (GHG) concentrations from 0.2 to 24 mgN_2O/L, 0.1 to 1 $mgCH_4/L$ and 1.8 to 52 $mgCO_2/L$ in effluent flows were detected. Specific carbon footprints resulted in the emissions of 0.04–0.20 $tonCO_{2eq}/PE/y$, varying as per the size of the plants. The most impactful categories were identified for indirect emissions, associated with dissolved GHGs discharged in the surface water body and due to energy consumption, which accounted for 13–70% and 10–40%, respectively. The overall territorial carbon footprint of the wastewater treatment service was also quantified to provide evidence-based decision support system (DSS) and prepare systemic mitigation strategies.

1. Introduction

Several regions are working towards low carbon and circular economy (Regional Policy, EC, 2014) and actions to decarbonise urban water management can have a relevant impact (Ballard et al., 2018) especially when territorial pathways are developed.

In recent years, environmental legislations such as the Urban Wastewater Treatment Directive (UWWTD) (DIRECTIVE 91/271/EC), currently under revision, introduced more stringent quality standards for the effluents of the wastewater treatment plants (WWTPs) that led to increase in energy consumption and greenhouse gas (GHG) emissions (Gu et al., 2016). In many European countries, urban water cycle accounts for 1–3% of the total electric energy consumption (Longo et al., 2016) and 3–10% of the global warming potential (GWP) by contributing towards GHG emissions into the atmosphere, both as direct and indirect footprints (Samuelsson et al., 2018). As per the U.S. Environmental Protection Agency (EPA), the emissions of CO_2, CH_4, and N_2O constitute the largest part of CF of WWTPs, and global GHG emissions of the wastewater sector are predicted to increase by up to 27% by 2030 (Caniani et al., 2019; Huang et al., 2020).

In this context, several national and international initiatives and activities have been started to support the transition to low-carbon regions and urban water utility (WACCLIM, 2020; Crippa et al., 2019). In Italy, the National Regulatory Authority for Energy, Networks, and Environment (ARERA, 2017) introduced the 'Carbon Footprint of the wastewater treatment service' as one of the key performance indicators to analyse and assess the technical quality standard of the activities carried out by water utilities. On one hand this regulatory driver can make a substantial shift towards the low-carbon water utilities, on the other hand the Authority could only guide the operators by indicating the adoption of the general ISO 14,064–1:2019. In fact, while for energy audits, a standardised European methodology was developed in the H2020 project ENERWATER to assess the energy footprint of WWTPs (Longo et al., 2019), for CF, same standard approach is missing. Today several tools and software have been developed in recent years to

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Nomenclature

AD	activity data
ASMN	activated sludge models for nitrogen
BSM2-e	benchmark simulation model No. 2 emission
BSM2G	benchmark simulation model No. 2 greenhouse gas
C	carbon
CF	carbon footprint
CFCT	carbon footprint calculation tool
CF-TOOL CTRL	carbon footprint control
CHEApet	carbon heat energy assessment plant evaluation tool
CO_{2eq}	equivalent CO_2
COD	chemical oxygen demand
COD_{eff}	effluent chemical oxygen demand
COD_{in}	influent chemical oxygen demand
COD_{rem}	removed chemical oxygen demand
DEEM	diffusive emissions estimation model
DSS	decision support system
ECAM	energy performance and carbon emissions assessment and monitoring
GHG	greenhouse gas
GWP	global warming potential
IA	intermittent aeration
K	potassium
MLE	modified Ludzack Ettinger
PE	population equivalent
SCENA	short cut enhanced nutrients abatement
SCF	specific carbon footprint
EF	emission factor
TN	total nitrogen
TN_{eff}	effluent total nitrogen
TN_{rem}	removed total nitrogen
TP	total phosphorus
TS	total solids
TSS	total suspended solids
WESTWeb	water energy sustainability tool
WWEECarb	water and waste environmental engineering carbon footprint;
WWTP	wastewater treatment plant

Table 1
Selected WWTPs for on-site measurements.

	Design capacity PE	Measured units -	Biological treatment -	COD influent tonCOD/y	TN influent tonTN/y	TP influent tonTP/y	COD removal %	TN removal %	TP removal %
WWTP1	73,000	Biological reactor	MLE	1919	198	30	93%	84%	90%
WWTP2	40,000	Biological reactor	MLE	1394	69	21.6	92%	84%	96%
WWTP3	40,000	Biological reactor, Via-nitrite supernatant treatment, Biofilter	MLE, via-nitrite supernatant treatment	2037	165	24	90%	70%	49%
WWTP4	70,000	Biological reactor, Biofilter	IA	1876	137	21.7	88%	71%	70%
WWTP5	32,000	Biological reactor	MLE	1253	103	21.1	93%	88%	79%
WWTP6	45,000	Biological reactor, Aerobic stabilisation	MLE	432	49	5.8	87%	85%	82%
WWTP7	9500	Biological reactor, Biofilter	MLE	273	34	3.9	93%	85%	72%
WWTP8	18,000	Biological reactor, Aerated degritting unit	MLE	318	44	4.9	86%	88%	58%
WWTP9	22,000	Biological reactor, Aerated degritting unit	MLE	444	40	7.8	90%	80%	60%
WWTP10	3000	Biological reactor	MLE	58	8.6	1.1	87%	88%	43%
WWTP11	4500	Biological reactor	MLE	93	11.7	1.1	93%	87%	60%
WWTP12	10,000	Biological reactor, Aerated degritting unit	IA	237	18.4	2.5	89%	88%	73%

WWTP wastewater treatment plant; PE population equivalent; MLE Modified Ludzack Ettinger; IA intermittent aeration; COD chemical oxygen demand; TN total nitrogen; TP total Phosphorus.

quantify CF although they do not follow any specific normalised or standard methodologies (Mannina et al., 2016). Moreover, one of the most critical aspects is the appropriate identification of representative and validated emission factors (EFs). In fact, some referenced databases were developed to collect and update the EFs, mainly at the international or national level, such as emission factor database (EFDB) by Intergovernmental Panel on Climate Change (IPCC). Nevertheless, the wastewater sector is typically characterised by wide local variations in influent characteristics and different process parameters or operative conditions (Vasilaki et al., 2019). Therefore, site-specific EFs need to be considered (Parravicini et al., 2016) through long-term sampling campaigns, especially for evaluating direct emissions from the main processes and indirect dissolved GHGs present in the final effluents.

This paper goes beyong the current state of the art because a new normalised methodological approach according to the guidelines of ISO 14,064 (WWEECarb) is proposed for the determination both of direct and indirect emissions in WWTPs and of the overall CF of regional wastewater service carried out by a water utility. Most of the considered emissions factors, were validated by site-specific measurements campaigns and emissions categories included both fossil and biogenic origin of the main GHGs. This validated approach can contribute to the standardization of the methodology for carbon footprint assessment in wastewater treatment service and to identify mitigation actions and priorities for regional decarbonisation.

This methodology was applied to territorial wastewater service (52 municipalities and 1376 km^2s) located in the northern Italy (411,484 PE) managed by a single public-owned water utility. The most accurate EF identification is presented by using the real operational data and appropriate measurements of emitted and dissolved GHGs from different operational units of 12 WWTPs.

2. Materials and methods

2.1. General guideline ISO 14,064 1

ISO standard (ISO 14064-1, 2019) provides '*specifications with*

Table 2
Comparison between existing carbon footprint tool and models and this specific case study methodology.

MODEL/ TOOL	Application	GHGs	Emissions Categories						Mitigation	EFs
			Direct GHGs	*Dissolved GHGs*	*Sludge Disposal*	*Energy*	*Chemicals*	*Transports*	*Carbon sequestration and minimisation*	
1 -CFCT	WWTP	CH_4, N_2O, fossil CO_2	*							Editable
2 -CF-TOOL CTRL	WWTP	N_2O, CO_2	**							Default
3-CHEApet	WWTP	CH_4, N_2O, CO_2	**	only N_2O						Default
4-WESTWeb	Water Service	CH_4, N_2O, CO_2, NO_x, PM, SO_x, VOC, CO	**							Default
5-ECAM	Water service	CH_4, N_2O, fossil CO_2	**	Not specified						Editable
6-DEEM	Biological Unit	N_2O, CO_2	**							Default
7-ASMN	Biological Unit	N_2O	**							Default
8-BSM2G	WWTP	CH_4, N_2O, CO_2	**							Default
9-BSM2-e	WWTP	CH_4, N_2O, CO_2	**							Default
10-WWEECarb	**WW Service**	**CH_4, N_2O, fossil and biogenic CO_2**								**Editable**

CFCT Carbon Footprint Calculation Tool; CF-TOOL CTRL Carbon Footprint Control; CHEApet Carbon Heat Energy Assessment Plant Evaluation Tool (CHEApet, 2011); WESTWeb Water-Energy Sustainability Tool (WESTWeb, 2021); ECAM Energy Performance and Carbon Emissions Assessment and Monitoring; DEEM Diffusive Emissions Estimation Model; ASMN Activated Sludge Models for Nitrogen; BSM2G Benchmark Simulation Model No 2 Greenhouse Gas; BSM2-*e* Benchmark Simulation Model No 2 Emission, WWEECarb Water and Waste Environmental Engineering Carbon Footprint; WWTP wastewater treatment plant; WW wastewater; GHGs greenhouse gases; EFs emission factors.

* Unique Direct EF from the whole water line.

** Direct EFs from biological reactor and sludge line.

Direct EFs from aerated de-sanding units, aerobic biological process, aerobic stabilization of sludge, fuggitive emissions from sludge line and biofilters 1 Gustavsson and Tumlin (2013), 2 Baeza et al. (2017), 3 https://www.waterrf.org/research/projects/demonstration-carbon-heat-energy-assessment-and-plant-evaluation-tool-cheapet, 4 https://west.berkeley.edu/model.php, 5 http://wacclim.org/ecam/sources.php, 6 Guo et al. (2012), Mannina et al. (2016), 7 Guo et al. (2012), Mannina et al. (2016), 8 Flores-Alsina et al. (2012), Mannina et al. (2016), 9 Mannina et al. (2016), Sweetapple et al. (2013), 10 This study.

guidance at the organization level for quantification and reporting of greenhouse gas emissions and removals . Several aspects and steps should be followed to apply the ISO standard at organisation level: (1) reporting boundaries should be defined; (2) direct and indirect emissions must be aggregated into inventory categories, including direct emissions and removals, and indirect emissions from imported energy, transportation, products used by the organisation, associated with the use of products from the organisation, and other sources; and (3) different GHGs (i.e. CO_2, CH_4, N_2O, NF_3, and SF_6) associated with the type of organisation have to be separately quantified, and (4) anthropogenic biogenic, non-anthropogenic biogenic, or fossil origin of CO_2 should be distinguished. Moreover, CF determination should be annually referred, and the result should be reported in tonnes of CO_{2eq} using appropriate GWPs (Towprayoon et al., 2019). Finally, based on the general principles of consistency and accuracy, ISO 14,064-1 requires the definition of quantitative and/or qualitative uncertainty associated with the method used for the quantification of the emissions.

2.2. Description of the territorial wastewater service

The proposed approach was applied to determine the CF of wastewater services in the region managed by the Italian water utility Alto Trevigiano Servizi, consisting of 52 municipalities in the province of Treviso(Italy), with a total served population of 411,484 PE. The regional wastewater treatment service is operated by 5 WWTPs with a design treatment capacity of more than 40,000 PE, by 3 WWTPs with the capacities range of 15,000 40,000 PE, 27 WWTPs with less than 15,000 PE capacity, and 28 septic tanks. The representative WWTPs to be monitored over the long-term, were selected by considering both the treatment capacity of the WWTPs and the representative characteristics. Therefore, site-specific EF measurements were carried out in 12 WWTPs (8 WWTPs with capacities higher than 15,000 PE and 4 smaller WWTPs), covering approximately 90% of the total served population. The characteristics of these WWTPs in terms of process configuration, influent loads and removals efficiencies are summarized in Table 1. The selected WWTPs included both conventional activated sludge in the Modified Ludzack Ettinger (MLE) configuration (n 10) and intermittent aeration (IA) processes (n 2). The innovative technology of Short Cut Enhanced Nutrients Abatement (SCENA) (n 1) (https://www.smart-plant.eu/) for the nitrite treatment of nutrient-rich anaerobic rejected liquor from the sludge line was studied. All the aerated units of different water and sludge lines (aerated degritting unit, biological reactor, aerobic stabilisation, via-nitrite supernatant treatment, and biofilter) were monitored. The operation data were collected for all the plants over one whole year. The CF results of the monitored WWTPs were reported as the entire data ($tonCO_{2eq}$/y) and specific coefficients (SCF, $tonCO_{2eq}$/PE/y). SCFs were used to quantify the GHG impacts of the unselected WWTPs.

Septic tank emissions were also calculated according to the methodology mentioned in Towprayoon et al. (2019), considering proposed EFs for both direct and indirect contributions of the dissolved GHGs in the effluent.

2.3. Direct emissions and dissolved gases measurements campaigns

Each selected WWTP was monitored for one month during the on-site campaign. Aerated points were considered as direct emission sources (aerated de-sanding units, aerobic biological processes, aerobic stabilisations of sludge, and biofilters). Measurements were performed for at least one week in each sampling unit. Monitoring was continuously carried out for the biggest WWTPs (WWTP1, 2, 3, 4, 5, and 6) and discontinuously for the smaller ones (WWTP7, 8, 9, 10, 11, and 12). Experimental equipment for the analysis of direct GHG mainly consisted of two devices.

For continuous measurements, the gas analyser (MIR 9000-CLD type, ENVEA, IT) with a membrane air dryer Mgf Sky 30/7 M was used under controlled thermal conditions with the online acquisition of emitted GHG concentrations (one data every 5 min). The measurements of CH_4, CO_2, and N_2O were performed using the standard infrared absorption (UNICHIM method and ISTISAN 91/41 Report). The system was also

Table 3
Emissions categories, including the distinction of CO_2 origin and the separate quantification for each GHG.

ISO 14,064 1:2019 Category	Proposed declined categories	CO_2 origin	Considered GHG
Direct emissions	Direct emission from combustion	Biogenic	$tonCO_{2eq}$/y, reported separately for N_2O, CH_4 and CO_2
	Direct emissions from WWTP processes in mainstream	Biogenic	
	Direct fugitive emissions	Biogenic	
Indirect emissions from imported energy	Indirect emissions from energy consumption	Non biogenic	$tonCO_{2eq}$/y
Indirect emissions from transportation	Indirect emissions from waste transport	Non biogenic	$tonCO_{2eq}$/y, reported separately for N_2O, CH_4 and CO_2
	Indirect emissions from chemical transport	Non biogenic	
Indirect emissions from products used by the organization	Indirect emissions from chemical consumption	Non biogenic	$tonCO_{2eq}$/y
Indirect emissions from other sources	Indirect emissions on the water body	Biogenic	$tonCO_{2eq}$/y, reported separately for N_2O, CH_4 and CO_2
	Indirect emissions from sludge composting	Biogenic	

calibrated each week using gas cylinders at the standard concentrations (CH_4 at 40.6 ppm, CO_2 at 2.33%, and N_2O at 160 ppm). The gaseous samples were conveyed to the analyser through a heated gas line at 120 C. A cooling device (HIREF solution, https://hiref.it/) was used to reduce the temperature of the gases to 4 C, thus, minimising the water vapour content. Every 3 h, a compressor was activated for ambient air sampling as a zero reference. In addition, a floating chamber was used to convey the samples to the analyser. For the design and construction of the chamber, guideline indications reported for similar measurements were followed (Spinelli et al., 2018; Caniani et al., 2019; Kwok et al., 2015). The floating system was made from high-density polyethylene with a total volume of 310 L and a bottom area of 1 m^2.

Some specific sampling campaigns were carried out in the discontinuous mode for WWTPs with less than 25,000 PE capacity. Specifically, peristaltic pump and gas bags (5 L) were used to acquire the gaseous samples (three replicates for each point). Subsequently, GHG concentrations were determined using photoacoustic spectroscopy (Brüel & Kjaer Multi-gas Monitor Type 1302).

Moreover, composite liquid samples from the influent and effluent of each WWTP were collected twice a week. The dissolved CO_2, CH_4, and N_2O were measured to calculate the possible indirect emissions of the GHG mass loads discharged in the water bodies. In this case, it was assumed that the dissolved gases are totally stripped after the discharge in the water body. Since the fate of dissolved GHGs is not uniquely predictable, the hypothesis allows to be conservative for the footprint calculation. Stripping the pre-treatment at 20 C (ultrasonic sonication SONOREX model), followed by photoacoustic spectroscopy determination (Brüel & Kjaer Multi-gas Monitor Type 1302) were carried out. Finally, the main conventional physical and chemical characterisations (chemical oxygen demand (COD), total suspended solids (TSS), total nitrogen (TN), and total phosphorus (TP)) of the wastewaters were analysed (APHA, 2015).

3. Results and discussion

3.1. Audit of predictive tool and models

Technical and scientific literature shows that several tools and

Table 4
Emissions categories, activity data and type of EFs.

Number	Declined category	Activity data	Emission factor (EF)	Ref.
(1)	Direct emission from combustion	Biogas produced (Nm^3/y), methane content ($\%CH_4$)	g CO_2/kg burnt CH_4 g CH_4/kg burnt CH_4 g N_2O/kg burnt CH_4	International databases (Bartram et al., 2019)
(2)	Direct emissions from WWTP processes in mainstream	Influent and effluent COD and TN loads (ton/y)	kg N_2O/ $kgTN_{rem}$ kg CH_4/kg COD_{in} kg CO_2/kg COD_{rem}	Measured in this study Literature, international databases (Aboobakar et al., 2013; Ahn et al., 2010; Caniani et al., 2019; Foley et al., 2010; Joss et al., 2009; Masuda et al., 2018; Ribera-Guardia et al., 2019; Wang et al., 2016);
(3)	Direct fugitive emissions	Biogas produced (Nm^3/y) and methane content ($\%CH_4$); sludge produced (ton/y) and characterisation (%TS,%N,%P,%K,%C)	kgN_2O/ tonTS $kgCH_4$/ tonTS $kgCO_2$/ tonTS	Literature (Kirkeby et al., 2005; Majumder et al., 2014; Scheutz and Fredenslund, 2019; Willen et al., 2016)
(4)	Indirect emissions for energy consumption	Electricity consumption (MWh/y), other fuel combustion also renewable (Nm^3/y or l/y)	$tonCO_{2eq}$/ GWh	National and International databases (ISPRA, 2018)
(5)	Indirect emissions for waste transport	km travelled	gN_2O/km gCH_4/km gCO_2/km	National databases (SINANET, 2017)
(6)	Indirect emissions for chemical transport	km travelled	gN_2O/km gCH_4/km gCO_2/km	National databases (SINANET, 2017)
(7)	Indirect emissions for chemical consumption	Quantity used (kg/y)	$kgCO_{2eq}$/ kg reagent	Literature, international databases (Gustavsson and Tumlin, 2013)
(8)	Indirect emissions on the water body	Effluent COD and TN loads (ton/y)	g CO_2/ $kgCOD_{eff}$ g CH_4/ $kgCOD_{eff}$ g N_2O/ $kgTN_{eff}$	Measured in this study
(9)	Indirect emissions and mitigation for sludge disposal	Dried sludge	kg N_2O /tonTS kg CH_4/ tonTS kg CO_2/ tonTS	Literature, international databases (Boldrin et al., 2009; Chai et al., 2015; Chen and Kuo, 2016; Han et al., 2018; Hergoualc h et al., 2019; Kirkeby et al., 2005; Piippo et al., 2018; Yuan et al.,

(*continued on next page*)

Table 4 (*continued*)

Number	Declined category	Activity data	Emission factor (EF)	Ref.
				2018; ClimFoot, 2018)
(10)	Indirect emissions from sludge reuse	Dried sludge	kg N_2O /tonTS kg CH_4/ tonTS kg CO_2/ tonTS	(Boldrin et al., 2009; Bruun et al., 2006; Hergoualc'h et al., 2019; Kirkeby et al., 2005)
(11)	Mitigations: carbon sequestration and synthetic fertiliser substitution	Dried sludge	kg N_2O /tonTS kg CH_4/ tonTS kg CO_2/ tonTS	(Foley et al., 2010; Kirkeby et al., 2005)

TS=total solids, COD_{in}= influent chemical oxygen demand; COD_{eff}= effluent chemical oxygen demand; COD_{rem}= removed chemical oxygen demand; TN_{rem}= removed total nitrogen; TN_{eff}= effluent total nitrogen; *K*=potassium; C=carbon.

software solutions have been developed for different purposes. Simple stationary (i.e., Carbon Footprint Calculation Tool (CFCT)) or dynamic calculations (Mannina et al., 2016) have been proposed in recent decades (Table 2). Simulation models (Models 6, 7, 8, and 9) were implemented to calculate the emissions generated mainly from biological processes, such as activated sludge model (ASM) and benchmark simulation model (BSM), while specific tools were implemented for determining the CF of a single WWTP (Tools 1, 2, and 3) or for the entire water and wastewater service (Tools 4, 5, and 10). Direct emissions in most of these existing tools are mainly considered from the biological processes of the water line and only in some few cases from biogas production. Other aerated stages, such as degritting units, aerobic stabilisations of sludge, biofilters, and anaerobic supernatant treatments, are not usually considered, notwithstanding their wide impact on the global emissions of the plants (Demir et al., 2019). Moreover, most of the analysed models and tools focus mainly on direct N_2O emissions, while CH_4 is generally considered only from the sludge line. Nevertheless, depending on the wastewater treatment configurations and influent characteristics, methane emissions could also represent a relevant contribution to the mainstream water line, even higher than N_2O emissions (Zhan et al., 2017). Moreover, CO_2 concentrations have typically accounted only for the fossil origin, while biogenic part, derived from microbial respiration during the biological processes has usually not been quantified. Finally, only a few studies have considered the dissolved fractions in the liquid of the gases, even when their contributions seemed relevant. In fact, the last report of the European Commission (JRC, 2020) also underlined the importance of considering the GHG impacts of an integrated system, including sewer network, wastewater treatment, sludge disposal, and final discharge into water bodies.

Additionally, even when emissions sources are located outside the physical boundaries of the WWTPs, they are strictly associated with the water utility management choices. Thus, indirect impacts due to energy consumption, chemical dosing, transport, and waste disposal have to be considered in wastewater CF assessment (Brown et al., 2010).

Currently, in the existing models, EFs are usually set by using internal libraries, without the possibility of editing default values and considering case-specific factors, which are crucial to achieve real evidence-based results in heterogeneous sectors such as the wastewater treatment service.

A few applications included CO_{2eq} mitigations, such as carbon sequestration from the soils and substitution of mineral fertiliser when sludge is applied in agriculture fields.

In contrast, normalised approach proposed in this study (WWEE-Carb) was applied to the entire wastewater service considering: (1) all main GHGs, both biogenic and of fossil origin; (2) direct emissions generated from different operational units of the WWTP; (3) indirect emissions due to energy and chemical consumptions and transportation; (4) dissolved gases present in the effluent; (5) emissions and removals related to sludge disposal and reuse; and (6) editable EFs, derived from both onsite measurement campaigns and technical literature libraries. Finally, the standard deviations of EFs were considered to evaluate the uncertainty and accuracy of the CF results.

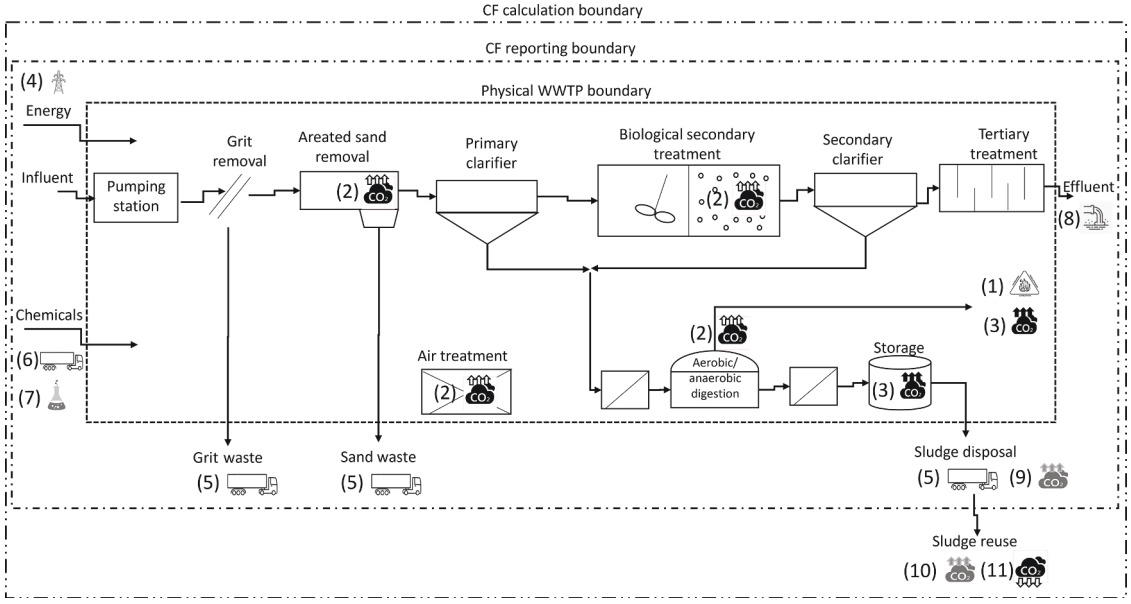


Fig. 1. General WWTP scheme and emissions categories in respect to operative, carbon footprint (CF) reporting and calculation boundaries.

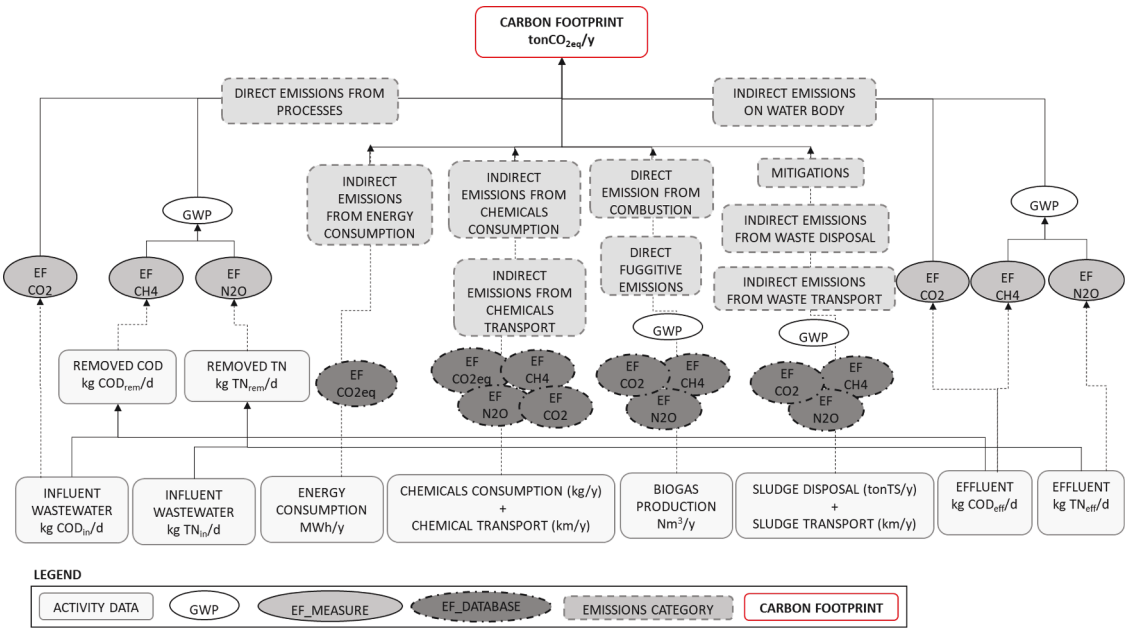


Fig. 2. Logical flow scheme of the proposed methodology.

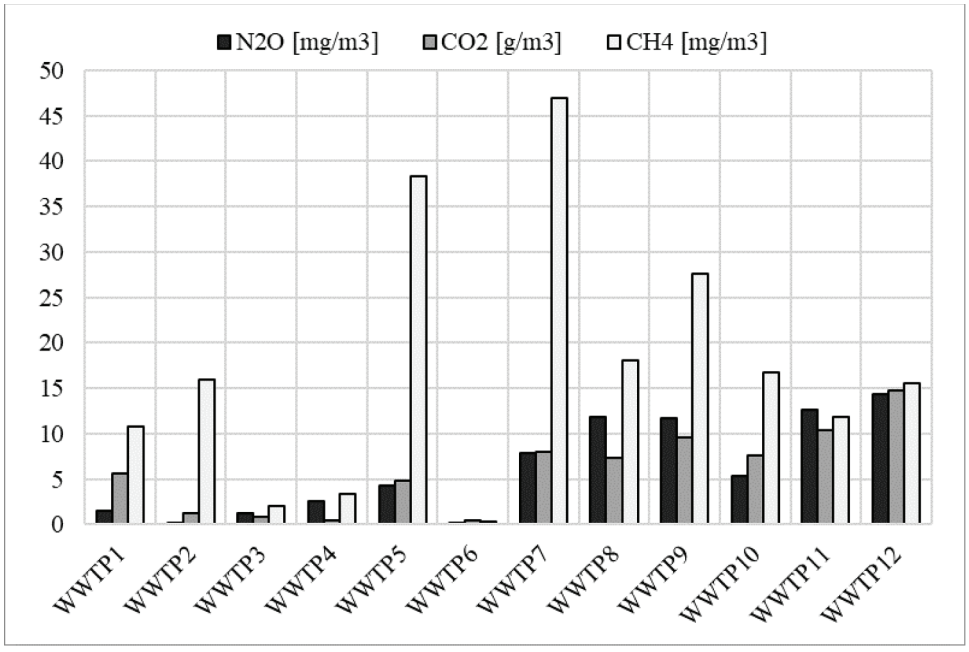


Fig. 3. Direct emitted GHGs concentrations from biological processes related to the capacity of the plants, from WWTP1 (highest capacity) to WWTP12 (smallest capacity).

3.2. Proposed methodology for carbon footprint estimation in wastewater service

The methodology was developed based on ISO 14,064–1:2019 standard and adapted to a wastewater service with systemic and territorial approaches. In this context, the operational control principle defined by UNI ISO 14,064–1:2019 was applied as a reporting boundary criterion for CF quantification. The reporting boundaries were set considering: (1) the physical operative limits of the WWTPs to define the direct emissions, (2) impacts of energy and chemical supplies, (3) waste and reagent transportations, and (4) emissions caused by the final sludge disposal or recovery/valorisation.

Specifically, direct emissions included the GHGs from: (i) biogas combustion, (ii) different aerated units (aerated degritting unit, biological reactor, aerobic sludge stabilisation, and biofilter) of the WWTP, and (iii) fugitive gases of the sludge line. In contrast, emissions from the dissolved gases on the water body, energy and chemical consumptions, transportation, and sludge disposal were considered as indirect. The indirect emissions from sludge reuse and related mitigations, such as carbon sequestration and synthetic fertiliser substitution, were estimated. CF was calculated based on the contribution of three main GHGs: methane, carbon dioxide, and nitrous oxide (Nguyen et al., 2019). Each relevant GHG contribution, fossil or biogenic CO_2 was distinguished based on the origin of the emission (Table 3).

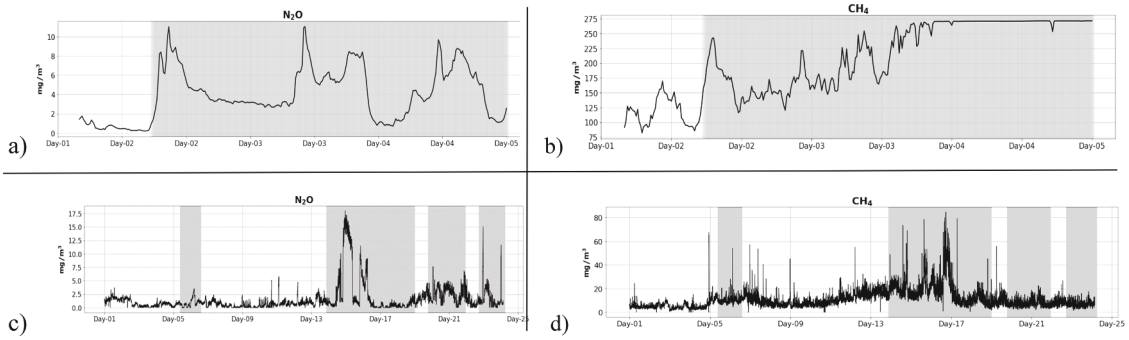


Fig. 4. Direct GHGs concentrations during the measurement campaigns, including wet period: **(**a) N_2O concentrations and (b) CH_4 concentrations from aerated degritting unit, (c) N_2O concentrations and (d) emitted CH_4 concentrations from biological treatment. **LEGEND:** xx Wet period Dried period.

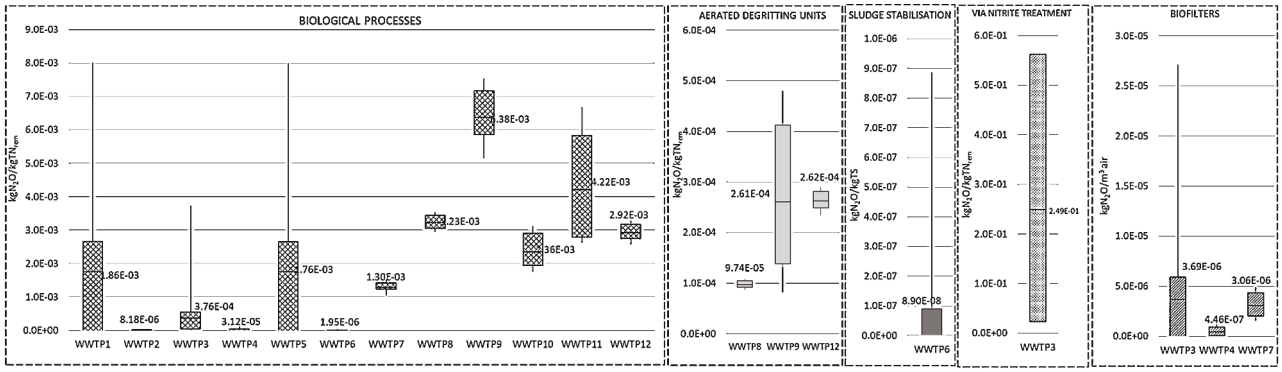


Fig. 5. Emission factors for direct N_2O emissions from WWTPs grouped for operational unit.

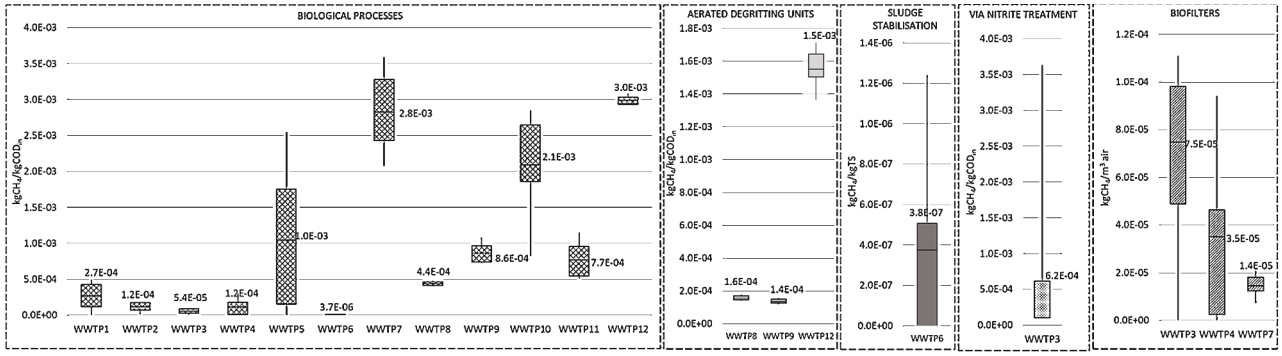


Fig. 6. Emission factors for direct CH_4 emissions from WWTPs grouped for operational unit.

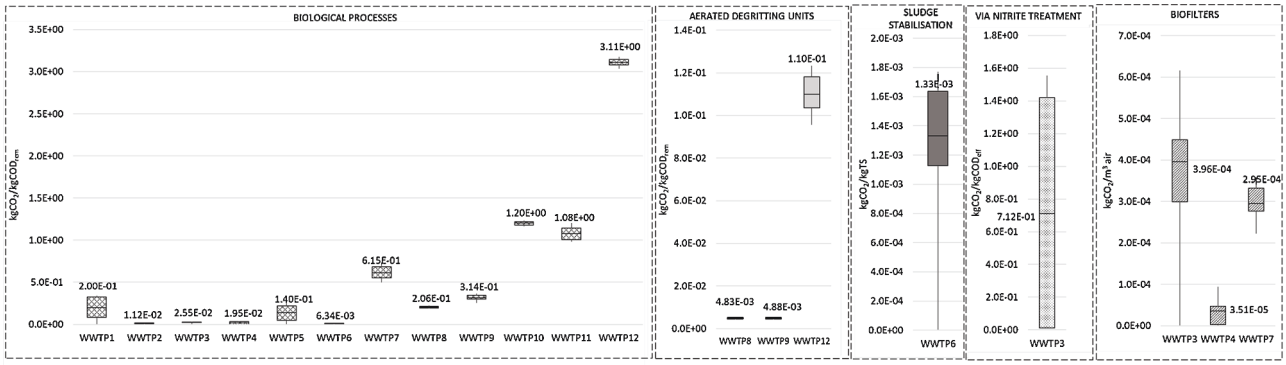


Fig. 7. Emission factors for direct CO_2 emissions from WWTPs grouped for operational unit.

Table 5
Emission factors (EFs) for indirect emissions on water body due to dissolve GHGs in WWTPs effluent and related standard deviations.

		WWTP1	WWTP2	WWTP3	WWTP4	WWTP5	WWTP6	WWTP7	WWTP8	WWTP9	WWTP10	WWTP11	WWTP12
DISSOLVED GHG_EF	**PE_COD**	44,499	31,819	46,511	53,713	30,645	20,166	6241	7258	10,139	1335	2125	5410
$gN_2O/kgTN_{eff}$	**Mean**	**46**	**53**	**33**	**108**	**782**	**305**	**98**	**129**	**538**	**346**	**140**	**122**
	Dev std	*23*	*23*	*21*	*147*	*330*	*171*	–	–	–	–	–	–
$gCH_4/kgCOD_{eff}$	**Mean**	**7**	**4**	**39**	**10**	**133**	**41**	**8**	**11**	**10**	**9**	**11**	**16**
	Dev std	*5*	*5*	*24*	*8*	*63*	*2*	–	–	–	–	–	–
$gCO_2/kgCOD_{eff}$	**Mean**	**790**	**1328**	**5358**	**1330**	**2546**	**3603**	**853**	**378**	**1014**	**735**	**553**	**3434**
	Dev std	*551*	*503*	*3082*	*1667*	*865*	*3508*	–	–	–	–	–	–

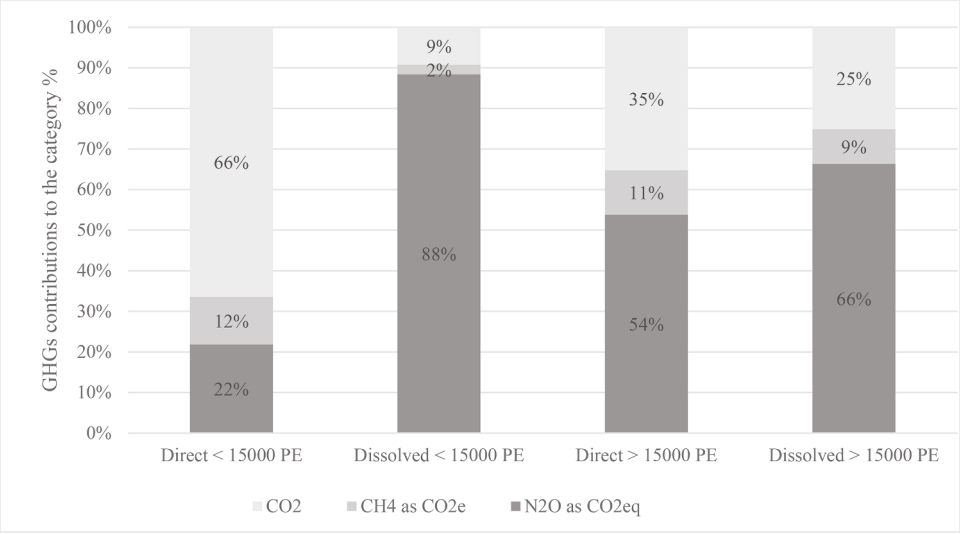


Fig. 8. GHGs contributions to the category's direct emissions from processes and indirect emission to the water body in relation with the WWTP size (< 15,000 PE and > 15,000 PE).

Emissions were determined based on Eq. (1).

$$Emission\ contribution\left(\frac{tonCO_{2eq}}{y}\right) = Activity\ data\left(\frac{quantity}{y}\right) * EF\left(\frac{tonGHGs}{quantity}\right) * GWPs\left(\frac{tonCO_{2eq}}{tonGHG}\right) \quad (1)$$

where GWPs are referred in IPCC Fifth Assessment Report, AR5 (Towprayoon et al., 2019).

In general, activity data (AD) represents the quantity, generated or used, of energy, mass, or volume, representing the key parameter for each emission category. The types of AD considered in this study are listed in Table 4. The EFs used were collected from the literature, guidelines, and databases or measured with specific on-site campaigns (Table 4 and Fig. 1).

The logical flow scheme of the proposed approach according to ISO 14,064–1 is reported in Fig. 2.

The overall calculation of CF, considering the on-site measured emissions, was carried out by following several phases: (1) elaboration of 1-year plant operation data of 12 WWTPs, such as temperature, wastewater flowrate, airflow rate, influent and effluent COD and TN mass loads; (2) calculation of the mass loads of emitted CO_2, CH_4, and N_2O; (3) normalisation at 20 °C; and (4) conversion into CO_2 equivalent.

3.3. Measurement campaigns: direct emitted GHG concentrations

Results from the measurement campaigns showed concentration variability in GHGs emitted from the biological processes of different WWTPs, as reported in previously literature studies (Tumendelger et al., 2019). The average values of N_2O concentrations were less than 4 mg/m^3 of air for the largest WWTPs (WWTP1–6), while they ranged from 4 to 15 mg/m^3 for the smallest plants (WWTP7–12) (Fig. 2). A similar behaviour was observed for CO_2 quantification. Values lower than 5 g CO_2/m^3 were detected for the largest WWTPs and in the range of 7–16 g CO_2/m^3 in other cases (Fig. 2). Especially for N_2O emissions, the difference in the contributions of the largest WWTPs could be justified by the usually higher efficiencies of the aeration systems and more elevated removals, as reported by Valkova et al. (2020). In contrast, for directly emitted CH_4 concentrations, a strict correlation was not identified with the size of the WWTPs (Fig. 3).

Furthermore, an important relationship was observed during the rain events that occurred during the sampling campaigns. It was evident (example shown for WWTP1 in Fig. 3) that wet periods affected the direct GHG emissions with higher concentrations, mainly for N_2O and CH_4, both emitting from the aerated degritting units and biological processes. It can be noticed that the emitted CH_4 and N_2O detected during the rain events were more than 3–6 times higher than the those detected during the dry period (Fig. 4a, c, b, and d). These variations were probably dependent on sewage system characteristics and a

Table 6
Carbon footprint of the selected monitored WWTPs.

	Direct emission from combustion	Direct emissions from WWTP processes in mainstream	Direct fugitive emissions	Indirect emissions from energy consumption	Indirect emissions from waste transport	Indirect emissions from chemical transport	Indirect emissions from chemical consumption	Indirect emissions on the water body	Indirect emissions from sludge composting	Indirect emissions from sludge reuse*	Mitigations from carbon sequestration and synthetic fertiliser substitution *	Total WWTP CF
	$tonCO_{2eq}/y$	$tonCO_{2eq}$ /y	$tonCO_{2eq}$ /y	$tonCO_{2eq}$ /y	$tonCO_{2eq}$ /y	$tonCO_{2eq}$ /y	$tonCO_{2eq}$ /y	$tonCO_{2eq}$ /y	$tonCO_{2eq}$ /y	$tonCO_{2eq}$/y	$tonCO_{2eq}$/y	$tonCO_{2eq}$ /y
WWTP1	0	862	3.2	881	30	2.2	61	511	777	220*	314*	3128
WWTP2	0	17	2.2	570	20	1.2	20	316	490	129*	190*	1436
WWTP3	398	310	252	770	14	3.2	110	1933	257	63*	101*	4047
WWTP4	530	87	335	370	28	4.1	189	1493	493	132*	204*	3528
WWTP5	0	244	1.9	627	20	1.4	49	3136	437	124*	186*	4517
WWTP6	0	7	2.7	679	30	3.1	210	872	598	139*	219*	2402
WWTP7	0	725	2.6	215	4	1.3	22	149	59	18*	29*	1179
WWTP8	0	79	1.9	189	8	1.5	16	215	116	31*	44*	627
WWTP9	0	185	2.2	187	6	0.9	42	1222	106	29*	42*	1752
WWTP10	0	66	0.0	39	2	0.2	4	100	0	0*	0*	210
WWTP11	0	103	0.0	69	3	0.3	4	61	0	0*	0*	240
WWTP12	0	717	1.9	99	5	0.5	19	174	71	20*	28*	1087
											TOTAL	**24,154**

* Emissions Categories excluded from reporting boundaries.

corresponding increase in the dissolved gases in the influent flow during the rain events. Urban sewer systems can cause several contaminant degradations, which can generate dissolved gaseous sub-products, as previously reported by Jin et al. (2019).

Moreover, from the measurement campaigns, the directly emitted GHG concentrations were also found to be spatially varying at different points of the same biological reactor, as discussed by Pan et al. (2016). For WWTP5, the average emitted concentrations at the beginning and end of the aerobic reaction volume decreased from 6 17 mgN_2O/m^3, 50 41 $mgCH_4/m^3$, and 5 2 gCO_2/m^3 to 3 5 mgN_2O/m^3, 22 28 mg CH_4/m^3, and 5 2 g CO_2/m^3, respectively. It was observed that for N_2O and CH_4, higher concentrations were observed in the initial section of the reaction volume, where the influent macro-contaminant mass loads (COD and TN) were also probably higher. On the other hand, CO_2 concentrations, mainly generated from biomass respiration, remained almost stable throughout the entire biological unit (Zhan et al., 2017). For the calculation of the emitted mass loads, average concentrations of GHGs for each unit were considered.

3.4. Measured direct emissions factors

GHG loads were calculated from the measured concentrations and normalised to identify different EFs reported in terms of average values and standard deviations in Figs. 5, 6, and 7 for N_2O, CH_4, and CO_2, respectively. Biological treatment was typically characterised by the highest emissions, with the values ranging from 6.7 10^{8} to 0.02 kg N_2O/kgN_{rem}, 2.2 10^{8} to 0.003 kg $CH_4/kgCOD_{in}$, and 1.8 10^{5} to 3.1 kg $CO_2/kgCOD_{rem}$. It was also observed that the EF values were inversely proportional to the WWTP size, resulting in higher specific values for the smallest WWTPs (WWTP7 12). In the short cut biological process treating nutrient-rich anaerobic rejected liquor via nitrite resulted in significantly high EFs in terms of N_2O (0.27 kg N_2O $/kgTN_{rem}$), while CH_4 and CO_2 impacts were consistent with other biological treatments. Aerated degritting units showed the values of less than 2.6 10^{4} kg N_2O $/kgN_{rem}$, in the range of 1.3 10^{4}- $1.5 * 10^{3}$ kg $CH_4/kgCOD_{in}$ and 4.1 10^{3} 0.1 $kgCO_2/kgCOD_{rem}$. Aerated sludge stabilisation, measured with a continuous monitoring campaign for WWTP 6, yielded the EFs for CO_2 (1.3 10^{3} kg CO_2/kgTS) higher than those of N_2O and CH_4 (8.9 10^{8} kg N_2O/kgTS and 3.8 10^{7} kg CH_4/kgTS, respectively). Finally, for biofilters, the normalised values per cubic meter of the treated air changed from 1.2 10^{6} to 6 10^{6} kg N_2O $/m^3$, 2.6 10^{5} to 7.5 10^{5} kg CH_4/m^3, and 4 10^{4} to 8.3 10^{4} kg CO_2/m^3, highlighting the significant impact of methane on the final global emission from these air treatment units.

3.5. Measured indirect emission factors

The dissolved GHG concentrations in the effluents of the WWTPs varied in the range of 0.2 24 mg/L for N_2O, 0.1 1 mg/L for CH_4, and 1.8 52 mg/L for CO_2. Specifically, for the largest WWTPs (from 1 to 6), the average values were 4.6 9.4 mg/L for N_2O, 0.4 0.3 mg/L for CH_4, and 30 19 mg/L for CO_2. Meanwhile, the values were generally found to be lower and respectively equal to 0.8 0.5, 0.3 0.2, and 16.4 2.5 mg/L for the smaller WWTPs (from 7 to 12). Scientific literature on the measured values of dissolved GHGs in the effluents of WWTPs is scarce. In general, these values have been found to range from 0.009 to 24 mg/L for N_2O, 0.009 to 4.5 mg/L for CH_4, and 245 to 1352 mg/L for CO_2 (Caniani et al., 2019; Masuda et al., 2015, 2018; Vieira et al., 2019). This variability indicates the need for site-specific campaigns for properly evaluating the indirect emissions due to dissolved GHG contributions.

Moreover, during the sampling campaigns, dissolved GHGs in the influent streams of the WWTPs from the sewage systems were monitored. Specifically, in the largest WWTPs (from 1 to 6), the dissolved GHG influent concentrations were found to be 12.5 26, 1.1 1.2, and 76 86 mg/L for N_2O, CH_4, and CO_2, respectively, while the values

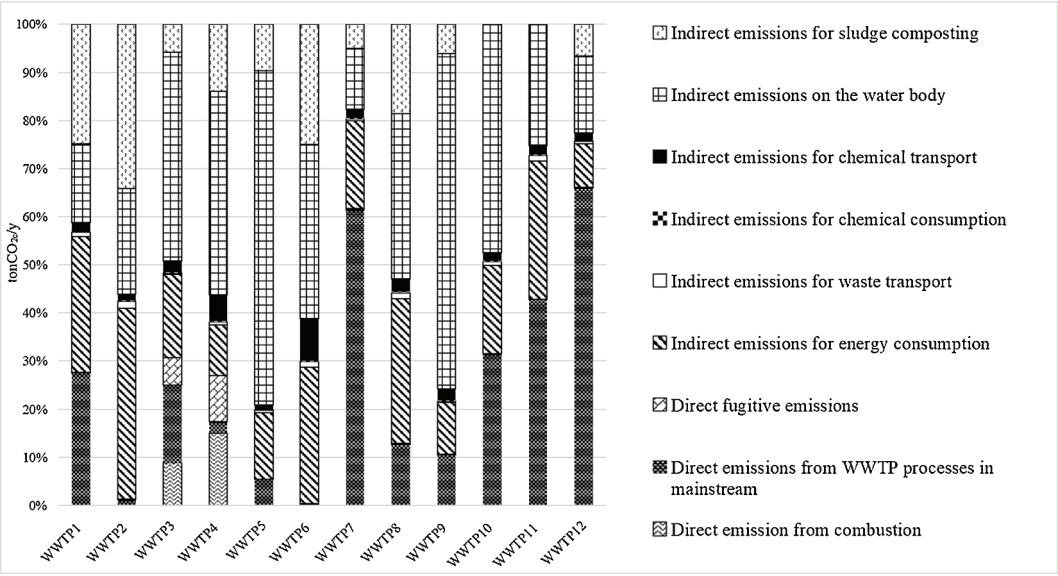


Fig. 9. Categories contributions (%) to the total carbon footprint for each selected WWTP.

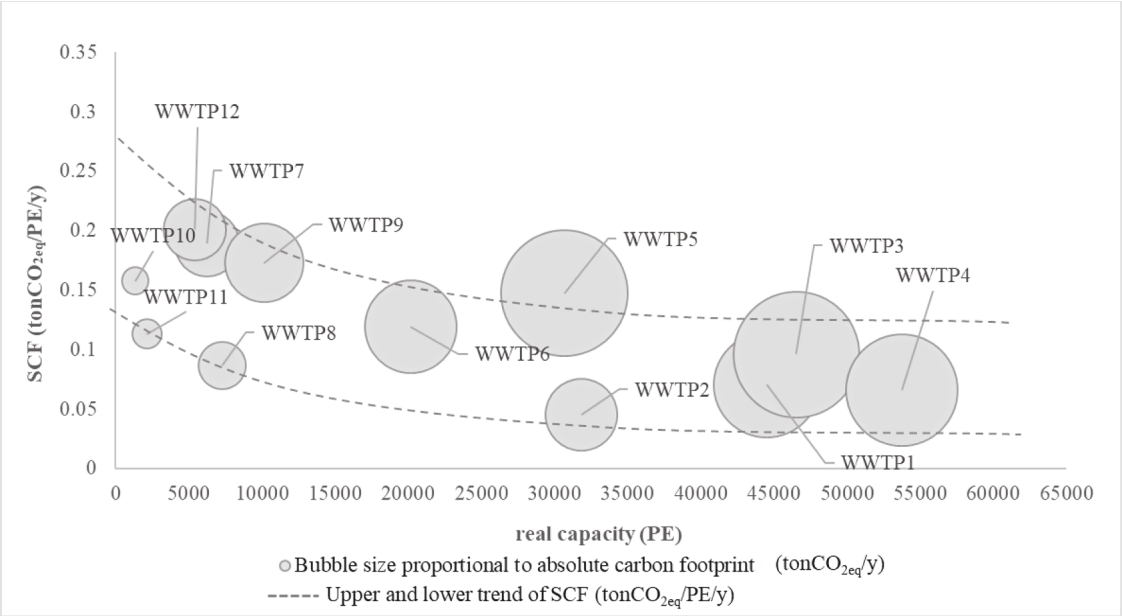


Fig. 10. Specific Carbon Footprints (SCF) of the selected WWTPs.

were found to be 0.5 ± 0.4 mg N_2O /L, 0.3 ± 0.2 mg CH_4/L, and 50 ± 16 mg CO_2/L for the smaller WWTPs (from 7 to 12). These aspects indicated that a relevant contribution of dissolved GHG comes directly from the sewage networks, especially for plants with higher capacities. Furthermore, for smaller WWTPs, the dissolved GHG concentrations in the effluent were higher than those of the influent stream. This confirmed that for smaller WWTPs, which are usually subjected to more limiting operative conditions (such as low carbon:nitrogen (C:N) ratio, unstable or optimised process parameters, and inefficient aeration supply) (Kumar et al., 2021), additional contributions of GHGs were generated during the biological processes, which remained dissolved in the liquid stream.

The EFs of dissolved GHG emission category varied in the ranges of 33–782 g N_2O /kgN_{eff}, 4–132 g CH_4/$kgCOD_{eff}$, and 553–358 g CO_2/$kgCOD_{eff}$ for N_2O, CH_4, and CO_2, respectively (Table 5). The standard deviations of the dissolved GHG EFs obtained for the six continuously monitored WWTPs were significantly relevant and heterogeneous, ranging from 34 to 136% with respect to the average values. Moreover, the EF values were found to be inversely proportional to the WWTP size, resulting in the higher specific factors for the smallest WWTPs (Table 5).

3.6. *Specific GHG contributions on measured direct and dissolved emissions*

The main contributions of different GHGs to the direct and dissolved emission categories were calculated as a percentage of the total emissions (Fig. 8). The reported percentages of different GHG contributions were expressed as the percentage of equivalent CO_2. The values, as the gaseous flows were directly transferred to the atmosphere, showed that the main impacts were attributed to CO_2 for smaller plants (< 15,000 PE), while N_2O was the main responsible in largest WWTPs (> 15,000 PE). On the other hand, CH_4 emissions accounted for about 11–12% for

all the sizes. For indirect emissions into the water body, the main contributor was N_2O, followed by CO_2 and CH_4.

3.7. Carbon footprint results

After the long-term sampling periods, the entire CF of each selected WWTP was calculated by adding the different contributions of the categories based on the normalised proposed approach (Table 6). The final values ranged from a minimum 210 tonCO_{2eq}/y (WWTP10) to a maximum 4047 tonCO_{2eq}/y (WWTP 3). In general, the most impactful categories (Fig. 9) were the indirect emissions associated with dissolved GHGs present in the water body, which influenced 13 70% of the CF of each WWTP. Indirect emissions due to energy consumption accounted for 10 40% and, as expected, this category is directly related to the carbon and nitrogen removed loads in the different WWTPs (Table 6). Moreover, direct emissions from treatment processes contribution approximately 19% on an average. The disposal of sewage sludge and use of chemicals affected 6 34% and 1 9%, respectively. The impacts and their variabilities, especially for direct and dissolved GHG contributions, further underlined the already discussed importance of the measurement campaigns to obtain more appropriate and proper data for the specific conditions of each plant.

The SCFs of the WWTPs were finally calculated and were found to be between 0.04 and 0.20 tonCO_{2eq}/PE/y (Fig. 10). The obtained SCFs were indirectly dependent on the size of the plants and were more widely distributed for the WWTPs with less than 15,000 PE capacity. Similarly, the average SCFs were found to be 0.091 0.038 tonCO_{2eq}/PE/y for the WWTPs with more than 15,000 PE capacity and 0.153 0.045 tonCO_{2eq}/PE/y for smaller facilities. These SCF results were found to be more variable and almost 1.7 times higher than those of the larger size (15,000 PE). Notwithstanding that the calculation procedure was not perfectly comparable, these results were consistent with those mentioned in other published research which reported the values in the range of 0.07 0.108 tonCO_{2eq}/PE/y (Gustavsson and Tumlin, 2013), 0.023 0.1 tonCO_{2eq}/PE/y (Maktabifard et al., 2020) and 0.062 0.161 tonCO_{2eq}/PE/y (Mamais et al., 2015).

The CF was finally extended at the regional level, including for all plants managed by the water utility, as well as the septic tanks, resulting in 2396 and 1588 tonCO_{2eq}/y emitted by the non-monitored WWTPs and septic tanks, respectively, yielding a total territorial CF of the wastewater service of 28,137 8497 tonCO_{2eq}/y. Biogenic CO2 had a 25% impact on total CF. The highest contribution to territorial CF was from WWTPs with more than 15,000 PE capacity, accounting for 68%. Nevertheless, the presence of several minor plants should be considered as their contributions were not found to be negligible, with the impacts of 16% from the plants with capacities ranging from 15,000 to 5000 PE and 10% for the smaller WWTPs. Finally, septic tank emissions accounted for 6% to the total CF.

4. Conclusion

Regional CF of wastewater treatment service was determined by properly applying for the first time the methodology ISO14064 1 to identify specific emission categories. The proposed new evidence-based methodology enables the calculation of the Carbon Footprint accounting all the emissions categories in clear operational and reporting boundaries within cities and regions. The calculations were validated measuring the most impacting categories of direct and dissolved GHGs emissions with specific sampling campaigns. The uncertainties related to the EFs let to long-term on-site measurements of relevant GHGs. In fact, calculations carried out using only the available EFs mentioned in the guidelines may significantly differ from the actual site conditions. The measurement campaigns mentioned in this study enabled us to define the site-specific EFs for the main direct sources from the treatment processes (degritting units, aerobic biological treatments, biofilters, and aerobic sludge stabilisations) in 12 different sized WWTPs. Furthermore, dissolved GHGs discharged in the final water bodies were analysed and were found to significantly impact the entire CF quantification. The results, in terms of the EFs (average values and standard deviations), showed a high variability in the emissions, based on the WWTP size and specific operative conditions, especially for biological processes. The average values were found to be 3.4 10 ^{3} kgN_2O/kgTN_{rem}, 1.7 10 ^{3} kgCH_4/kgCOD_{in}, and 1.1 kgCO_2/kgCOD_{rem} for the smallest (15,000 PE) WWTPs. On the other hand, the biggest (15,000 PE) WWTP EFs resulted in 6.6 10 ^{4} kgN_2O/kgTN_{rem}, 2.7 10 ^{4} kgCH_4/kgCOD_{in}, and 0.07 kgCO_2/kgCOD_{rem}. The analytical campaigns of this study also highlighted that biogenic CO_2 emitted from the biological processes significantly impacted the global CF of the 12 WWTPs, accounting for 35 66% of the total directly emitted CO_{2eq}. The overall territorial CF of the wastewater service was 28,137 8497 tonCO_{2eq}/y, including of the non-monitored WWTPs and septic tanks. The most impacting categories for most plants were: (i) indirect emissions associated with the dissolved GHGs present in the water body, (ii) indirect emissions due to energy consumption, followed by (iii) direct emissions from treatment processes, (iv) disposal of sewage sludge and (v) use of chemicals.

Finally, this approach can support territorial water utilities not only to assess their carbon footprint with normalized approach, but also to develop regional mitigation scenarios and decisions towards low-carbon water utilities. In fact, since the Specific Carbon Footprint coefficients change based on the plants size, mitigation actions could consider the population distribution in the territory and both centralized and decentralized systems. Moreover, mitigations for wastewater service decarbonisation according to the shown most impacting emission categories could be prioritised as following: (1) acquire renewable energy sources to reduce the indirect emissions from fossil primary energy production; (2) optimize efficiency and kinetics of biological removal of organic and nutrients loads and aeration efficiency in order to reduce dissolved GHGs in the final effluent, (3) reduce direct emissions mainly avoiding uncontrolled transitory phases or limiting operative conditions in the biological reactors; (4) promote less impacting sludge disposal destination especially avoiding landfill and (5) use chemical reagents characterized by lower Emission Factors for their primary production. The proposed methodological approach coupled with accurately planned site-specific long term measurement campaigns could further boost and address the decarbonization of the wastewater service in territories.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aboobakar, A., Cartmell, E., Stephenson, T., Jones, M., Vale, P., Dotro, G., 2013. Nitrous oxide emissions and dissolved oxygen profiling in a full-scale nitrifying activated sludge treatment plant. Water Res. 47 (2), 524 534. https://doi.org/10.1016/j.watres.2012.10.004.

Ahn, J.H., Kim, S., Park, H., Rahm, B., Pagilla, K., Chandran, K., 2010. N_2O emissions from activated sludge processes, 2008-2009: results of a national monitoring survey in the united states. Environ. Sci. Technol. 44 (12), 4505 4511. https://doi.org/10.1021/es903845y.

ARERA, 2017. *562/2017/R/IDR*, URL: https://www.arera.it/it/docs/17/562-17.htm (accessed 10 june 2021).
Baeza, J.A., Gabriel, D., Guisasola, A., Lafuente, F.J., Katsou, E., Massara, T., Noutsopoulos, C., Antoniou, K., Andreadakis, A., Mamais, D., Koumaki, E., Goldasi, M., Prado-Rubianes, O.J., Colon, J., Rosso, D., Krieg, G., Malamis, S., 2017. On-line monitoring, control and mitigation of greenhouse gases emissions in WWTPs. 7th International Conference on Biotechniques for Air Pollution Control and Bioenergy.
Ballard, S., Porro, J., Trommsdorff, C., 2018. The Roadmap to a Low-Carbon Urban Water Utility: An international guide to the WaCCliM approach. IWA Publishing. https://doi.org/10.2166/9781780409924.
Bartram, D.; Short, M.D.; Ebie, Y.; Farkas J.; Gueguen, C.; Peters, G.M.; Zanzotter N.M.; Karthik, M. Wastewater treatment and discharge. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, 2019, Vol 5, chap. 6.
Boldrin, A., Andersen, J.K., M ller, J., Christensen, T.H., Favoino, E., 2009. Composting and compost utilization: accounting of greenhouse gases and global warming contributions. Waste Manag. Res. 27 (8), 800 812. https://doi.org/10.1177/0734242X09345275.
Brown, S., Beecher, N., Carpenter, A., 2010. Calculator tool for determining greenhouse gas emissions for biosolids processing and end use. Environ. Sci. Technol. 44 (24), 9509 9515. https://doi.org/10.1021/es101210k.
Bruun, S., Hansen, T.L., Christensen, T.H., Magid, J., Jensen, L.S., 2006. Application of processed organic municipal solid waste on agricultural land - A scenario analysis. Environ. Model. Assess. 11 (3), 251 265. https://doi.org/10.1007/s10666-005-9028-0.
Caniani, D., Caivano, M., Pascale, R., Bianco, G., Mancini, I.M., Masi, S., Mazzone, G., Firouzian, M., Rosso, D., 2019. CO_2 and N_2O from water resource recovery facilities: evaluation of emissions from biological treatment, settling, disinfection, and receiving water body. Sci. Total Environ. 648, 1130 1140. https://doi.org/10.1016/j.scitotenv.2018.08.150.
Chai, C., Zhang, D., Yu, Y., Feng, Y., Wong, M.S., 2015. Carbon footprint analyses of mainstream wastewater treatment technologies under different sludge treatment scenarios in China. Water 7 (3), 918 938. https://doi.org/10.3390/w7030918 (Switzerland).
CHEApet, 2011. URL: https://www.waterrf.org/research/projects/demonstration-carbon-heat-energy-assessment-and-plant-evaluation-tool-cheapet, (Accessed 28 June 2021).
Chen, Y.C., Kuo, J., 2016. Potential of greenhouse gas emissions from sewage sludge management: a case study of Taiwan. J. Clean. Prod. 129, 196 201. https://doi.org/10.1016/j.jclepro.2016.04.084.
ClimFoot, 2018. https://www.climfoot-project.eu/. (Accessed 28 June 2021).
Crippa, M., Gabriel, O., Diego, G., Marilena, M., Edwin, S., Eleonora, L.V., Efisio, S., Fabio, M.F., Jos, O., 2019. Fossil CO_2 and GHG emissions of all world countries. J. Geophys. Res. Atmos. 105 (Issue D2) https://doi.org/10.2760/56420.
Demir, O., Yapıcıoglu, P., 2019. Investigation of GHG emission sources and reducing GHG emissions in a municipal wastewater treatment plant. Greenh. Gases Sci. Technol. 9 (5), 948 964. https://doi.org/10.1002/ghg.1912.
Flores-Alsina, X., Arnell, M., Amerlinck, Y., Corominas, L., Gernaey, K.V., Guo, L.S., 2012. A dynamic modelling approach to evaluate GHG emissions from wastewater treatment plants. Proc. World Congr. Water, Clim. Energy 1-8.
Foley, J., de Haas, D., Hartley, K., Lant, P., 2010a. Comprehensive life cycle inventories of alternative wastewater treatment systems. Water Res. 44 (5), 1654 1666. https://doi.org/10.1016/j.watres.2009.11.031.
Foley, J., de Haas, D., Yuan, Z., Lant, P., 2010b. Nitrous oxide generation in full-scale biological nutrient removal wastewater treatment plants. Water Res. 44 (3), 831 844. https://doi.org/10.1016/j.watres.2009.10.033.
Gu, Y., Dong, Y.N., Wang, H., Keller, A., Xu, J., Chiramba, T., Li, F., 2016. Quantification of the water, energy and carbon footprints of wastewater treatment plants in China considering a water-energy nexus perspective. Ecol. Indic. 60, 402 409. https://doi.org/10.1016/j.ecolind.2015.07.012.
Guo, L., Porro, J., Sharma, K.R., Amerlinck, Y., Benedetti, L., Nopens, I., Shaw, A., Van Hulle, S.W.H., Yuan, Z., Vanrolleghem, P.A., 2012. Towards a benchmarking tool for minimizing wastewater utility greenhouse gas footprints. Water Sci. Technol. 66, 2483 2495. https://doi.org/10.2166/wst.2012.495.
Gustavsson, D.J.I., Tumlin, S., 2013. Carbon footprints of Scandinavian wastewater treatment plants. Water Sci. Technol. 68 (4), 887 893. https://doi.org/10.2166/wst.2013.318.
Han, Z., Sun, D., Wang, H., Li, R., Bao, Z., Qi, F., 2018. Effects of ambient temperature and aeration frequency on emissions of ammonia and greenhouse gases from a sewage sludge aerobic composting plant. Bioresour. Technol. 270, 457 466. https://doi.org/10.1016/j.biortech.2018.09.048. September.
Hergoualc h, K.; Akiyama, H; Bernoux, M.; Chirinda, N.; Del Prado, A.; Kasimir, Å.; James MacDonald J. D.; Ogle, S.M.; Regina K.; Van der Weerden T.J. N_2O emissions from managed soils, and CO_2 emissions from lime and urea application. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, 2019, Vol 4, chap. 11.
Huang, F., Shen, W., Zhang, X., Seferlis, P., 2020. Impacts of dissolved oxygen control on different greenhouse gas emission sources in wastewater treatment process. J. Clean. Prod. 274, 123233 https://doi.org/10.1016/j.jclepro.2020.123233.
ISO 14064-1, 2019. *Greenhouse gases - Part 1: specification for the quantification, monitoring and reporting of organization emissions and removals.*
ISPRA, 2018. *Fattori di emissione atmosferica di gas a effetto serra e altri gas nel settore elettrico*, Rapporti 280/2018, ISBN: 978-88-448-0883-9.
Jin, P., Gu, Y., Shi, X., Yang, W., 2019. Non-negligible greenhouse gases from urban sewer system. Biotechnol. Biofuels 12 (1), 1 11. https://doi.org/10.1186/s13068-019-1441-8.
Joss, A., Salzgeber, D., Eugster, J., Konig, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J., Siegrist, H.R., 2009. Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR. Environ. Sci. Technol. 43 (14), 5301 5306. https://doi.org/10.1021/es900107w.
Kirkeby, J., Gabriel, S., Christensen, T.H., 2005. Milj vurdering af genanvendelse og slutdisponering af spildevandsslam: en livscyklus screening af fire scenarier. In: Danmarks Tekniske Universitet i samarbejde med Hedeselskabet, Århus og Fredericia Kommuner. Institut for Milj .
Kumar, A., Thanki, A., Padhiyar, H., Singh, N.K., Pandey, S., Yadav, M., Yu, Z.G., 2021. Greenhouse gases emission control in WWTS via potential operational strategies: a critical review. Chemosphere 273, 129694. https://doi.org/10.1016/j.chemosphere.2021.129694.
Kwok, Y.C.E., Müller, D., Caldow, C., Lebegue, B., M nster, J.G., Rella, C.W., Scheutz, C., Schmidt, M., Ramonet, M., Warneke, T., Broquet, G., Ciais, P., 2015. Methane emission estimates using chamber and tracer release experiments for a municipal waste water treatment plant. Atmos. Meas. Tech. 8 (7), 2853 2867. https://doi.org/10.5194/amt-8-2853-2015.
Longo, S., D Antoni, B.M., Bongards, M., Chaparro, A., Cronrath, A., Fatone, F., Lema, J. M., Mauricio-Iglesias, M., Soares, A., Hospido, A, 2016. Monitoring and diagnosis of energy consumption in wastewater treatment plants. A state of the art and proposals for improvement. Appl. Energy 179, 1251 1268. https://doi.org/10.1016/j.apenergy.2016.07.043.
Longo, S., Mauricio-Iglesias, M., Soares, A., Campo, P., Fatone, F., Eusebi, A.L., Akkersdijk, E., Stefani, L., Hospido, A., 2019. ENERWATER A standard method for assessing and improving the energy efficiency of wastewater treatment plants. Appl. Energy 242, 897 910. https://doi.org/10.1016/j.apenergy.2019.03.130.
Majumder, R., Livesley, S.J., Gregory, D., Arndt, S.K., 2014. Biosolid stockpiles are a significant point source for greenhouse gas emissions. J. Environ. Manag. 143, 34 43. https://doi.org/10.1016/j.jenvman.2014.04.016.
Maktabifard, M., Zaborowska, E., Makinia, J., 2020. Energy neutrality versus carbon footprint minimization in municipal wastewater treatment plants. Bioresour. Technol. 300, 122647 https://doi.org/10.1016/j.biortech.2019.122647. December 2019.
Mamais, D., Noutsopoulos, C., Dimopoulou, A., Stasinakis, A., Lekkas, T.D., 2015. Wastewater treatment process impact on energy savings and greenhouse gas emissions. Water Sci. Technol. 71 (2), 303 308. https://doi.org/10.2166/wst.2014.521.
Mannina, G., Ekama, G., Caniani, D., Cosenza, A., Esposito, G., Gori, R., Garrido-Baserba, M., Rosso, D., Olsson, G., 2016. Greenhouse gases from wastewater treatment - A review of modeling tools. Sci. Total Environ. 551-552, 254 270. https://doi.org/10.1016/j.scitotenv.2016.01.163.
Masuda, S., Suzuki, S., Sano, I., Li, Y.Y., Nishimura, O., 2015. The seasonal variation of emission of greenhouse gases from a full-scale sewage treatment plant. Chemosphere 140, 167 173. https://doi.org/10.1016/j.chemosphere.2014.09.042.
Masuda, S., Sano, I., Hojo, T., Li, Y.Y., Nishimura, O., 2018. The comparison of greenhouse gas emissions in sewage treatment plants with different treatment processes. Chemosphere 193, 581 590. https://doi.org/10.1016/j.chemosphere.2017.11.018.
Nguyen, T.K.L., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Nghiem, L.D., Liu, Y., Ni, B., Hai, F.I., 2019. Insight into greenhouse gases emissions from the two popular treatment technologies in municipal wastewater treatment processes. Sci. Total Environ. 671, 1302 1313. https://doi.org/10.1016/j.scitotenv.2019.03.386.
Pan, Y., Van Den Akker, B., Ye, L., Ni, B.J., Watts, S., Reid, K., Yuan, Z., 2016. Unravelling the spatial variation of nitrous oxide emissions from a step-feed plug-flow full scale wastewater treatment plant. Sci. Rep. 6, 1 10. https://doi.org/10.1038/srep20792. October 2015.
Parravicini, V., Svardal, K., Krampe, J., 2016. Greenhouse gas emissions from wastewater treatment plants. Energy Procedia 97, 246 253. https://doi.org/10.1016/j.egypro.2016.10.067.
Piippo, S., Lauronen, M., Postila, H., 2018. Greenhouse gas emissions from different sewage sludge treatment methods in north. J. Clean. Prod. 177, 483 492. https://doi.org/10.1016/j.jclepro.2017.12.232.
Regional Policy, EC, 2014. https://ec.europa.eu/regional_policy/en/policy/themes/low-carbon-economy/, (Accessed 28 June 2021).
Ribera-Guardia, A., Bosch, L., Corominas, L., Pijuan, M., 2019. Nitrous oxide and methane emissions from a plug-flow full-scale bioreactor and assessment of its carbon footprint. J. Clean. Prod. 212, 162 172. https://doi.org/10.1016/j.jclepro.2018.11.286.
Samuelsson, J., Delre, A., Tumlin, S., Hadi, S., Offerle, B., Scheutz, C., 2018. Optical technologies applied alongside on-site and remote approaches for climate gas emission quantification at a wastewater treatment plant. Water Res. 131, 299 309. https://doi.org/10.1016/j.watres.2017.12.018.
Scheutz, C., Fredenslund, A.M., 2019. Total methane emission rates and losses from 23 biogas plants. Waste Manag. 97, 38 46. https://doi.org/10.1016/j.wasman.2019.07.029.
SINANET, 2017. URL: http://www.sinanet.isprambiente.it/it, (Accessed 21 June 2021).
Spinelli, M., Eusebi, A.L., Vasilaki, V., Katsou, E., Frison, N., Cingolani, D., Fatone, F., 2018. Critical analyses of nitrous oxide emissions in a full scale activated sludge system treating low carbon-to-nitrogen ratio wastewater. J. Clean. Prod. 190, 517 524. https://doi.org/10.1016/j.jclepro.2018.04.178.
Sweetapple, C., Fu, G., Butler, D., 2013. Identifying key sources of uncertainty in the modelling of greenhouse gas emissions from wastewater treatment. Water Res. 47, 4652 4665.
Towprayoon, S.; Kim, S.; Jeon, E.C.; Ishigaki, T.; Amadou, S. N. Incineration and open burning of waste. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, 2019, Vol 5, chap. 5.

Tumendelger, A., Alshboul, Z., Lorke, A., 2019. Methane and nitrous oxide emission from different treatment units of municipal wastewater treatment plants in southwest Germany. PLoS ONE 14 (1), 1 17. https://doi.org/10.1371/journal.pone.0209763.

Valkova, T., Parravicini, V., Saracevic, E., Tauber, J., Svardal, K., Krampe, J., 2020. A method to estimate the direct nitrous oxide emissions of municipal wastewater treatment plants based on the degree of nitrogen removal. J. Environ. Manag. 279 https://doi.org/10.1016/j.jenvman.2020.111563. April 2020.

Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F., Katsou, E., 2019. A decade of nitrous oxide (N_2O) monitoring in full-scale wastewater treatment processes: a critical review. Water Res. 161, 392 412. https://doi.org/10.1016/j.watres.2019.04.022.

Vieira, A., Marques, R., Raposo, R., Martins, M., Alves, R., Povoa, P., Irizar, I., Beltran, S., Craamer, P., Urchegui, G., Oehmen, A., 2019. The impact of the art-ICA control technology on the performance, energy consumption and greenhouse gas emissions of full-scale wastewater treatment plants. J. Clean. Prod. 213, 680 687. https://doi.org/10.1016/j.jclepro.2018.12.229.

WACCLIM, 2020, URL: http://wacclim.org/ecam/sources.php, (Accessed 28 June 2021).

Wang, Y., Fang, H., Zhou, D., Han, H., Chen, J., 2016. Characterization of nitrous oxide and nitric oxide emissions from a full-scale biological aerated filter for secondary nitrification. Chem. Eng. J. 299, 304 313. https://doi.org/10.1016/j.cej.2016.04.050.

WESTWeb, 2021. URL: https://west.berkeley.edu/model.php, (Accessed 28 June 2021).

Willen, A., Rodhe, L., Pell, M., Jonsson, H., 2016. Nitrous oxide and methane emissions during storage of dewatered digested sewage sludge. J. Environ. Manag. 184, 560 568. https://doi.org/10.1016/j.jenvman.2016.10.025.

Yuan, J., Li, Y., Chen, S., Li, D., Tang, H., Chadwick, D., Li, S., Li, W., Li, G., 2018. Effects of phosphogypsum, superphosphate, and dicyandiamide on gaseous emission and compost quality during sewage sludge composting. Bioresour. Technol. 270 (September), 368 376. https://doi.org/10.1016/j.biortech.2018.09.023.

Zhan, X., Hu, Z., Wu, G., 2017. Greenhouse gas emission and mitigation in municipal wastewater treatment plants. IWA (Ed.). In: Water Intelligence Online, 16. IWA Publishing. https://doi.org/10.2166/9781780406312.

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Monitoring of cyanobacterial blooms and assessing polymer-enhanced microfiltration and ultrafiltration for microcystin removal in an Italian drinking water treatment plant☆

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ABSTRACT

The water intake of a drinking water treatment plant (DWTP) in Central Italy was monitored over six bloom seasons for cyanotoxin severity, which supplies drinking water from an oligo-mesotrophic lake with microcystin levels up to 10.3 μg/L. The historical data showed that the water temperature did not show extreme/large seasonal variation and it was not correlated either with cyanobacterial growth or microcystin concentration. Among all parameters, the cyanobacteria growth was negatively correlated with humidity and manganese and positively correlated with atmospheric temperature. No significant correlation was found between microcystin concentration and the climatic parameters. Polymer(chitosan)-enhanced microfiltration (PEMF) and ultrafiltration (PEUF) were further tested as an alternative microcystin removal approach from dense cyanobacteria-rich flows. The dominant cyanobacteria in the water intake, *Planktothrix rubescens*, was isolated and enriched to simulate cyanobacterial blooms in the lake. The PEMF and PEUF were separately applied to enriched *P. rubescens* culture (PC) (microcystin = 1.236 μg/L) as well as to the sand filter backwash water (SFBW) of the DWTP where microcystin concentration was higher than 12 μg/L. The overall microcystin removal rates from the final effluent of PC (always <0.15 μg/L) were between 90.1-94.7% and 89.5–95.4% using 4 and 20 mg chitosan/L, respectively. Meanwhile, after the PEMF and PEUF of SFBW, the final effluent contained only 0.099 and 0.057 μg microcystin/L with an overall removal >99%. The presented results are the first from the application of chitosan to remove *P. rubescens* as well as the implementation of PEMF and PEUF on SFBW to remove cyanobacterial cells and associated toxins.

1. Introduction

Harmful algal blooms (HABs) are becoming an aquatic stressor of great concern since the anthropogenic perturbations (e.g. climate change and eutrophication) increase continuously in coastal areas (Griffith and Gobler, 2020). Simply, biotoxins that are produced by many HAB species, called phycotoxins, can bioaccumulate in the tissues of bivalve shellfish in time and, when consumed by humans, can cause severe/fatal shellfish poisoning syndromes. Phycotoxins are also a serious threat to marine organisms including marine mammals, fish, sea birds, invertebrates, etc. (Huisman et al., 2018). Furthermore, the presence of toxins from cyanoHABs in drinking water reservoirs may represent serious health risks for the human population (Almuhtaram et al., 2018). The World Health Organization (WHO) set a guideline limit of 1 μg/L for the presence of microcystin-LR, one of the most harmful cyanotoxins known in drinking and recreational waters that inhibits

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protein phosphatase and causes liver failure and hepatic haemorrhage (WHO, 2020). Moreover, dissolved microcystin can be persistent in water for a long period without any change in its chemical structure, which makes it hard to degrade in natural ecosystems (He et al., 2021).

Planktothrix rubescens is one of the most frequently-detected cyanobacteria species in Europe with microcystins being the most frequent cyanotoxins in freshwaters (Bogialli et al., 2013). *P. rubescens* is a red-colored, filamentous cyanobacterium with gas vesicles, which can adjust its buoyancy and position in the water column to obtain its preferred low light environment. This metalimnic species can adapt to low underwater light climate, and usually forms deep-water maxima in the stratified lakes of temperate latitudes (Gallina et al., 2017). This has implications for planning monitoring activities, especially in the case of drinking water supplies, with offtakes typically at depths of some meters (Manganelli et al., 2016).

The over-enrichment of nutrients in watersheds is one of the main drivers of cyanoHABs. Additional drivers include stratification and associated changes in temperature, light as well as changes in the frequency and timing of extreme weather events (Bertani et al., 2017). While forecasting the effects of climate change is a challenge, the high probability that future climatic conditions will favor bloom formation poses an added challenge to develop effective mitigation strategies that consider both nutrient and climate drivers (Gobler, 2020). At this point, understanding long-term trends is important to inform forecasting efforts and to reduce uncertainty estimates while developing effective water and cyanoHAB management strategies and ensuring water safety (Urquhart et al., 2017).

In principle, drinking water treatment removes cyanobacteria in the final effluent without compromising cell integrity in order to simultaneously eliminate intracellular and extracellular toxins (Merel et al., 2013). However, when dense cyanobacterial colonies enter a drinking water treatment plant (DWTP), they can block filters and accumulate in these units due to the particulate load on the flocculation and clarification that can further cause the free toxin to pass through the system (Ghernaout et al., 2010). In fact, algal cell accumulation (over 1 10^5 cells/mL) in clarifier sludge and filter backwash samples were reported (Almuhtaram et al., 2018) with various cyanotoxin species at varying concentrations.

Conventional coagulation processes are effective to remove cyanobacteria cells but do not remove cyanotoxins efficiently, especially extracellular ones (Sorlini et al., 2013). Most of the DWTPs use conventional adsorbents such as powdered activated carbon (PAC) (Dixon et al., 2011) or coagulants such as polyaluminum chloride (PACl) (Sun et al., 2013) that both achieved high removal efficiencies for cyanobacteria cells, and even microcystins (Ho et al., 2011). However, in order to obtain complete cell removal, high dosages of coagulants must be added, which in return increase operational costs, produce high volumes of inorganic metallic sludges. Hence, the application of organic polymers as coagulants may be a more feasible and sustainable solution. Chitosan is considered as a new generation coagulant (Jin et al., 2017) since it is abundant, non-toxic, biodegradable, renewable, and ecologically sustainable as well as highly effective in removing algae from water (Xu et al., 2013). Chitosan can be easily derived from shellfish and crustaceans through alkaline deacetylation of chitin (Mucci et al., 2020). Its characteristics such as efficient charge neutralization and bridging effects give good coagulation/flocculation properties (Yang et al., 2016), thus it has been widely used in water and wastewater treatment in recent years (Miranda et al., 2017).

Coagulation and sedimentation processes are typically followed by rapid sand filtration in conventional DWTPs. No remarkable impact has been reported for the sand filter process to remove cyanobacteria and/or cyanotoxins (He et al., 2016). Moreover, the major concern for filtration underlies the possible disruption of cells that accumulate in the filter media, and the subsequent release of toxins via the effluent and/or backwashing. Almuhtaram et al. (2018) reported that over 70% of filter backwash samples contained cyanobacteria cell concentration greater than that of raw water, an order of magnitude higher than any other processes in DWTPs. Most of the time, sand filter backwash water (SFBW) is directly discharged or subjected to a sedimentation basin to recycle the water to the plant (Shafiquzzaman et al., 2018), which may not remove cyanotoxins.

Membrane processes (e.g., microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) are particularly efficient to remove cyanobacteria and cyanotoxins (Merel et al., 2013). Specifically, UF was reported to remove intracellular cyanotoxins higher than 98% and NF to remove extracellular toxins in the range of 96 99% (Gijsbertsen-Abrahamse et al., 2006). However, direct membrane application to high algae concentration brings challenges such as fouling due to algal cells and algae-derived organic matters (Qu et al., 2012) and/or cell rejection that causes an increase in extracellular toxins in the permeate due to cell breakage during filtration (Liu et al., 2017). Herein, the use of hybrid systems with enhanced coagulation has a high potential to remove both intra- and extracellular cyanotoxins and to reduce membrane fouling (Dixon et al., 2011; Yan et al., 2017). Polymer-enhanced ultrafiltration (PEUF) is effective and even sustainable in this sense, while it helps to remove foulants before filtration. In comparison to other membrane technologies, PEUF has the capability of separating low molecular weight compounds and a high permeability for solvent transport at relatively low transmembrane pressures, which is desirable from an energy and capital cost perspective (Huang and Feng, 2019).

A multi-barrier approach by developing a monitoring system to facilitate effective treatment is therefore highly applicable to minimize the effects of cyanotoxins in water bodies (He et al., 2016). This study reports the characteristics of cyanobacterial bloom and microcystin production through monitoring six bloom seasons in the water intake of a DWTP in Central Italy that supplies drinking water from an oligo-mesotrophic lake. Whereas the plant can efficiently remove cyanotoxins from the final effluent in the current configuration even in the bloom periods, we proposed PEUF and polymer-enhanced MF (PEMF) as alternative and more sustainable treatment strategies that can also be applied in case of potential toxin accumulation in treatment units. Although chitosan-aided coagulation/flocculation has been reported in a number of studies, to the best of our knowledge, no study has evaluated the removal of *P. rubescens* cells via chitosan. Moreover, we reported the findings obtained from both PEMF and PEUF on SFBW for the first time. Hence, the aims of this study can be listed as follow: i) to monitor and assess critical cyanobacterial blooms and microcystin variations in the DWTP of Lake Castreccioni; ii) to determine any correlation between cyanobacterial bloom/cyanotoxin production and environmental parameters; iii) to evaluate any potential cyanotoxin accumulation within the DWTP even in the absence of a bloom event; iv) to test and optimize PEUF to remove cyanobacterial cells and microcystin in comparison to PEMF; v) to perform PEUF and PEMF configurations to SFBW to mitigate the release cyanotoxins via these flows into water bodies.

2. Materials and methods

2.1. Site and plant description

Lake Castreccioni is in the municipality of Cingoli in the Marche Region, Italy, located across the Musone River near Monte San Vicino, about 70 km far from the coast. The biggest artificial lake in the region, Castreccioni covers about 2.4 km^2 and reaches depths of 16.8 m. The volume of the lake at the maximum altitude is approximately 50 million cubic meters. The DWTP (managed by Acquambiente Marche S.r.l.) is in the district of Castreccioni and responsible for drinking water for the member municipalities as shown in the **e-Supplementary file**. The plant supplies water for a total of about 65,000 inhabitants in the winter and about 95,000 in the summer with a maximum capacity of 500 L/s. The process steps include pre-disinfection with ozone (pre-ozonation), clariflocculation, sand filtration, ozonation, activated carbon filtration,

accumulation tanks, and final disinfection with chlorine dioxide.

2.2. Water intake monitoring

The water intake of the DWTP in Castreccioni was systematically monitored over six bloom seasons from January 2014 to November 2019. The water level of the lake varied between 332 and 343 m sea level during the monitoring period (**e-Supplementary file**). The physical-chemical parameters were monitored at monthly intervals; while total coliform, *E. coli*, cyanobacteria, and microcystin were measured 4 5 times a month. All physical-chemical parameters were measured according to the Standard Methods of the American Public Health Association (APHA, 2012). *E. coli* and total coliforms were determined using the membrane filtration method explained in Standard Methods 9221B (APHA, 2012). Cyanobacteria was measured according to a protocol described elsewhere (Lucentini and Ottaviani, 2011). Briefly, 1 L water sample taken from the water intake was fixed in Lugol s solution and an aliquot of 25 mL was sedimented and observed after 24 h in the Utermohl chamber under an inverted microscope for the quantitative determination of the cyanobacteria species. Cell dimensions were calculated using a transmitted light optical microscope with an immersion objective. Microcystin analysis was done via enzyme-linked immunosorbent assay (ELISA) as described in Section 2.6. The historical meteorological data of Cingoli were obtained as the average monthly data from a national website for the monitoring period.

2.3. Cyanobacteria isolation and cultivation

The isolation of single filaments of the cyanobacteria *P. rubescens* was made from raw water taken from the water intake of the Castreccioni DWTP on 15 November 2019 (water temperature 10 C, pH 7.9, turbidity 0.23 NTU), following the capillary pipette method (Hoshaw and Rosowski, 1973) under an inverted microscope (Zeiss Axiovert 135) equipped with phase contrast, at 200x magnification. After an initial growth in microplates, cells were cultured at 21 0.1 C under a 12:12 h L:D photoperiod and an irradiance di 90 100 mol m ^{2}s ^{1}, in BG11 medium. For the molecular identification, genomic DNA was extracted from the cells collected by centrifugation at 4000 *g* for 20 min from a 30 mL monoclonal culture of *P. rubescens* (strain PRMC1119) in logarithmic growth phase using the CTAB (N-cetyl-N,N,N-trimethylammoniumbromide) method (Richards et al., 2001). Extracted DNA was amplified by Polymerase Chain Reaction (PCR) using a SimpliAmpTM Thermal Cycler with the primers CYA371F and CYA783R (Janse et al., 2003) targeting the 16S rRNA gene. The PCR conditions were 94 C for 4 min, followed by 35 cycles of 94 C for 30 s, annealing at 60 C for 45 s, and elongation at 72 C for 1 min, followed by a further elongation at 72 C for 5 min. PCR products were visualized and quantified in 1.5% agarose gels stained with GelRedTM (Biotium, Hayward, CA, USA) using Low DNA Mass Ladder (Invitrogen, Carlsbad, CA, USA) as a reference, and visualized under UV light. PCR products with expected lengths and yields were purified and directly Sanger-sequenced by Macrogen Europe (Amsterdam, The Netherlands). The sequence was compared to the NCBI database by BLAST search with default settings (see **e-Supplementary file**). The culture was harvested at the final cell yield up to 10^5 cells/mL and further diluted to 10^4 cells/mL with the lake water of Castreccioni to simulate dense cyanobacteria period according to the historical data. The lake water was pre-filtered through a 0.45 m fiber membrane to remove any background algae before dilution.

2.4. Sampling and characterization for PEMF and PEUF experiments

The raw water sample was taken from the water intake of the Castreccioni DWTP. Based on the information gathered from the water utility, the current treatment configuration is highly effective to remove cyanobacteria and cyanotoxins in the final effluent. In addition, due to the accumulation of cyanobacteria and microcystin in time, the SFBW was also sampled and characterized together with the raw lake water for further bench-scale tests. Physical and chemical characterization of the water intake and SFBW are given in Table 1. All analyses were done according to the Standard Methods (APHA, 2012). pH, EC, and DO were measured on-site using a multi-parameter meter of pH/ISE/EC/DO, HI9829 (Hanna Instruments, US).

2.5. PEMF and PEUF experiments

Chitosan powder from crab shells was obtained from Sigma Aldrich Company Ltd. and used as the coagulant in the experiments. A chitosan solution of 1 mg/mL was prepared according to the protocol described elsewhere (Divakaran and Pillai, 2002). The experimental setup is depicted in the **e-Supplementary file**. The operating parameters (i.e. coagulant dose, rapid mixing velocity, and time) for the PEMF and PEUF experiments were initially optimized in 28 experimental systems. Briefly, chitosan was added to 500 mL of *P.rubescens* culture (10^4 cells/mL) at the concentrations of 1, 2, 3, 4, 5, 10, and 20 mg/L, and different rapid mixing conditions were tested for each concentration as follows: 100 rpm at 3 min, 150 rpm at 2 min, 200 rpm at 1.5 min and 250 rpm at 1.2 min. Then, the suspensions were slowly mixed at the velocity of 40 rpm for 20 min and finally left for sedimentation for 30 min. The supernatants were carefully collected and subjected to MF (fiber membrane filters - 0.45 m) and UF (Merck Millipore Amicon stirred cells 10 kDa) separately. The clarification efficiency (CE) of the supernatants after coagulation, MF, and UF were further determined via the measurement of the optical density at 750 nm (OD_{750}) with an ONDA UV-20 spectrophotometer. The CE was then calculated by CE (1 ODs/ODf) x 100% (Xu et al., 2013), where ODf is the OD of the feed sample, ODs is the OD of the supernatant after coagulation-flocculation-sedimentation, MF, or UF. Further, the PEMF and PEUF experiments were conducted at the pre-determined optimal conditions using two different cases: cyanobacterial culture (10^4 cells/mL) and SFBW. In addition to the chitosan dose of 4 mg/L, a high concentration of 20 mg/L was also tested to evaluate any possible enhanced removals of cyanobacteria cells and microcystin. In parallel, the effectiveness of the PEMF/PEUF was experimentally compared to that of conventionally used MF and UF, where the *P. rubescens* culture

Table 1
Characteristics of the water intake and sand filter backwash water (SFBW) in the Castreccioni DWTP during the sampling for PEUF tests (average values from duplicate measurements).

Parameter	Unit	Water intake	SFBW
pH		7.3	7.8
EC	s/cm^2	367	485
Alkalinity	mg$CaCO_3$/L	184	185
DO	mg/L	7.6	7.3
Chemical oxygen demand	mg/L	73	124
Total suspended solids	mg/L	5	60
Total dissolved solids	mg/L	680	520
Total phosphorus	mg/L	0.1	0.3
Nitrate	mg/L	2.89	0.1
Sulfate	mg/L	57.8	55.4
Chloride	mg/L	18.4	20.7
Sodium	mg/L	15.6	16.5
Potassium	mg/L	2.4	2.6
Magnesium	mg/L	14.3	14.6
Calcium	mg/L	96.8	99.2
Iron	mg/L	0.044	0.169
Copper	mg/L	0.044	0.123
Manganese	mg/L	0.005	0.034
Aluminium	mg/L	0.044	0.202
Zinc	mg/L	0.113	0.558
Barium	mg/L	0.124	0.069
Lead	mg/L	0.01	0.01
Chromium	mg/L	0.001	0.001
Nickel	mg/L	0.001	0.001
Cadmium	mg/L	0.0004	0.0004

and SFBW were directly filtered without prior coagulation. After each test, the samples were collected for microcystin analysis. The samples after PEUF and UF were also checked to ensure any microbiological contamination in the final effluents using the membrane filtration method. All experiments were carried out in duplicate and mean values are presented.

2.6. Microcystin analysis

Water samples of 10 mL were conditioned at room temperature, mixed by vortex for 60 s, and subjected to ultrasonication (180 W, 40 kHz DU-65, Argolab, Carpi, Italy) for 30 min for cell lysing (Foss and Aubel, 2015). The ELISA was performed by the commercial Microcystins-ADDA-Enzyme-Linked Immunosorbent Assay (Abraxis, Product No. 520011, Lot.No. 19GO113) following the manufacturer s instructions. All reagents, such as microtiter plate (12 8 strips) coated with an analog of microcystins conjugated to protein, ready-made Standards solutions at 0, 0.15, 0.40, 1.0, 2.0, 5.0 g/L, Control at 0.75 0.185 g/L, Sample Diluent, Antibody solution, Anti-sheep-HRP conjugate solution, Wash buffer (5x) concentrate, a substrate solution (color solution), and Stop solution, were provided by the manufacturer. The absorbances were read at 450 nm in a Multiskan FC, Labsystems microtiter plate reader (Thermofisher). Microcystin concentrations were calculated by interpolation using the standard curve constructed by plotting the %B/B0 for each standard versus the corresponding microcystins concentration (0, 0.15, 0.40, 1.0, 2.0, 5.0 g/L). The coefficient of variations (CVs) for standards was always ˂ 10%; for samples always ˂ 15%. Recovery for control standard (QCS, 0.75 0.185 g/L), were always between 75% and 125%. A final estimate for microcystins content was obtained and calculated as the mean of at least two subsamples (i.e. two replicates per subsample).

2.7. Statistical analysis

Correlations between environmental parameters and cyanobacterial blooms/microcystin concentrations during the monitoring period were determined by Pearson s correlation method. The significant differences were determined at the p 0.001, p 0.05, and p 0.1 level of significance. Principal component analysis (PCA) was applied to investigate the contribution of each considered variable. All analyses and visualizations were done in R ver. 3.6.3.

3. Results

3.1. Monitoring of environmental parameters and observation of cyanobacterial blooms

The physico-chemical parameters measured in the water intake between 2014 and 2019 are given in the **e-Supplementary file**. The water temperature measurements showed limited mean variations between different years, ranged between 8.4 and 10.9 C with a maximum value measured in 2019. The dissolved oxygen (DO) levels showed different patterns during the monitoring period (2014 2019). Whereas the highest average and maximum value were recorded in 2014 (8.6 mg/L and 8.8 mg/L, respectively), a remarkable decrease was detected in the following years with a minimum value of 0.1 mg/L. However, an increase in DO levels was observed in 2019 with an average concentration of 6.7 mg/L. The highest electrical conductivity (EC) values were measured in 2014 with an average concentration of 577 S/cm and a maximum of 1507 S/cm. A decreasing pattern was observed in the following years showing more stable characteristics. The lowest EC value was detected in 2019 as 416 S/cm. In general, the lake water was found to be slightly alkaline, the average pH was in the range of 7.68 and 7.85. The highest average and maximum pH values were measured in 2016 (7.3 and 8.2, respectively). The average alkalinity was in the range of 242 mg/L 274 mg/L until 2018, then lower values were detected. The average alkalinity was 183 mg/L in 2019. The average bicarbonate concentration was recorded in the range of 236 260 mg/L during the monitoring period without any significant variation. The average total organic carbon (TOC) levels were mostly under 4 mg/L. However, a high peak was detected in 2019 and the highest concentration was recorded for the monitoring period (8.1 mg/L). Ammonium concentration was steadily below the detection limit (0.1 mg/L) for most of the time except for a sharp increase in January 2019. Thus, it resulted in an increase in the average concentration in 2019. The average nitrate concentration ranged from 5 mg/L to 7.17 mg/L. The highest values were recorded in 2018 whereas the average and maximum concentrations were 7.17 mg/L and 12 mg/L, respectively. The phosphate concentration was measured steadily below 3 mg/L for the entire monitoring period. The highest iron concentration was measured in 2014 (82 g/L) and there was a tendency towards decreasing for the following years. However, higher concentrations were recorded in 2019 in which the average and maximum values were 27.3 g/L and 61 g/L, respectively. Similarly, there was a decreasing trend for manganese, the highest value was measured in 2015 as 136 g/L and it resulted to record the highest average value (63.5 g/L). The lower values were recorded in 2019 when the manganese levels ranged from 5 mg/L to 17 mg/L. There was not a significant difference in calcium and magnesium levels in the lake water, the variation between the average and the maximum levels was quite low. The average potassium concentration ranged from 3.07 mg/L to 3.87 mg/L and the highest levels were detected in 2019. There was not a high variation in the average sulfate levels in the last three years of the monitoring period (ranged from 54 mg/L to 58 mg/L). The highest sulfate concentration was observed in 2018. The chloride concentration varied between 21 mg/L and 25 mg/L, showing a constant pattern for the entire monitoring period. Since the average aluminum concentration varied from 33 g/L to 50 g/L between 2014 and 2016, the concentrations went up in 2017, and the highest levels were measured in this year whereas the minimum, average, and maximum levels were recorded as 34 mg/L, 83 mg/L, and 183 mg/L, respectively. Zinc concentrations were constantly measured below the detection limits (0.05 mg/L). The lead concentrations were very low during the monitoring period: it was measured under 2 g/L during the study period. A tendency towards decreasing was detected for barium and the average concentration ranged from 0.02 mg/L to 0.57 mg/L.

Concentrations of total coliforms ranged from 12 to 5.7 10^3 cfu/100 mL during the monitoring period. Until 2017, the highest total coliforms concentration was recorded as 3000 cfu/100 mL. Then, there was a sharp increase in the concentration in October 2017 (5700 cfu/100 mL). The highest average value was also detected in this year among the monitoring period (742 cfu/100 mL). Thereafter, the enhancement was observed in the water quality in terms of total coliform bacteria concentration. The lowest average value was measured in 2018 (138.75 cfu/100 mL). As expected, the trend of *E.coli* concentration mimicked that of total coliform bacteria, and the highest value was recorded in 2017 as 1.7 10^3 cfu/100 mL with an average of 145 cfu/100 mL. In the following years, the average concentrations were quite low and recorded as 5.33 cfu/100 mL and 8 cfu/100 mL for 2018 and 2019, respectively.

The cyanobacteria abundance and microcystin concentrations are presented in Fig. 1. In 2014, the abundance of cyanobacteria started to increase in winter and this trend continued until the beginning of spring. The cyanobacteria abundances reached the highest levels in March and April which revealed a bloom. A second and relatively not heavy bloom was observed in July and then the cyanobacteria abundance showed a tendency towards decreasing. In 2015, a sharp increase in the abundance occurred in June which was also the highest abundance detected during the entire monitoring period and a heavy bloom was formed. The abundance went down in the following months except for a slight increase in September. In 2016, the bloom was observed in August and the cyanobacteria abundances were quite low for the rest of the months. In 2017, the bloom was observed early compared to the previous years. The abundances increased significantly in April and May, then

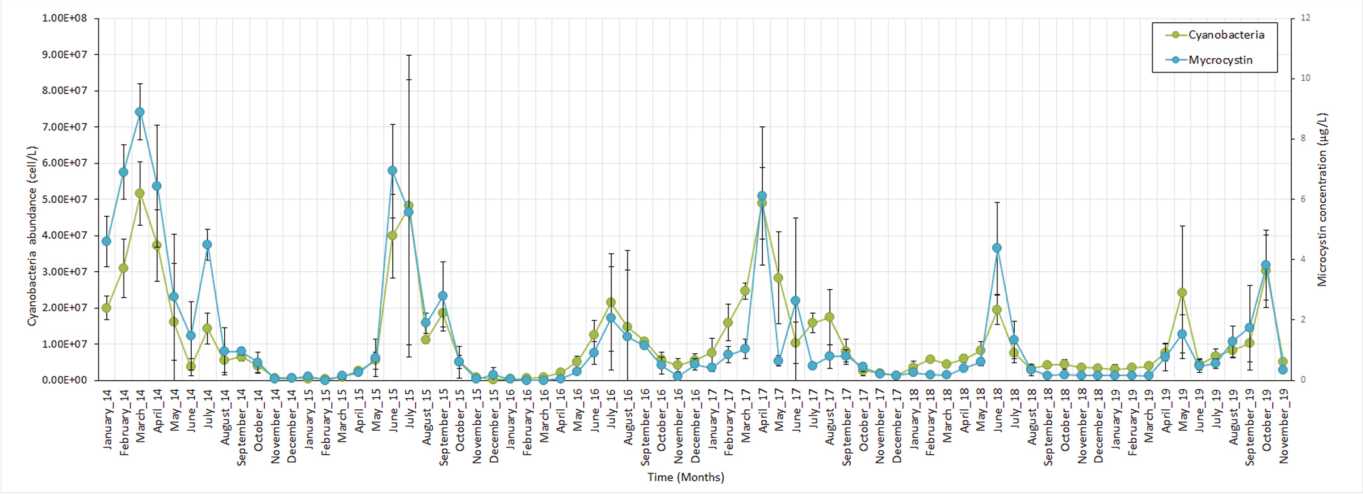


Fig. 1. Monthly data of cyanobacteria abundance and microcystin concentrations in the water intake of Castreccioni DWTP throughout the monitoring period (2014–2019).

decreased in the following seasons. In 2018, the bloom was formed in August and the abundance was lower than that of the previous cases. On the other hand, in 2019, the first bloom was recorded in May and the highest abundance was recorded all year round. A second bloom was observed in early autumn, but the abundance was not as high as the first one.

The contribution of *Plantothrix* to total phytoplankton abundance is also given in the **e-Supplementary file**. The phytoplankton community was comprised of diatoms, dinoflagellates, golden algae, green algae, and yellow algae. The genus *Planktothrix* constituted the prevalent part of the cyanobacteria (>97% in most cases), mainly represented by *P. rubescens*. During the bloom events especially in the summer period, the contribution decreased to around 70%.

The trend of microcystin concentrations in the water intake showed a similar pattern with cyanobacterial growth. As a result of the increase in cyanobacteria abundance, higher microcystin concentrations were detected during bloom formation. The highest microcystin level was detected as 10.3 µg/L in July 2015 in the monitoring period. The second peak concentration was recorded in 2014 (9.54 µg/L) followed by the levels observed in April and May 2017. After 2017, lower microcystin levels were detected in the water samples during the bloom events.

3.2. Correlation of environmental parameters to cyanobacterial blooms

3.2.1. Water quality changes and correlations with cyanobacteria and microcystin variations

To determine the relationship between the cyanobacterial growth, microcystin concentration, and major environmental parameters, the Pearson correlation was calculated and the results are displayed in Fig. 2A. As expected, a strong positive correlation was found between total coliform and *E.coli* concentrations ($p < 0.001$) as well as cyanobacterial growth and microcystin concentration ($p < 0.001$). Since the variation in the water temperature was quite small during the study period, it is apparent from the correlogram that very few parameters were negatively correlated with temperature (alkalinity ($p < 0.1$), pH ($p < 0.1$), and iron ($p < 0.05$)). Neither coliform bacteria nor *P. rubescence* was correlated with water temperature ($p > 0.1$). Whereas pH was negatively correlated with *E. coli* concentration ($p < 0.1$), there was a strong correlation between pH and total coliform bacteria levels ($p < 0.001$). The parameters, calcium, and sodium, showed a similar profile in the correlation analysis, they had a strong negative correlation with magnesium and alkalinity. Within all physico-chemical parameters, cyanobacteria and microcystin were only significantly correlated with manganese.

Fig. 2B provides an overview of the contribution of the parameters influencing the water quality in the water intake of Lake Castreccioni DWTP. The results revealed that the main drivers shaping the water quality throughout the monitoring period were microcystin and cyanobacteria concentrations as well as calcium. On the other hand, EC, nitrate, TOC, and water temperature were the least effective parameters showing the lowest contribution within the analysed parameters in the water samples.

3.2.2. Climatic conditions and correlations with cyanobacteria and microcystin variations

Meteorological factors have a key role in bloom formation. The effects of climatic variables on cyanobacterial growth and microcystin production were evaluated and the correlation analyses are shown in Fig. 2C. Although the atmospheric temperature showed significant changes among seasons, the water temperature did not differ significantly during the study period. Among all parameters, the cyanobacterial growth was only significantly negatively correlated with humidity ($p < 0.1$). Besides, there was a significant positive correlation between cyanobacterial growth and atmospheric temperature ($p < 0.1$). No significant correlation was found between microcystin concentration and the climatic parameters. The contributions of the climatic parameters on cyanobacteria abundance were shown in the variable factor map (Fig. 2D). The precipitation had the lowest contribution to the cyanobacterial growth. On the other hand, pressure and wind were not likely to be correlated with cyanobacterial abundance.

3.3. Existing treatment configuration to remove microcystin

The sample taken from the water intake of the plant for the experimental tests contained 0.099 µg/L microcystins since the sampling was done in a non-bloom period. In the meantime, the SFBW contained 12.32 µg microcystin/L, which highlighted that the sand filter unit is one of the main accumulation points for cyanotoxins in the treatment line, in particular microcystin. On the other hand, the microcystin concentration in the effluent of the sand filter was measured as 0.115 µg/L, which indicated that there was no re-release of microcystin in the effluent. In fact, considering the following treatment units in the plant, microcystin concentration in the final effluent is always much lower than the required limits (<1 µg/L). Consequently, we considered the SFBW as the most critical flow in the plant in terms of microcystin release into the environment, as well as in any other similar DWTPs, since these (waste) waters are often discharged without any treatment.

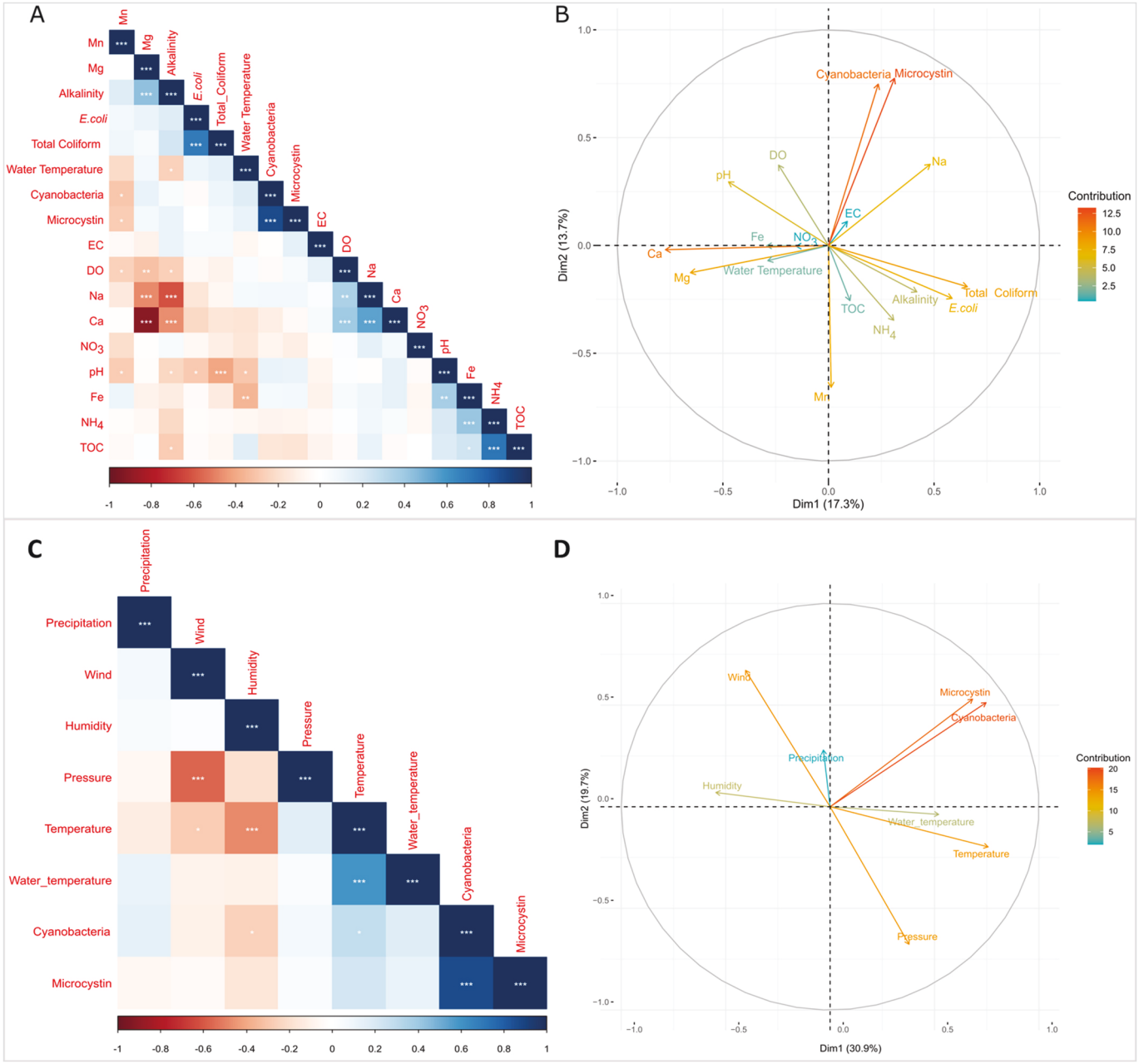


Fig. 2. A) Pearson correlation between water quality parameters and cyanobacteria and microcystin occurrence throughout the monitoring period (2014–2019). Positive significant correlations are highlighted in blue while negative significant correlations are highlighted in red. Lower significant positive and negative correlations are highlighted in lighter and lightest blue and red colors, in proportion to the correlation coefficient (*p < 0.1, **p < 0.05, ***p < 0.001). **B)** Variable-factor map. **C)** Pearson correlation between climatic parameters and cyanobacteria and microcystin occurrence throughout the monitoring period (2014–2019). **D)** Variable factor map. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Alternative mitigation strategies to remove P. rubescens and microcystin using PEMF and PEUF

The optimal coagulation condition in PEMF and PEUF tests was found at the chitosan dose of 4 mg/L and at the rapid mixing velocity at 200 rpm for 1.5 min (data not shown). The effect of two chitosan doses, optimal (4 mg/L) and extra high dosage (20 mg/L), on microcystin removal was further tested in pre-determined coagulation-flocculation conditions and subjected to MF and UF. Fig. 3 shows the results obtained from *P. rubescens* culture (PC) and SFBW with respect to microcystin concentration (Fig. 3A and B) and removal rate (Fig. 3C). PC had an initial microcystin concentration of 1.236 μg/L and decreased to 0.562 μg/L (54.3% removal) after the coagulation-flocculation at 4 mg/L of chitosan. After the MF and UF, the effluent has 0.065 μg/L (94.7% removal) and 0.122 μg/L (90.1% removal) of microcystin, respectively. Differently, 20 mg/L chitosan was achieved to obtain 0.073 μg/L (94.1% removal) of microcystin after the coagulation-flocculation. Following the MF and UF, the microcystin concentrations in the effluents were detected as 0.056 μg/L (overall 95.4% removal) and 0.13 μg/L (overall 89.5% removal), respectively. Meanwhile, the direct MF and UF of the PC (without prior coagulation step) resulted in the remaining microcystin concentrations of 0.364 μg/L (70.5%) and 0.125 μg/L (89.9% removal), respectively. The initial concentration of microcystin in the SFBW was measured as 12.32 μg/L, which is much greater than the PC. With the aid of 4 mg/L of chitosan, the effluent after coagulation-flocculation still contained 10.583 μg/L of microcystin (14.1%

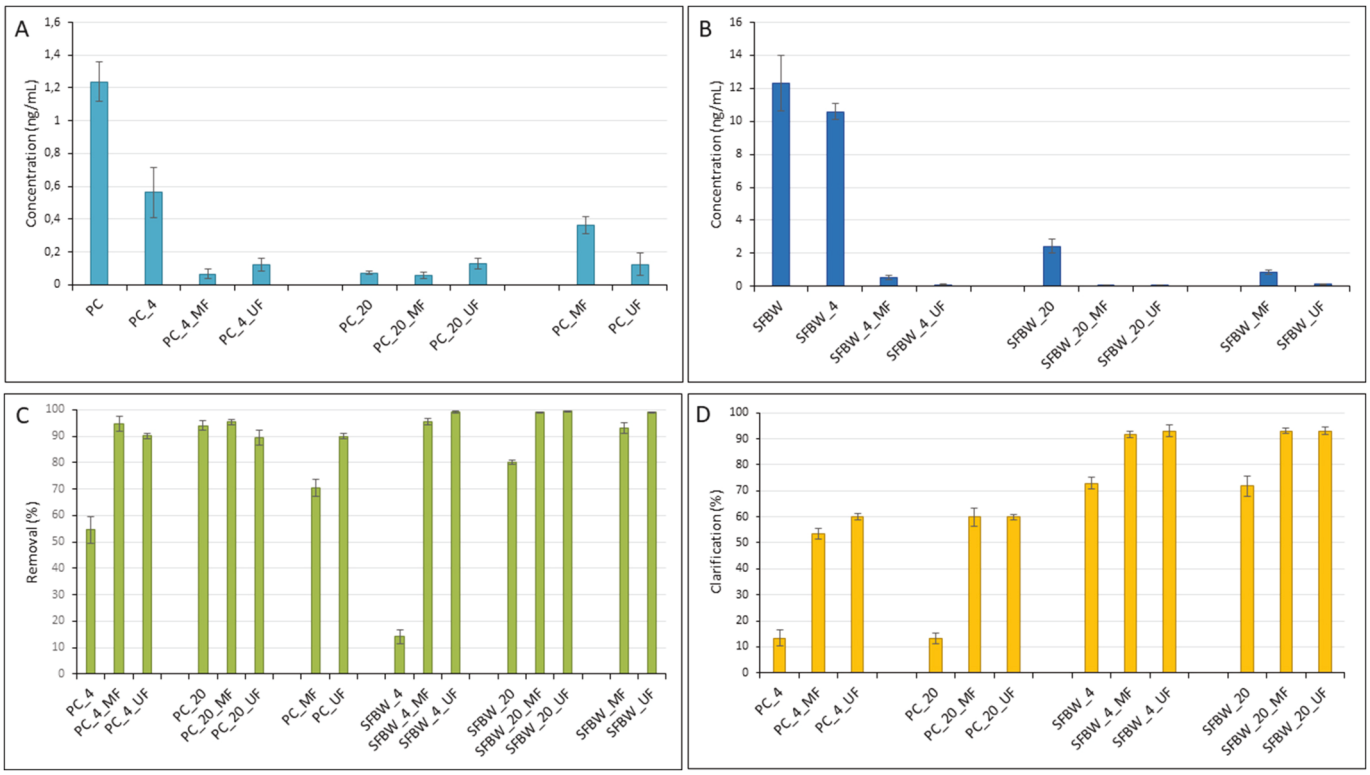


Fig. 3. Microcystin removal and clarification efficiencies after each process in PEMF and PEUF tests with the addition of 4 and 20 mg/L chitosan. **A)** Microcystin concentrations in pure culture (PC) after chitosan-enhanced coagulation-flocculation, MF, and UF. **B)** Microcystin concentrations in sand filter backwash water (SFBW) after chitosan-enhanced coagulation-flocculation, MF, and UF. **C)** Overall microcystin removal efficiencies of PC and SFBW samples. **D)** Clarification efficiencies of PC and SFBW samples.

removal). On the other hand, further MF and UF application ended up with microcystin concentration of 0.555 μg/L (overall 95.5% removal) and 0.106 μg/L (overall 99.1% removal), respectively. There was a significant contribution of the addition of 20 mg/L of chitosan compared to 4 mg/L, where the remaining concentration was 2.44 μg/L of microcystin (80.2% removal) after the coagulation-flocculation. After the MF and UF, there was only 0.099 μg/L (overall 99.2% removal) and 0.057 μg/L microcystin (overall 99.5% removal) in the final effluent, respectively, where the highest removal efficiencies were achieved. At the same time, the direct MF and UF of SFBW resulted in 0.842 μg/L (93.1% removal) and 0.119 μg/L of microcystin (99% removal) in the effluent, respectively.

As shown in Fig. 3D, the clarification efficiencies of the PEMF and PEUF tests confirmed the microcystin removals in the experiments. Chitosan-aided coagulation-flocculation had approximately 13.3% clarification efficiency in PC samples at both concentrations, and it increased to 53.3% and 60% after MF at the concentrations of 4 and 20 mg/L, respectively. There was not a remarkable contribution of UF to the clarification, since the efficiency was measured as 60% in both flows. Similarly, there was not a difference in the clarification efficiency between 4 and 20 mg/L of chitosan in the SFBW samples after the coagulation-flocculation process, calculated as 72.9% and 71.8%, respectively. When MF and UF were applied to SFBW samples after the coagulation-flocculation, the maximum clarification efficiencies were obtained as 91.8% and 92.9%, respectively.

4. Discussion

According to the historical data, the cyanobacteria proliferated multiple times throughout the year in Lake Castreccioni reaching a bloom usually in spring-summer periods. Furthermore, there were some periods when the microcystin concentrations reached up to 10 μg/L with respect to the dense cyanobacteria occurrence and the average concentration ranged from 0.6 μg/L to 3.81 μg/L, which often exceeded the limit set by WHO (1 μg/L). Our results are in line with the previous study carried out by Manganelli and co-authors (2016), where *P. rubescens* was found to be dominant in another meso-eutrophic lake in Central Italy (Vico Lake) with 0.5–5 μg/L of microcystin in the drinking water collection point. The authors also found no relevant correlation between *P. rubescens* density nor toxicity and total phosphorus, phosphate, and inorganic nitrogen. In another Italian drinking water basin, high levels of cyanobacteria (up to 160 × 10^6 cells/L) and microcystin (28.4 μg/L) were recorded over 28 months of monitoring, which was mostly represented by *P. rubescens* (Bogialli et al., 2013). A discharge of microcystin-contaminated freshwater into a coastal environment primarily originated from *P. rubescens*, has also been reported in the Adriatic coast, Italy (Rita et al., 2014). It is among the predominant cyanobacterial species in many Italian regions (Funari et al., 2017). *P. rubescens* produces mainly variants of the hepatotoxin microcystin, known to be associated with oncological and endocrinological risks. At high levels of exposure (representative of acute intoxication), microcystin produces a cascade of events (e.g. cytoskeleton alterations, lipid peroxidation, oxidative stress, apoptosis), while at low doses (typical of long-term exposure), phosphatases inhibition induces cellular proliferation and hepatic hypertrophy (Funari et al., 2008).

Lake Castreccioni is characterized by relatively low nutrient values (oligo-mesotrophic lake), which is typical for most Italian lakes (Rogora et al., 2018). DO is one of the key parameter indications for a healthy aquatic ecosystem and 4–6 mg/L of DO indicates a good water quality (Avvannavar and Shrihari, 2008). The oxygen levels in Lake Castreccioni also pointed a healthy ecosystem. According to the data obtained in our study during the monitoring period, there were not any notable changes either in phosphorous nor in nitrate concentration (except in several cases) in the water intake which resulted to have no

significant interaction between the cyanobacterial growth as well as microcystin concentration and nutrient concentration. Carvalho et al. (2011) also found that the nutrient concentrations were not the key drivers for cyanobacteria growth. Our results also reflect those of Zamyadi et al. (2012) who could not determine any correlation between the physico-chemical parameters such as pH, turbidity, dissolved oxygen, conductivity, and microcystin concentration.

Like other organisms, cyanobacteria also require trace metals for their growth especially as cofactors in electron transfer apparatus (e.g. cytochromes, plastocyanin) in photosynthesis, and a sufficient amount of trace metals are necessary. Despite manganese is required for the metabolic functions in the organism, it is mostly found at high levels in aquatic environments and is not considered as a limiting factor (Facey et al., 2019). Contrarily, high levels can be toxic for cyanobacteria which results in a reduction of growth rates (Rueter and Petersen, 1987). In fact, we found a significant negative correlation between cyanobacteria abundance and manganese concentration. In accordance with our findings, Giles et al. (2016) also recorded an increased manganese concentration during the bloom collapse period. Although the blooms were correlated with atmospheric temperature in our study, we could not determine any correlation between the algal bloom and water temperature.

P. rubescens is stenoterm species, its optimal growth temperature is 12 C (Walsby et al., 2006), and Lake Castreccioni provides a favorable temperature for its growth. Many factors have a role in the phytoplankton succession such as ecological requirements and competition between species (Hadas et al., 2002) and *P. rubescens* is superior in competition with other phytoplankton species thanks to regulating its buoyancy (Akçaalan et al., 2014). When the environmental conditions are not convenient for their growth, they can migrate to the appropriate depth and benefit from the conditions and proliferate. A strong correlation between the cyanobacteria abundance and microcystin concentration was inherently found in our study, which is also introduced by the WHO. Since the toxin analysis is quite costly and cannot be performed regularly, the WHO proposed two threshold levels for cyanobacteria density (Merel et al., 2013). Our correlation analysis also revealed that within all climatic parameters, cyanobacteria density was only in relation to humidity and atmospheric temperature. In a study carried out by Zhang et al. (2016), significant correlations were found for algal bloom area, wind speed, and air pressure. On the other hand, similar to our study, they could not determine any correlation between algal growth and precipitation, also the main contributors in the lake were atmospheric temperature, air pressure, and wind speed.

There are many studies (summarized in the **e-Supplementary file)** on the removal of various cyanobacteria species and/or cyanotoxins via coagulation using chitosan (Pei et al., 2014; Xu et al., 2013) as well as other coagulants (Sun et al., 2013). Whereas this approach is common, with and without membrane processes, it was mainly used to remove *M. aeruginosa* and not *P. rubescens*. Moreover, depending on the quantity and quality of the cyanotoxins, membrane technologies have been reported with high removal rates on cyanobacteria and/or cyanotoxins (Campinas and Rosa, 2010; Gijsbertsen-Abrahamse et al., 2006; Sorlini et al., 2013). The most common type is UF, while MF was not often applied and compared to UF, as we demonstrated in our study. Finally, either enriched pure culture or real lake water was used in these studies and SFBW was not tested so far.

Sorlini et al. (2013) obtained high removals of cyanobacteria (*Anabaena lemmermannii*) (98%) and total algae (99%) using a pilot-scale MF and further reported severe fouling that caused a rapid increase in the transmembrane pressure. In addition, UF has also been used to treat SFBW to enable safe reuse in a DWTP (Reissmann and Uhl, 2006). Regarding membrane processes, such as UF that shows superb separation properties while treating dense-cyanobacteria water of lakes or reservoirs, they still face challenges due to membrane fouling and poor rejection of soluble organics (Liu et al., 2017). A combination of coagulation and membrane processes (e.g. PEMF, PEUF) can effectively remove cyanobacteria and associated cyanotoxins as proposed and confirmed with the results of this study while alleviating the fouling on the membranes (Yan et al., 2017). At this point, the determination of proper coagulant dosage is an important factor controlling membrane fouling in the membrane process of algal-rich water as highlighted by Park et al. (2019) that tested the combination of PACl-coagulation and MF.

Our results remarked that there was an apparent difference in the microcystin concentrations in both effluents, PC, and SFBW, after the coagulation at 4 and 20 mg chitosan/L. Although 4 mg chitosan/L was comparatively more efficient to remove microcystin in PC, a high concentration of microcystin, as well as the more complex nature of SFBW, required a higher dosage of chitosan. After the coagulation-flocculation-clarification steps, 20 mg chitosan/L achieved relatively 0.73 and 4.69-fold higher microcystin removal rates in PC and SFBW compared to 4 mg/L, respectively. The difference in the increment of removal efficiency between these two water matrices can be due to the better formation of flocs via the suspended matter in SFBW. In the study of Jin et al. (2017), chitosan quaternary ammonium salt (HTTC) was used to coagulate *Microcystis aeruginosa* cells in different coagulation conditions. The authors found complete removal of algal cells at the HTCC dosage of 1.5 mg/L, rapid mixing for 0.5 min at 5.04 g, and slow mixing for 30 min at 0.20 g. On the contrary, chitosan was found to be effective in the removal of *Microcystis* spp. but not *Cylindrospermopsis* spp. (Miranda et al., 2017). This finding points out the importance of algal species and the selection of relevant coagulants.

Furthermore, there was not a remarkable difference between PEMF and PEUF in terms of overall microcystin removal and clarification efficiencies. In all conditions, the microcystin concentration in the final effluents was 0.15 g/L. A slight increase in microcystin concentration was even observed in the final PEUF effluents in PC samples. This can be contributed to high transmembrane pressure that may have caused a slight release of microcystin (Gijsbertsen-Abrahamse et al., 2006). Meanwhile, direct MF and UF applications without prior coagulation resulted in poorer removal efficiencies. Although PEMF and PEUF resulted in similar removal efficiencies, the superiority of UF over MF in terms of microbial elimination should also be noted. PAC-aided coagulation and UF were investigated by (Dixon et al., 2011) with above 90% removal of intracellular and extracellular cyanobacterial toxins. The authors highlighted the importance of PAC-coagulation on cell removal and improving the flux of the UF. Similarly, up to 94% of microcystin was effectively removed when the coagulation/flocculation and PAC systems were combined with UF membranes, while coagulation/-flocculation alone could not lead to an appreciable removal of microcystin (Şengül et al., 2018). Eventually, the adaptation and optimization of such hybrid systems are crucial since their performance is governed by multiple mechanisms.

Although polymer-enhanced membrane treatment is a promising approach for the removal of cyanobacteria and cyanotoxins from drinking water, most studies on PEUF were carried out on a small scale to date. The biggest obstacle in membrane-based systems is high capital expenditure (CAPEX) as well as a high operational expenditure (OPEX) for membrane replacements due to fouling. For instance, a pilot test was conducted by Lermontov et al. (2014) to make a comparative cost assessment of conventional water treatment (with coagulation, flocculation, settling, filtration steps followed by chlorine for disinfection) and UF unit. Even though UF comprises a higher CAPEX, its net present value shows a lower cost than the conventional option. When considering the land required and its cost for each solution, the UF solution indicates an equivalent or even more financially feasible option, since the UF-based DWTP requires eight times less land than a conventional DWTP. Hybrid systems that include polymer-aided coagulation/flocculation before UF can ease the membrane fouling and thus help to decrease OPEX in the long term. However, water-soluble polymers added to the feed solution to carry out PEUF can often cause severe foulant in some cases (Huang and Feng, 2019). From the coagulant point of view, the unit price of

chitosan is still much higher than that of traditional inorganic flocculants (Yang et al., 2016), but its environmental benefits can encompass the high operational cost. Farid et al. (2013) indicated that flocculant chitosan for water treatment on the industrial scale can be provided with the price of about $1.5/kg, while calculated the cost of coagulation/-flocculation process of microalgae around $0.0246/(kg dry biomass) including (nano)chitosan and acetic acid. Hence, novel and simplified techniques can be developed to improve the functionality and to decrease the cost of chitosan-based flocculants (Yang et al., 2016).

5. Conclusion

The historical data of the water intake supplied from Lake Castreccioni revealed unconventional water quality (manganese) and climatic (humidity and atmospheric temperature) correlations with the dense cyanobacteria periods while no correlation was found with conventional indicators such as nutrients and water temperature. This can lead to alternative choices of parameters for future decision support systems to control cyanotoxins in the drinking water supply chain and put different management/treatment options in place if needed. In addition to the occurrence of microcystin in the water intake of the DWTP, SFBW was found to be another critical flow (microcysin 12 g/L). Such intermediate waste flows during drinking water treatment often exhibit a high level of cyanotoxin accumulation and therefore should not be neglected and thoroughly treated before being discharged into water bodies or recirculated within the plant. The overall PEMF and PEUF test results enabled to reach the concentration of microcystin for national drinking water standards and also indicated great removal efficiencies (99%) to eliminate microcystin in the final effluent of the SFBW. Although PEMF can be applied for water flows with low concentrations of microcystin, PEUF can be more effective at higher concentrations and/or on more complex water matrices like SFBW. In addition, higher chitosan dosage (i.e. 20 mg/L) performed better in terms of microcystin removal in both water flows, while it did not have a major effect on the overall removal performance of the further membrane units. Thus, the required chitosan dosage may also vary with respect to water matrix and/or initial microcystin concentration. The superior removal efficiency of PEUF systems as well as their help to alleviate membrane fouling highlights the cruciality of multiple barrier approaches for removing cyanotoxins from drinking water. However, further research is needed to test bench- and/or pilot-scale PEUF units in continuous operation and to understand the relationship between cyanobacterial removal and possible re-release of cyanotoxins as well as the fouling mechanism of membranes before these results can be generalized.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117535.

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References

Akçaalan, R., Koker, L., Gürevin, C., Albay, M., 2014. Planktothrix rubescens: a perennial presence and toxicity in Lake Sapanca. Turk. J. Bot. 38, 782 789. https://doi.org/10.3906/bot-1401-26.

Almuhtaram, H., Cui, Y., Zamyadi, A., Hofmann, R., 2018. Cyanotoxins and cyanobacteria cell accumulations in drinking water treatment plants with a low risk of bloom formation at the source. Toxins 10. https://doi.org/10.3390/toxins10110430.

APHA, 2012. Standard methods for the examination of water and wastewater, 22nd edn. American Public Health Association, Washington, DC. https://doi.org/ISBN 9780875532356.

Avvannavar, S.M., Shrihari, S., 2008. Evaluation of water quality index for drinking purposes for river Netravathi, Mangalore, South India. Environ. Monit. Assess. 143, 279 290. https://doi.org/10.1007/s10661-007-9977-7.

Bertani, I., Steger, C.E., Obenour, D.R., Fahnenstiel, G.L., Bridgeman, T.B., Johengen, T. H., Sayers, M.J., Shuchman, R.A., Scavia, D., 2017. Tracking cyanobacteria blooms: do different monitoring approaches tell the same story? Sci. Total Environ. 575, 294 308. https://doi.org/10.1016/j.scitotenv.2016.10.023.

Bogialli, S., Nigro Di Gregorio, F., Lucentini, L., Ferretti, E., Ottaviani, M., Ungaro, N., Abis, P.P., Cannarozzi De Grazia, M., 2013. Management of a toxic cyanobacterium bloom (Planktothrix rubescens) affecting an Italian drinking water basin: a case study. Environ. Sci. Technol. 47, 574 583. https://doi.org/10.1021/es302260p.

Campinas, M., Rosa, M.J., 2010. Evaluation of cyanobacterial cells removal and lysis by ultrafiltration. Separ. Purif. Technol. 70, 345 353. https://doi.org/10.1016/j.seppur.2009.10.021.

Carvalho, L., Miller, C.A., Scott, E.M., Codd, G.A., Davies, P.S., Tyler, A.N., 2011. Cyanobacterial blooms: statistical models describing risk factors for national-scale lake assessment and lake management. Sci. Total Environ. 409, 5353 5358. https://doi.org/10.1016/j.scitotenv.2011.09.030.

Divakaran, R., Pillai, V.N.S., 2002. Flocculation of algae using chitosan. J. Appl. Phycol. 14, 419 422. https://doi.org/10.1023/A:1022137023257.

Dixon, M.B., Richard, Y., Ho, L., Chow, C.W.K., O Neill, B.K., Newcombe, G., 2011. A coagulation-powdered activated carbon-ultrafiltration - multiple barrier approach for removing toxins from two Australian cyanobacterial blooms. J. Hazard Mater. 186, 1553 1559. https://doi.org/10.1016/j.jhazmat.2010.12.049.

Facey, J.A., Apte, S.C., Mitrovic, S.M., 2019. A review of the effect of trace metals on freshwater cyanobacterial growth and toxin production. Toxins 11, 1 18. https://doi.org/10.3390/toxins11110643.

Farid, M.S., Shariati, A., Badakhshan, A., Anvaripour, B., 2013. Using nano-chitosan for harvesting microalga Nannochloropsis sp. Bioresour. Technol. 131, 555 559. https://doi.org/10.1016/j.biortech.2013.01.058.

Foss, A.J., Aubel, M.T., 2015. Using the MMPB technique to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS). Toxicon 104, 91 101. https://doi.org/10.1016/j.toxicon.2015.07.332.

Funari, E., Manganelli, M., Buratti, F.M., Testai, E., 2017. Cyanobacteria blooms in water: Italian guidelines to assess and manage the risk associated to bathing and recreational activities. Sci. Total Environ. 598, 867 880. https://doi.org/10.1016/j.scitotenv.2017.03.232.

Funari, E., Testai, E., Classification, C., 2008. Human Health Risk Assessment Related to Cyanotoxins Exposure 97 125. https://doi.org/10.1080/10408440701749454.

Gallina, N., Beniston, M., Jacquet, S., 2017. Estimating future cyanobacterial occurrence and importance in lakes: a case study with Planktothrix rubescens in Lake Geneva. Aquat. Sci. 79, 249 263. https://doi.org/10.1007/s00027-016-0494-z.

Ghernaout, B., Ghernaout, D., Saiba, A., 2010. Algae and cyanotoxins removal by coagulation/flocculation: a review. Desalin. Water Treat 20, 133 143. https://doi.org/10.5004/dwt.2010.1202.

Gijsbertsen-Abrahamse, A.J., Schmidt, W., Chorus, I., Heijman, S.G.J., 2006. Removal of cyanotoxins by ultrafiltration and nanofiltration. J. Membr. Sci. 276, 252 259. https://doi.org/10.1016/j.memsci.2005.09.053.

Giles, C.D., Isles, P.D.F., Manley, T., Xu, Y., Druschel, G.K., Schroth, A.W., 2016. The mobility of phosphorus, iron, and manganese through the sediment water continuum of a shallow eutrophic freshwater lake under stratified and mixed water-column conditions. Biogeochemistry 127, 15 34. https://doi.org/10.1007/s10533-015-0144-x.

Gobler, C.J., 2020. Climate change and harmful algal blooms: insights and perspective. Harmful Algae 91, 101731. https://doi.org/10.1016/j.hal.2019.101731.

Griffith, A.W., Gobler, C.J., 2020. Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. Harmful Algae 91, 101590. https://doi.org/10.1016/j.hal.2019.03.008.

Hadas, O., Pinkas, R., Malinsky-Rushansky, N., Shalev-Alon, G., Delphine, E., Berner, T., Sukenik, A., K, A., 2002. Physiological variables determined under laboratory conditions may explain the bloom of Aphanizomenon ovalisporum in Lake Kinneret. Eur. J. Phycol. 259 267.

He, X., Liu, Y.L., Conklin, A., Westrick, J., Weavers, L.K., Dionysiou, D.D., Lenhart, J.J., Mouser, P.J., Szlag, D., Walker, H.W., 2016. Toxic cyanobacteria and drinking water: impacts, detection, and treatment. Harmful Algae 54, 174 193. https://doi.org/10.1016/j.hal.2016.01.001.

He, X., Wang, H., Zhuang, W., Liang, D., Ao, Y., 2021. Risk prediction of microcystins based on water quality surrogates: a case study in a eutrophicated urban river network. Environ. Pollut. 275, 116651. https://doi.org/10.1016/j.envpol.2021.116651.

Ho, L., Lambling, P., Bustamante, H., Duker, P., Newcombe, G., 2011. Application of powdered activated carbon for the adsorption of cylindrospermopsin and microcystin toxins from drinking water supplies. Water Res. 45, 2954 2964. https://doi.org/10.1016/j.watres.2011.03.014.

Hoshaw, R.W., Rosowski, J.R., 1973. Methods for microscopic algae. Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, pp. 53 68.

Huang, Y., Feng, X., 2019. Polymer-enhanced ultrafiltration: fundamentals, applications and recent developments. J. Membr. Sci. 586, 53 83. https://doi.org/10.1016/j.memsci.2019.05.037.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16, 471 483. https://doi.org/10.1038/s41579-018-0040-1.

Janse, I., Meima, M., Kardinaal, W.E.A., Zwart, G., 2003. High-resolution differentiation of cyanobacteria by using rRNA-internal transcribed spacer denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 69, 6634 6643. https://doi.org/10.1128/AEM.69.11.6634-6643.2003.

Jin, Y., Pei, H., Hu, W., Zhu, Y., Xu, H., Ma, C., Sun, J., Li, H., 2017. A promising application of chitosan quaternary ammonium salt to removal of Microcystis aeruginosa cells from drinking water. Sci. Total Environ. 583, 496 504. https://doi.org/10.1016/j.scitotenv.2017.01.104.

Lermontov, A., Dos Santos Pereira, R.A., Nagaoka, I.A., Philipps, K., Singh, M., 2014. An economic analysis on ultrafiltration membranes in water treatment: Brazilian cases. 87th Annu. Water Environ. Fed. Tech. Exhib. Conf. WEFTEC 2014 7, 787 798. https://doi.org/10.2175/193864714815940136.

Liu, B., Qu, F., Liang, H., Van der Bruggen, B., Cheng, X., Yu, H., Xu, G., Li, G., 2017. Microcystis aeruginosa-laden surface water treatment using ultrafiltration: membrane fouling, cell integrity and extracellular organic matter rejection. Water Res. 112, 83 92. https://doi.org/10.1016/j.watres.2017.01.033.

Lucentini, L., Ottaviani, M., 2011. Cianobatteri in acque destinate a consumo umano. Stato delle conoscenze per la valutazione del rischio, vol. 1. Rapporti ISTISAN 11/35 Pt. 1.

Manganelli, M., Stefanelli, M., Vichi, S., Andreani, P., Nascetti, G., Scialanca, F., Scardala, S., Testai, E., Funari, E., 2016. Cyanobacteria biennal dynamic in a volcanic mesotrophic lake in central Italy: strategies to prevent dangerous human exposures to cyanotoxins. Toxicon 115, 28 40. https://doi.org/10.1016/j.toxicon.2016.03.004.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E., Thomas, O., 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ. Int. 59, 303 327. https://doi.org/10.1016/j.envint.2013.06.013.

Miranda, M., Noyma, N., Pacheco, F.S., de Magalhaes, L., Pinto, E., Santos, S., Soares, M. F.A., Huszar, V.L., Lürling, M., Marinho, M.M., 2017. The efficiency of combined coagulant and ballast to remove harmful cyanobacterial blooms in a tropical shallow system. Harmful Algae 65, 27 39. https://doi.org/10.1016/j.hal.2017.04.007.

Mucci, M., Guedes, I.A., Faassen, E.J., Lürling, M., 2020. Chitosan as a coagulant to remove cyanobacteria can cause microcystin release. Toxins 12. https://doi.org/10.3390/toxins12110711.

Park, K., Kim, P., Kim, H.G., Kim, J.H., 2019. Membrane fouling mechanisms in combined microfiltration-coagulation of algal rich water applying ceramic membranes. Membranes 9. https://doi.org/10.3390/membranes9020033.

Pei, H.Y., Ma, C.X., Hu, W.R., Sun, F., 2014. The behaviors of microcystis aeruginosa cells and extracellular microcystins during chitosan flocculation and flocs storage processes. Bioresour. Technol. 151, 314 322. https://doi.org/10.1016/j.biortech.2013.10.077.

Qu, F., Liang, H., He, J., Ma, J., Wang, Z., Yu, H., Li, G., 2012. Characterization of dissolved extracellular organic matter (dEOM) and bound extracellular organic matter (bEOM) of Microcystis aeruginosa and their impacts on UF membrane fouling. Water Res. 46, 2881 2890. https://doi.org/10.1016/j.watres.2012.02.045.

Reissmann, F.G., Uhl, W., 2006. Ultrafiltration for the reuse of spent filter backwash water from drinking water treatment. Desalination 198, 225 235. https://doi.org/10.1016/j.desal.2006.03.517.

Richards, E., Reichardt, M., Rogers, S., 2001. Preparation of genomic DNA from plant tissue. Curr. Protoc. Mol. Biol. Chapter 2. https://doi.org/10.1002/0471142727.mb0203s27. Unit2.3.

Rita, D.P., Valeria, V., Silvia, B.M., Pasquale, G., Milena, B., 2014. Microcystin contamination in sea mussel farms from the Italian southern adriatic coast following cyanobacterial blooms in an artificial reservoir. J. Ecosyst. 2014, 1 11. https://doi.org/10.1155/2014/374027.

Rogora, M, Buzzi, F, Dresti, C, Leoni, B, Lepori, F, Mosello, R, Patelli, M, Salmaso, N, 2018. Climatic effects on vertical mixing and deep-water oxygen content in the subalpine lakes in Italy. Hydrobiologia 824, 33 50. https://doi.org/10.1007/s10750-018-3623-y.

Rueter, J.G., Petersen, R.R., 1987. Micronutrient effects on cyanobacterial growth and physiology. N. Z. J. Mar. Freshw. Res. 21, 435 445. https://doi.org/10.1080/00288330.1987.9516239.

Şengül, A.B., Ersan, G., Tüfekçi, N., 2018. Removal of intra- and extracellular microcystin by submerged ultrafiltration (UF) membrane combined with coagulation/flocculation and powdered activated carbon (PAC) adsorption. J. Hazard Mater. 343, 29 35. https://doi.org/10.1016/j.jhazmat.2017.09.018.

Shafiquzzaman, M., Al-Mahmud, A., Al-Saleem, S., Haider, H., 2018. Application of a low cost ceramic filter for recycling sand filter backwash water. Water 10. https://doi.org/10.3390/w10020150.

Sorlini, S., Gialdini, F., Collivignarelli, C., 2013. Removal of cyanobacterial cells and Microcystin-LR from drinking water using a hollow fiber microfiltration pilot plant. Desalination 309, 106 112. https://doi.org/10.1016/j.desal.2012.09.028.

Sun, F., Pei, H.Y., Hu, W.R., Li, X.Q., Ma, C.X., Pei, R.T., 2013. The cell damage of Microcystis aeruginosa in PACl coagulation and floc storage processes. Separ. Purif. Technol. 115, 123 128. https://doi.org/10.1016/j.seppur.2013.05.004.

Urquhart, E.A., Schaeffer, B.A., Stumpf, R.P., Loftin, K.A., Werdell, P.J., 2017. A method for examining temporal changes in cyanobacterial harmful algal bloom spatial extent using satellite remote sensing. Harmful Algae 67, 144 152. https://doi.org/10.1016/j.hal.2017.06.001.

Walsby, A. E, Schanz, F, Schmid, M, 2006. The Burgundy-blood phenomenon: a model of buoyancy change explains autumnal waterblooms of Planktothrix rubescens in Lake Zürich. New Phytologist 169 (1), 109 122. https://doi.org/10.1111/j.1469-8137.2005.01567.x.

WHO, 2020. Cyanobacterial Toxins : Microcystins. WHO/HEP/ECH/WSH/2020.6.

Xu, Y., Purton, S., Baganz, F., 2013. Chitosan flocculation to aid the harvesting of the microalga Chlorella sorokiniana. Bioresour. Technol. 129, 296 301. https://doi.org/10.1016/j.biortech.2012.11.068.

Yan, Z., Liu, B., Qu, F., Ding, A., Liang, H., Zhao, Y., Li, G., 2017. Control of ultrafiltration membrane fouling caused by algal extracellular organic matter (EOM) using enhanced Al coagulation with permanganate. Separ. Purif. Technol. 172, 51 58. https://doi.org/10.1016/j.seppur.2016.07.054.

Yang, R., Li, H., Huang, M., Yang, H., Li, A., 2016. A review on chitosan-based flocculants and their applications in water treatment. Water Res. 95, 59 89. https://doi.org/10.1016/j.watres.2016.02.068.

Zamyadi, A., McQuaid, N., Prevost, M., Dorner, S., 2012. Monitoring of potentially toxic cyanobacteria using an online multi-probe in drinking water sources. J. Environ. Monit. 14, 579 588. https://doi.org/10.1039/c1em10819k.

Zhang, Y., Shi, K., Liu, J., Deng, J., Qin, B., Zhu, G., Zhou, Y., 2016. Meteorological and hydrological conditions driving the formation and disappearance of black blooms, an ecological disaster phenomena of eutrophication and algal blooms. Sci. Total Environ. 569 (570), 1517 1529. https://doi.org/10.1016/j.scitotenv.2016.06.244.