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In-situ trace metal (Cd, Pb, Cu) speciation along the Po River plume (Northern Adriatic Sea) using submersible systems

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Abstract

Information on the distribution and speciation of trace metals is of critical importance for our ability to interpret the links between the bioavailability and uptake of an element, and its biogeochemical cycle in coastal environments. Within the framework of the European Project “In-situ automated Monitoring of Trace metal speciation in Estuaries and Coastal zones in relation with the biogeochemical processes (IMTEC)”, the chemical speciation of Cd, Pb and Cu was carried out along the Po River plume in the period 27 October – 2 November 2002. During the cruise, five Voltammetric In-situ Profiling systems and one Multi Physical Chemical Profiler, as well as conventional voltammetric instruments, were successfully applied in order to evaluate the distribution of Cd, Pb and Cu between different fractions (free ion, dynamic, colloidal, dissolved and particulate fractions) and to assess the evolution of these fractions during estuarine mixing and in the water column. Dynamic concentrations were $0.05\text{--}0.2\text{ nmol L}^{-1}$ Cd, $0.02\text{--}0.2\text{ nmol L}^{-1}$ Pb, and $0.15\text{--}4.0\text{ nmol L}^{-1}$ Cu. Cd was mainly present as dynamic fraction (40–100% of the dissolved Cd). High proportions of Pb (~70%) and Cu (~80%) were present as colloids probably of biogenic origin. Principal components analysis reveals a strong influence of the Po River discharge on the spatial and vertical distributions of metal species. Almost all the metal fractions globally decreased following the salinity gradient. Metal concentrations are far below (at least one order of magnitude lower) the Environmental Quality Standard established by the Italian law. However, the Cu dynamic fraction showed concentrations likely to be toxic to sensitive phytoplankton community and to have negative effects on larva development of coastal macroinvertebrate species (toxicity data extracted from literature).

Keywords: Metals; Speciation; Adriatic Sea; Voltammetry

1.1 Introduction

The Adriatic Sea is a semi-enclosed shelf basin located in the northeast part of the Mediterranean Sea and it is strongly influenced by several riverine inputs. In its northern sub-basin, the freshwater plume of the Po River plays a fundamental role in driving the coastal dynamics and the physical and biogeochemical processes of the whole basin. Crossing the entire northern part of the Italian country, which is one of the most industrialized areas in Europe, the Po river discharges in the Adriatic Sea a remarkable load of pollutants, which in turn affects the Italian coasts even to approximately the city of Ancona (following the prevailing southward marine currents).

The Northern Adriatic Sea has been intensely investigated by studying the physical structure of the water column, as well as the circulation patterns (Russo and Artegiani, 1996; Falcieri et al., 2014), the plankton community (Totti et al., 2005; Cabrini et al., 2012; Godrijan et al., 2013), the nutrients (Boldrin et al., 2005; Grilli et al., 2013) and trace elements distribution in seawater (Annibaldi et al., 2009, 2011, 2015; Cindrić et al., 2015; Tankere and Statham, 1996; Zago et al., 2000, 2002).

Nowadays, following the enactment of specific EU Directives, there is a growing need to control the quality of coastal waters which are affected by several anthropogenic activities (building of infrastructure for human settlement, habitat modification, tourism, transport by sea and disposal of industrial and domestic effluents). Combined efforts are requested to develop monitoring and assessment programs in order to survey and guarantee a good chemical and ecological status of the coastal ecosystems, including heavy metals. Heavy metals are ubiquitous in the environment and their concentrations are increased with respect to natural pre-industrial values due to anthropogenic activities, which have also modified the metal biogeochemical cycles on a regional and global scale (Sadiq, 1992). It is well known that the toxicity, bioavailability and mobility of trace metals in seawater depend on their speciation rather than on only their total concentration (Allen and Hansen, 1996). Dissolved trace metals in seawater can exist in different chemical species (forms), such as free hydrated ions, organic and inorganic complexes. The study of the metal species distribution in seawater gives insights on their cycles, as each metal species is characterized by different reactivity and different interactions with organisms. Free hydrated ions are the most reactive and highly bioavailable, but their reactivity, as well as their toxicity can be greatly modified by the presence of organic and inorganic compounds. Thus, to better understand the biogeochemical cycle of a trace metal and its overall mobility in the water column, it is essential to identify and quantify the various species that make up its total concentration.

Across much of the world, regulatory limit values (e.g., Environmental Quality Standards in the EU, Water Quality Criteria in the US, Australia, Canada, etc.) for metals in water bodies do not take metal speciation into account. They are mainly based on dissolved metal, since this fraction more closely approximates the bioavailable fraction of the metal in the water column. Recently, thanks to a major refinement in the scientific understanding of the behavior, fate and toxicology of metals in the environment, and to a series of statutory and voluntary risk assessments performed under the existing regulations on pollutant monitoring programs, several public administrations have started to implement the in-force water quality assessments by introducing a speciation-based approach. The revised Priority Substances Daughter Directive, 2013/39/EU (2013) now includes annual average Environmental Quality Standards (EQS) for nickel and lead in the freshwater environment that refer to bioavailable concentrations. These bioavailable EQS are based on Biotic Ligand Models (BLM), but at the time of the development of the latest draft of the Water Framework Directive, there was no validated and accepted BLM for some metals (Slaveykova and Wilkinson, 2005), therefore complimentary availability-based approaches were adopted to define the EQS bioavailable. For example, the EQS for lead are based on the availability correction for the dissolved organic carbon (SCHEER-EC, 2017).

Several studies have reported chemical speciation of trace metals in the Adriatic Sea, the majority of which deal with the horizontal and vertical partitioning between dissolved and particulate phases (Tankere and Statham, 1996; Tankere et al., 2000; Zago et al., 2000, 2002; Annibaldi et al., 2011, 2015; Cindrić et al., 2015). Some authors (Scarponi et al., 1995, 1998, Chapman et al., 2009; Louis et al., 2009; Plavšić et al., 2009) studied the distribution of dissolved trace metals between the labile fraction (mainly hydrated free ions or metal ions bound to inorganic or organic complexes whose dissociation kinetics are rapid for the applied method) and bound fraction (organically complexed metal), as well as the content of ligands complexing the metal and the related conditional stability constants. These approaches are mainly based on voltammetric titrations usually carried out in well-equipped laboratories. However, such studies are often affected by the risks of contaminations or chemical species modifications due to sampling, sample storage and handling (Buffle and Tercier-Waeber, 2005). Few efforts have been devoted to developing voltammetric equipments for performing voltammetry on field or on board ship in order to minimize sample perturbation problems. Luther III et al. (1999) mounted a solid-state Au/Hg voltammetric electrode on microprofiling instruments attached to a Remote Operated Vehicle (ROV) to measure in real-time O₂, Mn, Fe and sulfide present at µM concentrations in sedimentary pore-waters of Rartan Bay (NY). Nevertheless, the development of rough, reliable, submersible probes coupled with a new generation of voltammetric microelectrodes is essential to perform in-situ voltammetric trace metal speciation that maintains high-sensitivity (sub-nanomolar detection limit) and reliability (long-term monitoring). An integrated in-situ monitoring approach of the evolution of hazardous trace metals in relation to master bio-physicochemical variables is indeed of primary importance. This would help to identify the main sources of target metal species, to better understand their behavior and fate, and to evaluate the impact of human activities on aquatic ecosystems.

Within the framework of the European Project “In-situ automated Monitoring of Trace metal speciation in Estuaries and Coastal zones in relation with the biogeochemical processes (IMTEC)”, several studies on metal speciation were carried out in different European coastal areas by the partners of the project (University of Geneva, University of Göteborg, University of Plymouth, University of Ancona, University of Neuchatel). Tercier-Waeber et al. (2005) and Braungardt et al. (2009) reported preliminary results of speciation studies carried out by applying submersible voltammetric probes that were developed and tested within the IMTEC project for trace element monitoring in natural environments. Tercier-Waeber et al. (2005) focused on the in-situ deployment of a new technology to study trace metal speciation. Braungardt et al. (2009) reported results of inter-comparison exercises undertaken to test the reliability and performance of the new technology implemented on voltammetric probes. Here, a more integrated and systematic speciation study of Cd, Pb and Cu along the Po River plume, in relation to several hydrological, chemical and biological processes is presented. The in-situ speciation was carried out by applying two voltammetric probes, the Voltammetric In-Situ Profiler (VIP) and the Multi Physical Chemical Profiler (MPCP).

The VIP probe is the first commercially available instrument (Idronaut, Italy) developed by Tercier and co-workers (Tercier et al., 1998) that combines trace metal speciation analysis of high sensitivity and resolution with automated in-situ operation and options for remote deployment. The VIP allows the long-term, real-time and simultaneous determination of a fraction of the conventional total dissolved trace metal, the so-called “dynamic fraction”, of several trace metals, directly in the water column, with no pretreatments. The dynamic fraction is defined as the sum of the free metal ions and the sufficiently labile (high dissociation rate) and mobile (high diffusion rate) inorganic and organic complexes of a few nanometers in size (Buffle and Tercier-Waeber, 2005). This fraction (equivalent to the so-called truly-dissolved metal fraction with size of few nanometers) is of great importance, as it represents the maximum concentration of metals potentially bioavailable. Indeed, if the metal mass transport in the external medium (e.g. the diffusion) is the rate limiting step, instead of the transport of metal through the biological membrane, the labile and mobile metal complexes contribute to the metal bioavailability (Buffle and Tercier-Waeber, 2005; Tercier-Waeber et al., 2012; Zhao et al., 2016). This is achieved with a specifically designed Gel-Integrated MicroElectrode (GIME sensor) that consists of an array of 100 interconnected Ir-based micro-disc electrodes, coated with an antifouling gel membrane of 300 µm thickness (for more details see Belmont-Hebert et al., 1998). The fouling material as well as the metal associated with biopolymers, macromolecules and inorganic and organic colloids of size > 35 nm are excluded from the gel and therefore cannot be determined by the VIP system (Buffle and Tercier-Waeber, 2005). The non-dynamic fraction of trace metals, hereafter operationally defined as the colloidal metal fraction, can be deduced from the difference between the total dissolved metal concentration and the dynamic concentration, as in Braungardt et al., 2011. This fraction includes strong inert organic complexes and labile, but non-mobile (size > few nm), metal complexes. A more sophisticated system, called Multi Physical Chemical Profiler (MPCP), has also been developed to extend the capability of the VIP to in situ monitoring of trace metal speciation (Tercier-Waeber et al., 2005). The MPCP allows the simultaneous in situ, autonomous monitoring and profiling (down to 150 m) of three major metal fractions: i) the free metal ion concentration, (i.e. the metal species related to biological uptake) by means of a particular sensor, the Complexing Gel Integrated Micro-Electrode (CGIME sensor), which is covered by a thin 3.5 µm layer of a Microchelex chelating resin which in turn is covered by a thick 300 µm antifouling agarose gel (details of CGIME measurement principles and preparation in Noel et al., 2006); ii) the dynamic metal species (metal species related to biological availability) by a GIME sensor; and iii) the total acid-extractable metal concentration by means of a GIME sensor coupled to a submersible flow-injection analysis (FIA) system (Tercier-Waeber et al., 2005, 2008). The MPCP is coupled with a CTD probe for the measurement of the master variables (pressure, temperature, pH, oxygen, conductivity, salinity, redox potential, turbidity and chlorophyll-a). Both the VIP and the MPCP have been successfully applied in several environments, i.e. freshwater (Tercier-Waeber et al., 1998, 2002, 2009; Tercier-Waeber and Buffle, 2000), groundwater, fjord water (Tercier-Waeber et al., 1999), macro-tidal estuaries (Tercier-Waeber et al., 2005; Braungardt et al., 2009) and coastal marine waters (Tercier-Waeber et al., 1999, 2005; Howell et al., 2003; Braungardt et al., 2009).

In the autumn of 2002, a joint oceanographic cruise was carried out along the Po river plume in order i) to evaluate the metal distribution between different fractions (free ion, dynamic, colloidal, dissolved and particulate fractions) and their relationship with the total content; ii) to assess the evolution of the different metal fractions during the estuarine mixing and in the water column; and iii) to evaluate the effect of the hydrological characteristics of the water column, as well as of phytoplankton, on metal speciation. Moreover, preliminary tests of the CGIME sensor on discrete samples were also carried out. As the validation of new technique measurements in complex media is not straightforward, hollow fiber permeation liquid membrane (HF-PLM) coupled to an inductively coupled plasma mass spectrometer (ICP-MS) were used in parallel to CGIME measurements for comparison purposes. This technique

allows the measurement of free metal ions and some lipophilic contribution (Parthasarathy et al., 1997). At present, the data set monitored in situ and presented here is the first for the Adriatic Sea.

2.2 Materials and Methods

2.1.2.1 Laboratory, apparatus and reagents

A clean room laboratory ISO 14644-1 Class 6 (or US Fed. Std. 209e Class 1000), with areas at ISO Class 5 (or US F. S. 209e Class 100) under laminar flow cabinets, was available for trace metal clean working conditions. A portable laminar flow hood was available on board for sample treatments and analyses carried out on-site. Clean room garments, masks and gloves were worn by the personnel, who strictly followed clean room procedures during the most critical analytical steps.

Seawater sampling bottles were 8-L and 10-L GO-FLO from General Oceanics (Florida, USA). CTD probes, mod. Ocean Seven 316 CTD and Ocean Seven 301 were from Idronaut (Milan, Italy). The Handheld Salinity, Conductivity and Temperature System, Mod. 30, was from YSI (Yellow Springs, OH, USA). The Portable Turbidimeter Model 966 was from Orbeco-Hellige (New York, USA).

Filtration systems were the Sulfoflo from Nalgene (Rochester, New York) equipped with 0.45 μm pore size membrane filters (cellulose mixed esters \varnothing 47 mm, Schleicher & Schuell, Dassel, Germany) and the glassware apparatus with pre-weighted filter membranes. The UV digestion apparatus was the Mod. 705 UV-digester from Metrohm, (Herisau, Switzerland), equipped with twelve 12-mL quartz vessels and a 500 W mercury lamp. The Mod. 290A pH-meter, equipped with an Orion epoxy pH electrode Sure-Flow (Mod. 9165BN) was from Orion (Beverly, MA, USA). For phytoplankton analyses, the inverted microscope, Mod. Axiovert 135, and the epifluorescence microscope Mod. Axioplan were from Zeiss (Milan, Italy). For nutrients, the Technicon Autoanalyzer Traacs 800 system was from Seal Analytical (Norderstedt, Germany). Plastic containers were of low-density polyethylene material (Kartell, Italy). The polyethylene bottles, the quartz vessels, the sampling equipment, the filtration apparatus and all other plastic containers were decontaminated following the procedure reported elsewhere (Illuminati et al., 2015). Details of the decontamination procedure are briefly described in Supporting Information, text S1.

The instrumentation for metal speciation consisted of (i) two Metrohm systems (Herisau, Switzerland), comprised of a 746 VA Trace Analyser and a 747 VA Stand, each one equipped with a Teflon PFA (perfluoroalkoxy copolymer) cell and a three-electrode system, which includes an epoxy-impregnated graphite rotating disk working electrode (as a support for the thin mercury film electrode, TMFE), a Ag/AgCl, KCl 3 mol L⁻¹ reference electrode (to which all potentials are referred throughout) and a glassy carbon rod counter electrode; (ii) five Voltammetric In-situ Profiling (VIP) systems (Idronaut, Italy), each one equipped with a pressure compensated mini flow-through Plexiglas voltammetric cell comprising a GIME sensor, an Ag/AgCl, KCl saturated gel reference electrode and a built-in platinum ring auxiliary electrode (Tercier-Waeber et al., 2002, 2008); (iii) one MPCP equipped with two VIP systems and one submersible Flow-Injection Analysis (FIA) system; (iv) one Amel potentiostat equipped with a Metrohm cell based on a three-electrode configuration which includes a CGIME sensor, an Ag/AgCl/KCl_(saturated) reference electrode and a platinum rod counter electrode; and (v) one Hollow Fiber Permeation Liquid Membrane (HF-PLM) device coupled to an ICP-MS. Transferpette variable volume micropipettes from Brand (Wertheim, Germany) and neutral tips were used.

Ultrapure water was Milli-Q from Millipore (Bedford, MA, USA). Ultrapure HCl (34.5%), HNO₃ (70%), H₂O₂ (ca. 30%), and superpure HClO₄ (65%) were from Romil (Cambridge, England; UpA grade). Superpure KCl, Hg(CH₃COO)₂, hexadistilled mercury, KSCN and NaNO₃ were from Merck (Darmstadt, Germany). 1.5% LGL agarose gel was from Biofinex (Neuchâtel, Switzerland). Atomic absorption standards of Cd (II), Pb (II) and Cu (II) were from Carlo Erba (Milan, Italy). Research-grade nitrogen, purity \geq 99.999%, was from Sol (Monza, Italy). The estuarine water reference material SLEW-3, the nearshore-seawater reference material CASS-4, the seawater reference material NASS-5 for trace metals were from the National Research Council of Canada.

2.2.2.2 Study area

The Adriatic Sea can be divided into three regional basins (North, Central and South), differing in bathymetry, hydrology, morphology and biogeochemical features (Russo and Artegiani, 1996). The northern basin, with a width of about 75 nautical miles and a mean depth of 30 m (maximum depth about 70 m), is the shallower part of the Adriatic epicontinental shelf. It is characterized by large inputs of freshwaters from several rivers mainly concentrated along the northern and the northwestern coast of Italy. The Po River is Italy's largest river (with a length of 673 km and drainage basin of 71,000 km²) and it flows through one of the most industrialized regions of the country. It has an annual outflow rate of 1500–1700 m³ s⁻¹, accounting for about one third of the total riverine freshwater input into the Adriatic Sea (Marini et al., 2010). Discharge peaks of about 2000 m³ s⁻¹ are generally observed in spring (May–June) following the snow melting, and in autumn (October–November) when intense rainfall contributes to Po river discharge (Boldrin et al., 2005; Marini et al., 2008).

The oceanographic cruise in the Northern Adriatic Sea was carried out on board of *G. Dallaporta* vessel (CNR, Ancona, Italy) from October 27th to November 4th 2002, along a transect of six stations from the Po mouth toward the open sea, in direction East - South East (Fig. 1). About five stations were located in the plume while the remaining one was outside. For a better representation of data, the study area was divided into three different zones. According to Falcieri et al., 2014 (who carried out a 8-year simulation of the Po plume mean spatial variability based on data of temperature, salinity and currents collected during September 2002 as initial conditions), we marked the

limit for the front between the plume and the sea water at the surface salinity of ~ 36 . Here, we called “pro-delta area” the first segment of the transect, which was 10-km away from the Po mouth. This segment was characterized by high fluctuations of salinity (from ~ 18 to ~ 29) and it included Stns. 1 and 2. The second segment, called “frontal area”, extended from 10 km to ~ 40 km from the Po mouth. It was characterized by more stable salinity values (from ~ 33 to ~ 36) and it includes Stns. from 3 to 5. Finally, the third segment was called “marine area”, which included Stn. 6 and it was characterized by marine water masses not influenced by the Po River (Fig. 1). Worthy of note is that Stn. 5 had a surface salinity around 36 hence it should be part of the marine area. Nevertheless, it was included in the second segment of the transect because at this station most of all the variables studied had a behaviour comparable to the frontal area instead of the marine one.

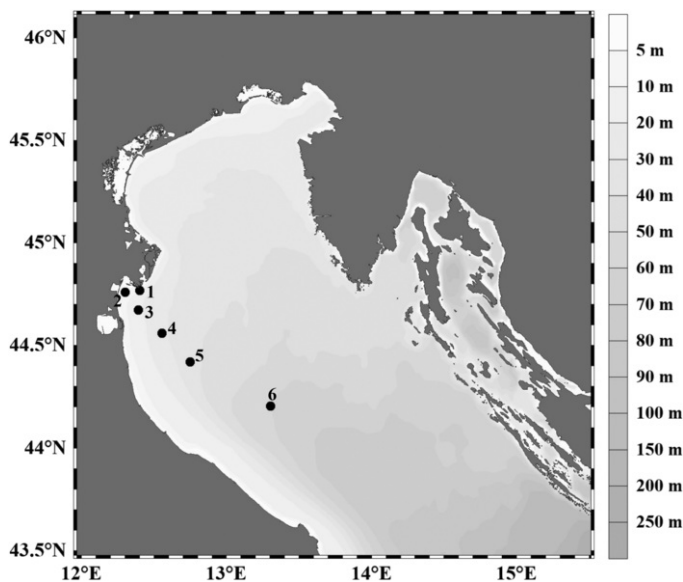


Fig. 1 Study area and location of the sampling stations from the Po mouth to the open sea.

alt-text: Fig. 1

2.3.2.3 Sample collection and treatments

During the oceanographic cruise, two kinds of speciation were carried out. The first type dealt with the in situ-based speciation using several VIPs and one MPCP (equipped with two VIPs and one GIME-FIA system) for the determination of the dynamic (Me_{dyn}) and the total acid-extractable ($Me_{ac,extr}$) fractions of Cd, Pb and Cu in the water column. The second type regarded the on board/laboratory-based speciation carried out on discrete samples collected at the same depths of the VIP and MPCP deployment, by means of the conventional Metrohm instrumentations for the total and dissolved metal concentration measurements. Discrete samples were also collected for immediate on-board analysis of both dynamic and total extractable fractions on surface samples, since the VIP and MPCP probes, due to their manufacture characteristics, cannot be used in depths < 1 m (Tercier et al., 1998; Tercier-Waeber et al., 2005).

Surface samples were collected manually in polyethylene bottles by a rubber dinghy at ~ 1 km from the oceanographic vessel. Immediately after collection, the samples were divided in various aliquots that were subjected to different treatments in order to obtain the different metal fractions. The first was acidified (raw sample) with ultrapure HCl (2:1000, pH ~ 2) for the determination of the total metal concentration (Me_{tot}). An aliquot was filtered through 0.45- μ m pore-size membrane filter and then acidified with ultrapure HCl (2:1000, pH ~ 2) for the determination of the dissolved metal contents (Me_{diss}). Before analysis, all the acidified samples (both raw and filtered) were subjected to photo-oxidative digestion, by UV irradiation for 12 h after the addition of 1:1000 ultrapure H_2O_2 , in order to destroy organic matter, to leach bound metals, and to avoid possible adverse effects on the voltammetric determination (Kolb et al., 1992). Total particulate metal concentration (Me_{part}) was obtained by the difference between the total and the dissolved concentrations, while the metal concentration in the colloidal fraction (Me_{coll}) was computed as the difference between the dynamic and the total dissolved concentration.

Aliquots of seawater samples were acidified with ultrapure HCl (2:1000, pH ~ 2) and then analysed on board without UV digestion for the determination of the total HCl-extractable metal fraction to compare it with that measured by the GIME-FIA system. Other seawater aliquots were used, without any treatments, for the determination on board of the free metal ion concentration (Me_{free}) using both CGIME sensor and the PLM technique. Finally,

other seawater aliquots were used to measure turbidity, DOC and nutrients.

2.4.2.4 Metal speciation methodology

Cd, Pb and Cu were determined simultaneously using background subtractive Square-Wave Anodic Stripping Voltammetry (SWASV) (Tercier-Waeber et al. 1998) implemented both on conventional laboratory instrumentation and submersible voltammetric probes for the in-situ measurements. As stated in Buffle and Tercier-Waeber (2005), the word “conventional” is used to designate well-established techniques and instrumentations, in contrast to the recent developments.

2.4.1.2.4.1 In situ-based speciation

The dynamic fraction and the in-situ total acid extractable concentration of Cd, Pb and Cu were determined by using the VIP and the MPCP systems. Analytical procedure for preparation of the GIME sensors and instrumental parameters of the voltammetric analysis are the same as those reported in Braungardt et al., 2009 and Tercier-Waeber et al., 2002, 2005.

The quantification of both the dynamic and the total extractable concentration was obtained using the calibration curve method, in N₂ degassed 0.1 mol L⁻¹ NaNO₃ standard solutions spiked with various concentrations of Cd, Pb and Cu. The resulting slopes were normalized (Tercier-Waeber et al., 1999) after: (1) smoothing of the currents (using Golay-Savitzky moving average); (2) subtraction of the background current from the current signal; (3) peak height quantification; and, where necessary, (4) temperature effect correction on peak current measured in situ (Tercier-Waeber et al., 1999; Howell et al., 2003). The calibration procedure was performed before and at the end of the oceanographic campaign, while the validity of slopes was tested during the cruise, on board, by analysing a N₂-degassed standard solution previously used in the calibration curve procedure.

2.4.2.2.4.2 On board/laboratory-based speciation

~~2.4.2.1.2.4.2.1 (Is it possible to delete the number of hierarchy? as in the sent paper? If not, the hierachy is correct.) (delete number)~~ **Metrohm system** Total and dissolved metal fractions were determined in a land-based laboratory (within 1–3 months after the sample collection) by conventional voltammetric instrumentation. The procedure involved the pouring of a 10-mL UV-digested sample aliquot into a pre-cleaned voltammetric cell, where the thin mercury film electrode (TMFE) was already prepared and tested (Annibaldi et al., 2007; Illuminati et al., 2013, 2015). The Hg(NO₃)₂ solution (2.5 × 10⁻² mol L⁻¹) for the TMFE was obtained by oxidation of hexadistilled mercury with ultrapure nitric acid. The voltammetric analyses were carried out in the background subtraction technique, using the following instrumental parameters: $E_{\text{dep}} = -975 \text{ mV}$; $t_{\text{dep}} = 3$ to 10 min (as a function of the metal concentrations, during in situ deployments); $E_{\text{fin}} = 0 \text{ mV}$; $E_{\text{equil}} = -975 \text{ mV}$; $t_{\text{equil}} = 7.5 \text{ s}$; $E_{\text{precl}} = -50 \text{ mV}$; $t_{\text{precl}} = 5 \text{ min}$; $E_{\text{SW}} = 25 \text{ mV}$; $f = 100 \text{ Hz}$; $\Delta E_{\text{step}} = 8 \text{ mV}$; $t_{\text{step}} = 150 \text{ ms}$. Two-three replicates were carried out with the sample in the cell, after which quantification was obtained using the multiple standard addition method.

~~2.4.2.2.2.4.2.2 (delete number hierarchy)~~ **CGIME sensor** Preliminary tests for the determination of the free metal ion concentration with the complexing gel integrated microelectrode (CGIME) were carried out on discrete samples on board, by using a standard potentiostat with a three-electrode cell. Preparation of the CGIME sensor was as reported previously (Noel et al., 2006; Tercier-Waeber et al., 2005). The measurement of free metal ion concentrations was performed in two steps: (1) equilibration of the sensor with the sample to accumulate trace metals on the Microchelex resin in proportion to free metal ion concentrations in seawater; (2) transfer of the CGIME sensor in a glass cell filled with an acid solution (0.1 mol L⁻¹ NaNO₃ + 0.1 mol L⁻¹ superpure HNO₃). Here, the accumulated metals were released by acid and then immediately detected by SWASV using the following instrumental parameters (Noel et al., 2006; Tercier-Waeber et al., 2005): accumulation time = 1 to 2 h with a renewal of the sample in the cell every 15 min; $E_{\text{dep}} = -900 \text{ mV}$; $t_{\text{dep}} = 10$ to 30 min; $E_{\text{fin}} = +100 \text{ mV}$; $E_{\text{SW}} = 25 \text{ mV}$; $\Delta E_{\text{step}} = 8 \text{ mV}$; $f = 50 \text{ Hz}$.

~~2.4.2.3.2.4.2.3 (Delete number)~~ **HF-PLM technique** Hollow Fiber Permeation Liquid Membrane (HF-PLM) coupled to ICP-MS detection was used for laboratory measurements performed on some discrete samples for comparison purposes with the CGIME measurements of the free metal ion concentrations. The analytical procedure used for the HF-PLM preparation and measurements included different steps (Parthasarathy et al., 1997, 2001): (1) preparation of the hollow fiber which was a loosely, coil, single polypropylene hollow fiber with an inner diameter of 600 μm; (2) impregnating of hollow fiber with the organic metal carrier (0.1 mol L⁻¹ 1,10-didecyl diaza 18-crown-6 and 0.1 mol L⁻¹ lauric acid dissolved in a mixture of phenylhexane and toluene); (3) metal separation and pre-concentration, in which the impregnated hollow fiber membrane was immersed in the sample solution placed in a plastic beaker and held vertical by means of a clamp; (4) filling of the lumen side of the hollow fiber with 5 × 10⁻⁴ mol L⁻¹ CDTA (trans-cyclohexanediamine tetra-acetic acid) strip solution adjusted to pH 6.4 with NaOH; (5) collection of the strip solution by pushing the solution by means of a peristaltic pump; (6) laboratory metal determination by ICP-MS in both the sample and strip solutions.

2.5.2.5 Accuracy tests

To ascertain accuracy and to assure comparability of data produced during the cruise, analytical quality control of Cd, Pb and Cu measurements were carried out by analysing several certified reference materials (the SLEW-3 for estuarine water, the CASS-4 for nearshore seawater, and the NASS-5 for open seawater) with all the instrumentations available. Certified reference materials were UV irradiated prior to analysis, as for the seawater samples collected.

The results of the systematic measurements carried out on reference materials during the entire period of work, using all the instrumentations available on board and in laboratory, gave ($n = 5-7$) mean values (\pm SD) of SLEW-3, Cd $0.43 \pm 0.01 \text{ nmol L}^{-1}$; Pb $0.046 \pm 0.010 \text{ nmol L}^{-1}$; Cu $20 \pm 2 \text{ nmol L}^{-1}$; CASS-4, Cd $0.24 \pm 0.02 \text{ nmol L}^{-1}$; Pb $0.045 \pm 0.008 \text{ nmol L}^{-1}$; Cu $9.3 \pm 0.8 \text{ nmol L}^{-1}$; NASS-5 Cd, $0.21 \pm 0.02 \text{ nmol L}^{-1}$; Pb $0.038 \pm 0.006 \text{ nmol L}^{-1}$; Cu $4.6 \pm 0.3 \text{ nmol L}^{-1}$, against certified mean values (\pm 95% confidence interval) of SLEW-3 Cd $0.43 \pm 0.04 \text{ nmol L}^{-1}$; Pb $0.043 \pm 0.007 \text{ nmol L}^{-1}$; Cu $24 \pm 2 \text{ nmol L}^{-1}$; CASS-4 Cd $0.23 \pm 0.03 \text{ nmol L}^{-1}$; Pb $0.047 \pm 0.017 \text{ nmol L}^{-1}$; Cu $9.3 \pm 0.9 \text{ nmol L}^{-1}$; NASS-5 Cd $0.20 \pm 0.03 \text{ nmol L}^{-1}$; Pb $0.040 \pm 0.003 \text{ nmol L}^{-1}$; Cu $4.7 \pm 0.7 \text{ nmol L}^{-1}$. Results were in good agreement with certified reference values within the experimental errors, showing a good accuracy for all the measurements.

2.6.2.6 Inter-comparison exercises

During the cruise, laboratory and field-based inter-comparison exercises of all the analytical techniques available were carried out in order to validate the developed analytical tools with common analytical procedures. Three principal types of field-based inter-comparisons were carried out: (i) the comparison between the different dynamic fractions determined by the GIME sensors available on board; (ii) the comparison between the total and the total HCl-extractable concentrations measured by conventional instrumentations, on one side, and the in-situ total acid extractable concentration measured by the GIME-FIA system, on the other; and (iii) the comparison between the free metal ion concentrations determined by the CGIME sensor and the HF-PLM technique. Some results of the inter-comparison exercises were reported in Braungardt et al. (2009) and Tercier-Waeber et al. (2005). Braungardt et al. (2009) compared the metal dynamic concentrations measured at 5-m depth of the first two stations of the study transect. Tercier-Waeber et al. (2005) compared the results obtained for i) total extractable metal and total dissolved concentration detected, respectively, by the in-situ application of the GIME-FIA and the conventional Metrohm instrumentation, and ii) the free metal ion concentrations monitored on-board by the CGIME sensor and the HF-PLM technique. Here we extended the comparison between the metal fractions monitored by voltammetry with the various instrumentations to all the samples collected during the cruise. Results are fully reported in the Supporting Information (Text S2; Tables S1 and S2).

As shown in Table S1, a general good agreement was observed between the dynamic fraction data measured by different VIPs available on board. Cd dynamic fraction determined by the VIP-A represented the only exception, showing at the surface very high values, probably related to laboratory contamination. Concerning the total concentrations (see Table S2 in the Supporting Information), although some values are high due to possible contamination problems, the in-situ total extractable fraction measured by the GIME-FIA procedure was often consistent with the total metal concentration for Pb. On the contrary, the in-situ total extractable fraction of Cu was comparable to the total acid-extractable fraction measured by conventional Metrohm instrumentation. The few data of GIME-FIA available for Cd were higher than the total concentrations, probably due to contamination problems.

The very few data obtained for the free metal ion concentration by the CGIME sensor (values available only for Cu) seemed to be in agreement with values measured by the HF-PLM technique.

2.7.2.7 Ancillary measurements

At each station of the 2002-Adriatic cruise transect, continuous water column profiles (vertical resolution of 0.5 m), were sampled for the master hydrographic variables (i.e. temperature, salinity, dissolved oxygen, oxygen saturation, pH, redox potential) using a CTD probe fitted with a fluorometer for the chlorophyll-a vertical profile.

Temperature and salinity data obtained from the CTD measurements were used to calculate the density (ρ), and the water column stability. The latter is proportional to the Brunt-Värsälä buoyancy frequency $N^2(z)$, which represents the strength of density stratification (Agusti and Duarte, 1999). The depth at which the Brunt-Värsälä buoyancy frequency was estimated to be maximal, which also corresponds approximately to the middle of the pycnocline, represents the Upper Mixed Layer (UML). The value of $N^2(z)$ was also taken as a stability index of the water column (i.e. what is commonly called pycnocline strength).

At each station, several discrete samples were also collected at different depths for the determination of turbidity, dissolved organic carbon (DOC), dissolved inorganic nutrients (nitrate- NO_3^- , nitrite- NO_2^- , ammonium- NH_4^+ , orthophosphate- PO_4^{3-} and orthosilicate- $\text{Si}(\text{OH})_4$), and phytoplankton abundance and composition. Nutrient concentrations were measured following modified procedures developed by Strickland and Parsons (1972). Detailed description of the nutrient analysis is reported elsewhere (Campanelli et al., 2012; Marini et al., 2008). Total dissolved inorganic nitrogen (DIN) was calculated as the sum of the NO_3^- , NO_2^- and NH_4^+ concentrations.

The analysis of phytoplankton was carried out following the Utermöhl method (Elder and Elbrachter, 2010). Immediately after collection, seawater samples were preserved in dark glass bottles by adding 0.8% formaldehyde neutralized with hexamethylenetetramine and stored at 4 °C. In laboratory, 40-100 mL sub-samples were homogenized, settled in a cylinder-chamber complex and then observed using the inverted microscope. During the counting procedure, 30 random fields were examined at 400-x magnification. All phytoplankton cells larger than 2 μm were identified and counted. Then the entire chamber was observed at 200-x to assess the larger and less frequent organisms. The bio-volume was measured to evaluate the biomass (expressed in $\mu\text{g C L}^{-1}$) following Menden-Deuer and Lessard (2000).

2.8.2.8 Statistical analysis

Countered sections are plotted using master variable and nutrient data that were gridded using the Data-interpolating Variational Analysis (DiVA) coupled to the software Ocean Data View 4.7.10 (Schiltzer, 2017).

Experimental data were elaborated by principal component analysis (PCA), which was carried out on standardized data; significant components were obtained through the Wold cross-validation procedure (Wold, 1978). The 5 m samples of Stns. 1 and 2 were excluded from the dataset because only the dynamic fractions were determined at this depth. Only three depths of Stn. 6 (0.2 m, 5 m and 25 m) were considered, since metal dynamic fractions were measured only at those depths. The high (probably contaminated) values of dynamic Cd were substituted by the HCl-extractable dissolved fraction (obtained by filtering seawater samples on 0.45 μm pore membranes, acidifying with superpure HCl 2:1000 and analysing without UV pre-treatment) which was very close to the Cd dynamic fraction (see data in Supplementary Information, Table S3), and when this fraction was not available (we determined HCl-extractable dissolved fraction only in surface samples), the dissolved Cd values (10- and 15-m depth values of Stn. 3) were used. The free metal ion concentrations were also excluded because of the very few and sporadic results obtained. The statistical analysis and the PCA were carried out using Statistica package (StatSoft; vers. 8.0) and the differences were deemed statistically significant at $p_i < 0.05$.

3.3 Results

3.1.3.1 Hydrography, dissolved nutrients and phytoplankton

Data on the master hydrographic variables, dissolved organic carbon (DOC), dissolved nutrients and phytoplankton obtained during the cruise in the 2002-Adriatic Sea cruise are shown in Figures 2-4, S1 and Table S4.

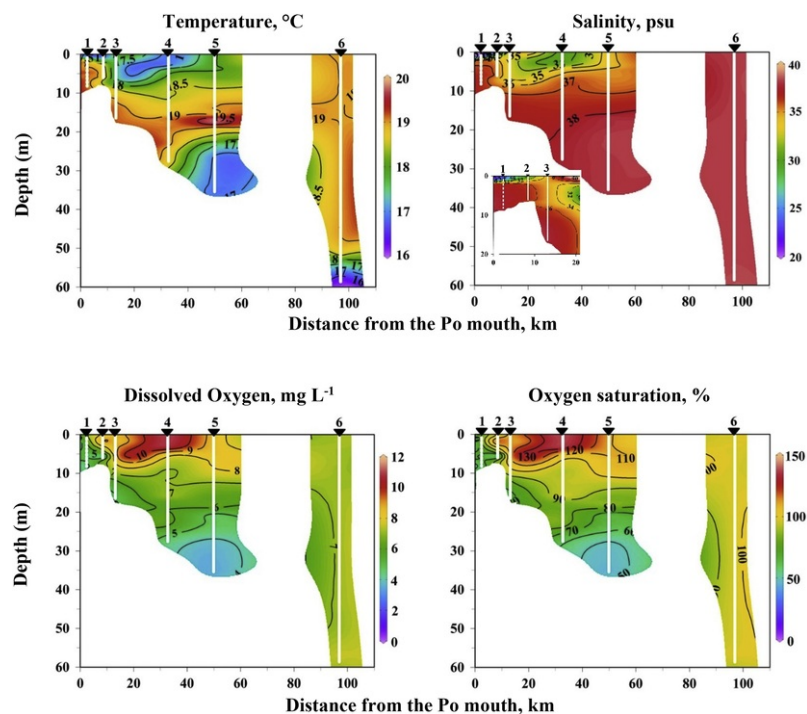


Fig. 2 Contour plots of temperature, salinity, dissolved oxygen concentration, and oxygen saturation percentage during the 2002-Adriatic Sea cruise. The white dots represent the sampling points. The transect position is plotted in Fig. 1.

alt-text: Fig. 2

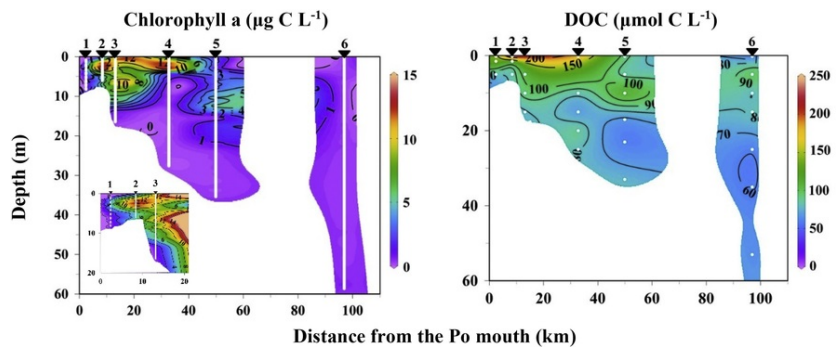


Fig. 3 Contour plots of chlorophyll-a and DOC during the 2002-Adriatic cruise. The white dots represent the sampling points. The transect position is plotted in Fig. 1.

alt-text: Fig. 3

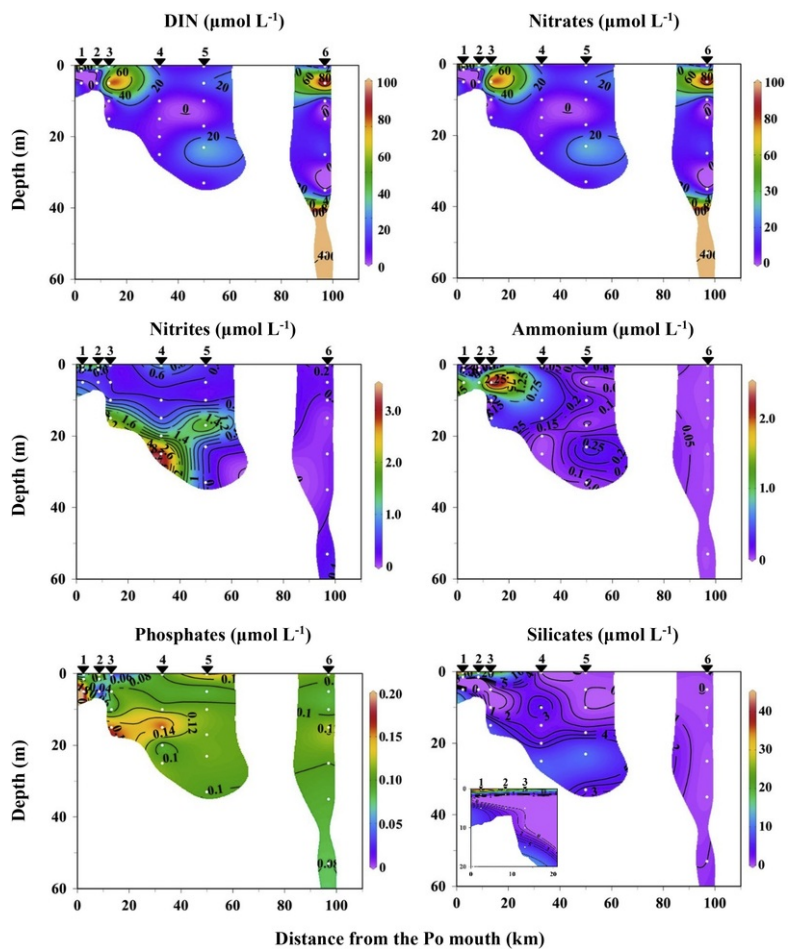


Fig. 4 Contour plots of DIN, nitrates, nitrites, ammonium, phosphates, and silicates during the 2002-Adriatic cruise. The white dots represent the sampling points. The transect position is plotted in Fig. 1.

Temperature and salinity varied from 16.28°C to 19.74°C and from 25.50 to 38.90, respectively (Fig. 2). Density anomalies varied from 18.12 kg m⁻³ to 28.66 kg m⁻³ (Fig. S1). Temperature varied within a narrow range (up to 18% variability), thus density variations were essentially due to salinity variations (also confirmed by the high positive correlation coefficient, $r=0.9798$, Table S4). Within the Po River plume, a stratification of the water column was observed with a very shallow UML (up to 5-m depth) and with a sharp salinity gradient. At this depth, the pycnocline showed a high strength ($N^2(z) = 557 \times 10^{-4} \text{ s}^{-2}$), which progressively decreased seawards. In the marine area (Stn. 6), the water column became quite homogeneous, the UML extended deeper (~50 m), the halocline disappeared, while the thermocline still persisted and was responsible for a very weak pycnocline ($N^2(z) = 15 \times 10^{-4} \text{ s}^{-2}$) (Fig. 2).

Dissolved oxygen showed concentrations in the range 3.5–11 mg L⁻¹, slightly over saturation levels (100%) with percentages ranging between 100 and 138% within the stratified layer (except for Stn.1, Fig. 2). Then, it gradually decreased down to the bottom reaching saturation values of ~60%. Values slightly lower than 50%, indicative of hypoxia, were observed only at Stn. 5 close to the bottom, while in the marine area, dissolved oxygen showed a homogenous vertical distribution and well-oxygenated waters (~100% of saturation). Along the longitudinal profile, dissolved oxygen values (as well as saturation percentages) showed a surface maximum at Stn. 4, after which it decreased seawards to values very close to those of the pro-delta stations.

The pH ranged from 7.94 to 8.39 and showed a longitudinal profile similar to that observed for dissolved oxygen (Fig. 2). In the pro-delta area, the pH slightly decreased with depth, except in Stn.1 where it showed an opposite trend in the water column. In the frontal and marine areas, the pH remained almost constant within the mixed layer, and then it sharply decreased with depth, even though at Stn. 6 this decrease was less remarkable (Fig. S1).

Turbidity was high (~16 NTU) near the Po mouth, but sharply decreased seawards reaching values close to the detection limit of the instrument. In all the stations studied, the turbidity showed a similar trend in the water column; it decreased with depth, but proceeding seawards, higher values (sometimes 2–3 times higher than the surface) were recorded in the proximity of the bottom (Fig. S1).

Chlorophyll-a (Chl-a) concentration measured in situ by the CTD probe ranged between 0.3 and 13.3 mg L⁻¹ and sharply decreased seawards. A clearly defined deep chlorophyll maximum (DCM) of ~10 mg m⁻³ was observed, closely following the pycnocline. In the marine area, Chl-a vertical distribution was homogeneous with very low values, ~0.3 mg L⁻¹ (Fig. 3).

The total phytoplankton abundance ranged between $8.3 \times 10^6 \text{ cell L}^{-1}$ and $3.4 \times 10^5 \text{ cell L}^{-1}$ (Table S4). Spatial distribution of micro-phytoplankton showed a general seaward-decreasing trend, even if a peak (principally due to diatoms) of abundance was observed in the frontal area, with densities similar to those of the stations closer to the Po mouth. The most abundant groups were represented by diatoms and phytoflagellates (ranging from 0.014 to $4.8 \times 10^6 \text{ cells L}^{-1}$ and from 0.25 to $3.8 \times 10^6 \text{ cells L}^{-1}$, respectively, Table S4) in agreement with several previous studies on phytoplankton composition in the northern Adriatic Sea (Totti et al., 2005).

DOC concentrations decreased along the Po plume, with values that ranged between ~140 μmol L⁻¹ at the Po mouth and ~60 μmol L⁻¹ in open sea, with a variation of ~60% (Fig. 3). This horizontal decreasing trend of DOC was also observed in the water column, with bottom values that were 40–60% lower than those of the surface. This distribution changed in the marine area, where after a slightly increase within the first 15-m depth, it was quite homogeneous down to the bottom.

DIN (ranging from ~1 μmol L⁻¹ to ~90 μmol L⁻¹) and orthosilicates Si(OH)₄ (ranging from 0.02 to 42 μmol L⁻¹), showed a decreasing trend (~98%) seawards (Fig. 4). DIN was represented for the most part by nitrates (60–100% of the total). A very small fraction (ranging from 0.1% to ~15%) of the DIN was represented by ammonium, while nitrites represented a more remarkable quote (1–40% of the total). Both DIN and orthosilicates were characterized by a general drawdown of concentrations within the stratified layer. This decreasing trend was more marked in the stations closer to the Po, because of the river influence. Afterward, concentrations remained almost constant with depth, while at the bottom they arose again (the anomalous high seafloor value of nitrates at Stn. 6 was probably due to contamination problems, and thus, it was not considered in the computation of DIN) (Fig. 4).

Orthophosphates were generally low, with values ranging from 0.04 μmol L⁻¹ to ~0.12 μmol L⁻¹. They showed an opposite trend with respect to nitrogen species and silicates, with concentrations increasing by ~50% along the transect (Fig. 4). Phosphate distribution in the water column varied greatly from station to station. In Stns. 1 and 3 it increased gradually with depth, while at Stn. 2 a gradual decrease was observed. From Stn. 4 to the open sea, phosphates showed a maximum at the pycnocline; afterwards they slightly decreased down to the bottom.

3.2.3.2 Metal speciation

The distribution of the different metal fractions along the study transect is reported in Figures 5–8, divided for each metal and for each sampling station. Total, dissolved, particulate, dynamic and colloidal concentrations are given for each element. Also, free-ion metal concentrations are discussed, even if these data are reported only in Table S2, due to the paucity of the values measured. Data on dynamic fraction are the mean of measurements carried out by each of the VIP systems available on board with the exception of contaminated samples. It is to be noted that preliminary results on free and dynamic metal ion concentrations have been previously reported in Terrier-Waeber et al. (2005). Here, we provide a more exhaustive description, which includes such data presented previously, as well as the comparison with the other metal fractions and master variables. The metal concentrations here measured are also compared to literature data obtained from several estuaries, deltas, coastal and oceanic waters, worldwide (Table 1).

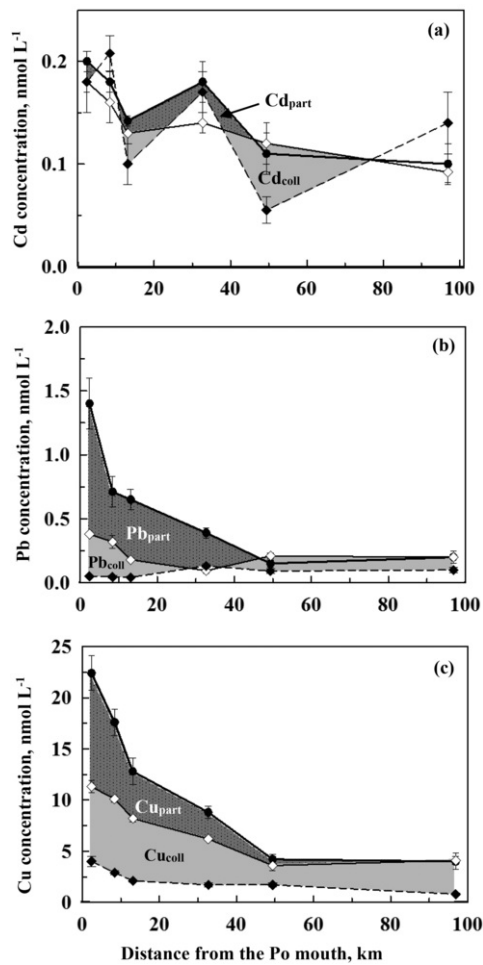


Fig. 5 Horizontal profiles of Cd (a), Pb (b), and Cu (c) speciation during the 2002-Adriatic cruise. The figures report the total concentration (—●—), the dissolved (---◇---) and the dynamic (---◆---) fraction distribution in the water column, for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations.

alt-text: Fig. 5

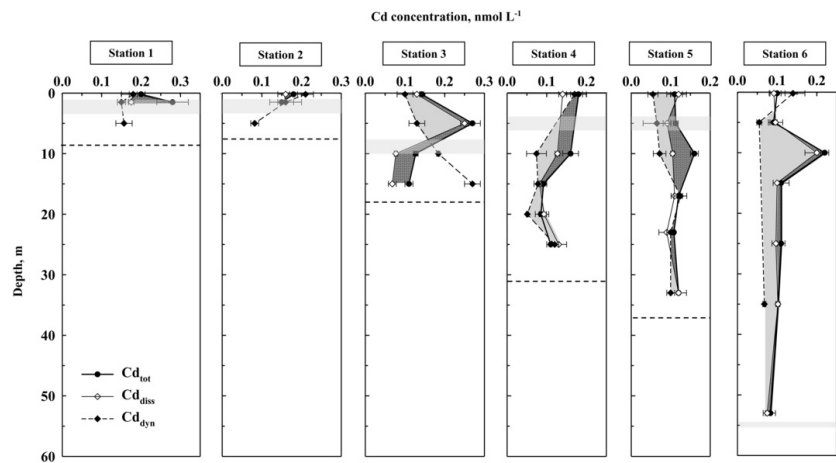





Fig. 6 Vertical profiles of Cd speciation during the 2022-Adriatic cruise. The figure reports the total concentration (), the dissolved () and the dynamic () fraction distribution in the water column for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations. The transparent grey stripes represent the pycnocline zones.

alt-text: Fig. 6

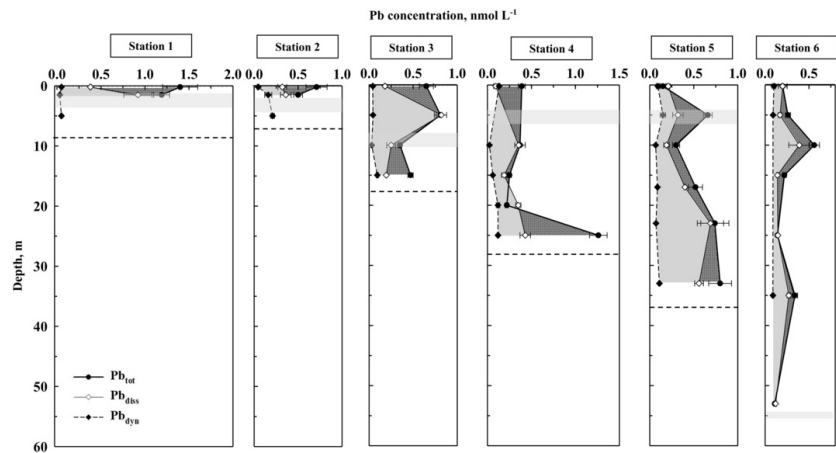





Fig. 7 Vertical profiles of Pb speciation during the 2022-Adriatic cruise. The figure reports the total concentration (), the dissolved () and the dynamic () fraction distribution in the water column for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations. The transparent grey stripes represent the pycnocline zones.

alt-text: Fig. 7

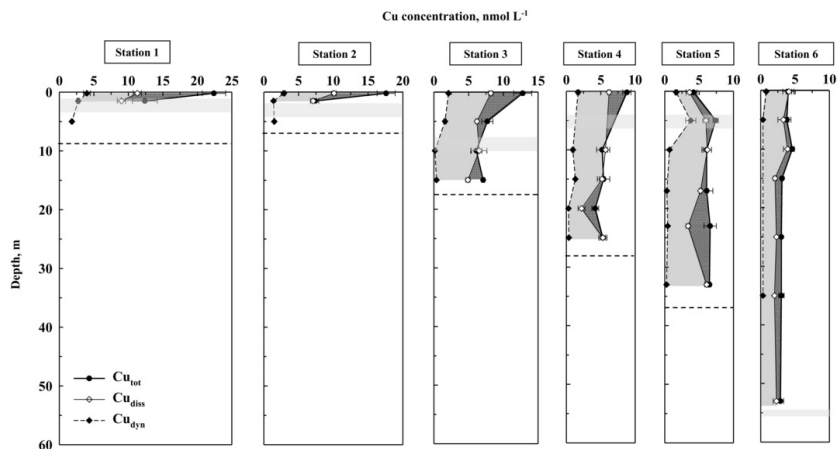


Fig. 8 Vertical profiles of Cu speciation during the 2002-Adriatic cruise. The figure reports the total concentration (—●—), the dissolved (---○---) and the dynamic (---◆---) fraction distribution in the water column for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations. The transparent grey stripes represent the pycnocline zones.

alt-text: Fig. 8

Table 1 Concentrations of trace metals along the Po River plume, selected Mediterranean rivers and open Mediterranean Sea.

alt-text: Table 1

| Site | Dissolved and (total) concentrations (nmol L ⁻¹) | | | Reference |
|--------------------------|--|---------------------------|-----------------------|---------------------------|
| | Cd | Pb | Cu | |
| Po plume 2002 | 0.12 ± 0.04 (0.14 ± 0.05) | 0.33 ± 0.21 (0.52 ± 0.35) | 5.3 ± 2.5 (7.1 ± 4.6) | This study |
| Po river | | | | (Tankere et al., 2000) |
| Summer 1994 | | 1.2 ± 0.92 | 41 ± 20 | |
| Winter 1995 | | 1.5 ± 0.48 | 57 ± 18 | |
| Po river 1991 | 0.58 | 0.72 | 25.7 | (Dorten et al., 1991) |
| European estuaries | | | | |
| Marche estuaries (Italy) | 0.089–0.18 (0.18–0.36) | 0.34–0.72 (0.48–9.7) | 6.3–31(7.9–47) | (Annibaldi et al., 2015) |
| Arno (Italy) | 0.89 | 1.01 | 27.5 | (Dorten et al., 1991) |
| Krka (Croatia) | 0.015 (0.020) | 0.03(0.14) | 4.44 (6.51) | (Cindrić et al., 2015) |
| Aber-Wrac'h (France) | ~0–0.36 (~0–0.44) | | 6.3–9.4(6.3–14) | (L'Her Roux et al., 1998) |
| Gironde (France) | 0.04–0.84 | | 2.2–20 | (Michel et al., 2000) |
| Loire (France) | 0.08–0.29 | | 8.0–21 | (Waeles et al., 2004) |
| Rhone (France) | | 0.33 | 32.7 | (Ollivier et al., 2011) |
| Huveaunne (France) | 0.07 | 0.64 | 27 | (Oursel et al., 2013) |
| Jarret (France) | 0.07 | 0.37 | 28 | (Oursel et al., 2013) |

| | | | | |
|---------------------------------|-------------|-------------|-----------|----------------------------------|
| Ebro (Spain) | 1.07 | 0.75 | 15.3 | (Dorten et al., 1991) |
| Severn (UK) | 0.98–3.6 | ~1.9 | 63–79 | (Harper, 1991) |
| Tay (UK) | 0.11 | 0.65 | 13 | (Owens and Balls, 1997) |
| Scheldt (Netherlands) | 0.03–1.35 | 0.2–2.6 | 8–42 | (Baeyens et al., 2005) |
| World average river | 0.71 | 0.38 | 23 | (Gaillardet et al., 2003) |
| Northern Adriatic Sea 1994 | 0.083 | | 7.14 | (Tankere and Statham, 1996) |
| Northern Adriatic Sea, 1996 | | | 5.4 ± 2.5 | (Zago et al., 2002) |
| Northern Adriatic Sea, 1997 | | | 6.4 ± 2.8 | (Zago et al., 2002) |
| Central Adriatic Sea, 2000–2004 | 0.14 ± 0.06 | 0.24 ± 0.14 | 7.1 ± 3.6 | (Annibaldi et al., 2009) |
| Southern Adriatic Sea 1994 | 0.076 | | 2.95 | (Tankere and Statham, 1996) |
| Ligurian Sea | | 0.12 ± 0.04 | | (Migon and Nicolas, 1998) |
| Mediterranean Sea | 0.062 | | 1.7 | (Tankere and Statham, 1996) |
| Worldwide oceans | | | | |
| Arctic Ocean | ~0.4 | | ~3.0 | (Aparicio-González et al., 2012) |
| Atlantic Ocean | ~0.1 | ~0.1 | ~1.5 | (Aparicio-González et al., 2012) |
| Indian Ocean | ~0.2 | ~0.04 | ~1.5 | (Aparicio-González et al., 2012) |
| Pacific Ocean | ~0.3 | ~0.04 | ~1.4 | (Aparicio-González et al., 2012) |
| Southern Ocean | ~0.6 | ~0.03 | ~2.0 | (Aparicio-González et al., 2012) |

3.2.1.3.2.1 Cadmium

The Cd content in the waters of the transect was very low with an overall mean (interquartile range) of 0.14 (0.11–0.16) nmol L⁻¹. Our values are lower than those recorded in previous surveys on the Po river and on other Italian rivers, while they are similar to Cd concentrations measured in the whole basin of the Adriatic Sea and in general in the Mediterranean Sea. Further, our values are in the same order of magnitude as those found elsewhere in European estuaries and, in general, as those measured in oceanic waters worldwide (Table 1).

A general longitudinal decreasing trend can be observed seaward for all the Cd fractions detected (Fig. 5a). Dissolved Cd (from 0.068 nmol L⁻¹ to 0.25 nmol L⁻¹) constituted the most important fraction of the total (concentrations ranging between 0.080 nmol L⁻¹ and 0.26 nmol L⁻¹). The only exception was present in the stations closer to the Po mouth where a more marked contribution by particulate fraction can be detected, though Cd_{diss} percentages still remained high (70–90% of the total). The vertical profile of dissolved Cd showed an almost homogeneous behaviour over the transect, with a maximum (excepting in the pro-delta area) at the DCM (5–10 m depth).

Apart from the high values at the surface of Stns. 2, 4 and 6 and at the bottom of Stn. 3, due to possible contamination problems, the Cd dynamic fraction accounted for ~40% to ~100% of Cd_{diss} (values ranging from 0.05 nmol L⁻¹ to 0.20 nmol L⁻¹). The percentage of this fraction with respect to the dissolved concentration was high (90–100%) in the pro-delta area, while it decreased seawards within the UML, reaching values about half those of Cd_{diss} (Fig. 6) in the open sea. Below the pycnocline, the concentration of Cd_{dyn} increased down to the seafloor, where its percentage was again 90–100% of Cd_{diss}. In the open sea, a quite homogeneous distribution of Cd_{dyn} with depth was observed, with a contribution to the dissolved fraction of about 60–70%. An opposite trend was observed for the colloidal Cd fraction that increased seaward, reaching values ~40–50% of Cd_{diss}, and sharply decreased with depth to values close to zero (Fig. 6).

Free Cd-ion [Cd²⁺] concentrations were found to be below the detection limit (~60 pmol L⁻¹), as reported in Noel et al., 2006) for both the CGIME sensor and the HF-PLM system (Table S-2).

3.2.2.3.2.2 Lead

Lead speciation changed greatly along the transect (Fig. 5b and Fig. 7). The total concentration of Pb was generally high at the Po river mouth (~1.4 nmol L⁻¹) and then decreased seaward, to reach asymptotically an open sea value of ~0.2 nmol L⁻¹, with an 85% decreasing trend. At the surface of the pro-delta and frontal areas, Pb content was dominated by the particulate fraction (accounting for ~70% of the total), since dissolved Pb showed low concentrations (~0.40 nmol L⁻¹). Pb_{diss} initially

decreased in the frontal area, passing from $\sim 0.4 \text{ nmol L}^{-1}$ at the Po River mouth to $\sim 0.09 \text{ nmol L}^{-1}$ up to $\sim 30 \text{ km}$ off shore from the river. Proceeding seaward, it increased both in absolute and in relative terms reaching values close to those of Pb_{tot} ($\sim 0.2 \text{ nmol L}^{-1}$). Therefore, Pb_{part} greatly decreased along the Po plume, reaching values close to zero in the marine area. Both the total and dissolved Pb concentrations were in good agreement with data reported in the literature for the same area and for other rivers worldwide (Table 1). Obviously, our data are higher than those referred to oceanic waters (sometimes about one order of magnitude) with the exception of the seawater end-member values, which fall within the same order of magnitude of literature data.

In the water column, Pb was mainly present in its dissolved form (70–100% of the total) which was also responsible for the maximum of Pb_{tot} at the DCM (5–10 m depths). The particulate fraction returned significant at the seafloor, where its contribution to the total increased up to $\sim 70\%$.

The dynamic fraction of Pb ranged between $\sim 0.02 \text{ nmol L}^{-1}$ and $\sim 0.2 \text{ nmol L}^{-1}$. In general, Pb_{dyn} represented a very small and constant fraction of Pb_{diss} ($\sim 15\text{--}20\%$) within the first 10 km far from the Po mouth, further offshore it greatly increased in both its concentration and percentage reaching values up to the 50% of the dissolved concentration at the surface. The contribution of Pb_{dyn} with respect to the dissolved concentrations increased with depth in the stations close to the Po mouth, while it decreased in the water column with the increasing distance from the river. Hence, the colloidal fraction of Pb was dominant ($\sim 80\%$ of the Pb_{diss}) in the pro-delta area and then decreased in the open sea to values half of those of Pb_{diss} (Fig. 7).

The free-ion Pb fraction (no CGIME data are available) showed very low concentration, varying from about 2 pmol L^{-1} near the Po river to $\sim 10 \text{ pmol L}^{-1}$ in the open sea (Table S-2). It represented a few percentage of the Pb_{diss} (from $\sim 1\%$ to $\sim 10\%$), but the contribution to Pb_{dyn} was much higher (from $\sim 5\%$ to $\sim 40\%$). Due to the little data available for this fraction, we are not able to describe in detail the behaviour of $[\text{Pb}^{2+}]$ in the study area. We can only observe an increasing trend of $[\text{Pb}^{2+}]$, both in absolute and in relative terms with the distance from the river and with depth.

3.2.3.3.2.3 Copper

Copper showed a marked decreasing trend for all the fractions along the study transect (Fig. 5c, 8).

Total Cu concentration varied from $\sim 3 \text{ nmol L}^{-1}$ to $\sim 20 \text{ nmol L}^{-1}$, and was mainly constituted of the dissolved fraction that ranged from 2 nmol L^{-1} to $\sim 11 \text{ nmol L}^{-1}$. The contribution of Cu_{diss} to Cu_{tot} at the surface increased along the study transect, Cu_{diss} being $\sim 100\%$ of the total in the open sea. Both total and dissolved Cu values are similar to those recorded in previous surveys for the northern and central Adriatic Sea (Table 1), with the only exception of Tankere et al., 2000 who reported higher Cu values. For the Southern Adriatic Sea and the rest of the Mediterranean Sea (Tankere and Statham, 1996) lower values have been reported as well as for the oceanic waters (Table 1). Dissolved Cu concentrations were generally lower than those measured in other European estuaries and the average value of the rivers worldwide (Table 1).

A vertical decreasing trend of Cu_{tot} was also observed within the Po plume. At the boundary of the frontal area (Stn. 5) and in the marine area, Cu_{tot} showed a maximum in the upper 10–15 m of the water column, and then it remained almost constant with depth (Fig. 8). Moreover, Cu_{diss} decreased within the stratified layer; and below it remained almost constant down to the seafloor with values close to Cu_{tot} . Therefore, the Cu_{diss} proportion increased with depth reaching values up to $\sim 90\%$ of the total. Total particulate Cu was high at the surface in the stations of the pro-delta and decreased seaward and with depth reaching low values (up to $\sim 30\%$ in the open sea) (Fig. 8).

As for Pb, the dynamic fraction of Cu represented a small fraction (up to $\sim 50\%$ Fig. 5c) of the dissolved concentration, with values ranging between ~ 0.15 and $\sim 4.0 \text{ nmol L}^{-1}$. The fraction of dissolved Cu associated with colloidal material was much more significant ($\sim 70\text{--}100\%$ of the Cu_{diss}). Both Cu_{dyn} and Cu_{coll} concentrations decreased seaward, but the contribution of the Cu_{coll} to the dissolved Cu slightly increased along the transect (Fig. 5c).

Considering the water column, Cu_{dyn} decreased at all the stations within the stratified layer (with the exception of Stn. 5 where a maximum at 5-m depth was observed); afterwards it remained almost constant down to the seafloor (Fig. 8). The proportion of Cu_{dyn} vs. Cu_{diss} was $\sim 30\%$ within the stratified layer, for all the stations, and then it drastically fell to $\sim 7\%$ near the seafloor. Also Cu_{coll} decreased within the stratified layer, but contrary to Cu_{dyn} , below the pycnocline it increased with depth. Its percentage to Cu_{diss} was generally higher than Cu_{dyn} and slightly increased in the water column. In the marine area (Stn. 6), although little data is available on dynamic Cu, a sub-surface decrease and a subsequent homogeneous distribution with depth can be recognized (Fig. 8).

The Cu free-ion fraction measured by the CGIME sensor showed very low values ranging between $\sim 0.03 \text{ nmol L}^{-1}$ to $\sim 0.62 \text{ nmol L}^{-1}$ (Table S-2). $[\text{Cu}^{2+}]$ decreased by $\sim 95\%$ along the transect, and the proportion of this fraction with respect to the dissolved concentration decreased by $\sim 90\%$ seawards. Data available for this fraction are scarce, thus a proper description of Cu_{free} variation in the water column cannot be defined, even if an apparent decrease can be noted.

3.3.3.3 Multivariate statistical data analysis

The correlation matrix (Pearson's linear coefficients) is reported in Table S5.

Among nutrients, only NO_3^- and Si(OH)_4 co-varied positively (highly significant correlation), whereas PO_4^{3-} showed no correlations with the other nutrients, even in the surface layers. Chl-a was associated with O_2 , pH, total phytoplankton abundance and biomass and negatively correlated with phosphates. Total phytoplankton abundance and biomass were in contrast with the main hydrographic parameters (depth, temperature, salinity and density) and

with the distance from the Po, while they were positively correlated with turbidity and DOC.

Nearly all the metal fractions were in contrast with salinity (although some of the correlations were not significant), indicating the influence of the river waters on their distribution, as subsequently confirmed by PCA (see below). Only Pb_{dyn} and Cd_{coll} showed a different behaviour, co-varying (but not significantly) with salinity. Both Cd and Cu (except Cd_{part} and Cd_{coll}) showed significant correlations with phytoplankton abundance and biomass. Pb_{tot} was positively correlated with phytoplankton community as well. Moreover, Cd_{diss} was correlated positively only with nitrogen species, while Cd_{dyn} co-varied with silicates and not with nitrates. Pb_{dyn} showed no significant correlations with any physical, chemical or biological variable, while Pb_{tot} was statistically correlated with NO_2^- and $Si(OH)_4$, and Pb_{diss} with NH_4^+ . Cu was strong correlated with $Si(OH)_4$ and, partly (only Cu_{tot} and Cu_{part}) with NO_3^- . No significant correlations were found between any of the metals and PO_4^{3-} , overall. All Cu fractions were correlated with total, dissolved and dynamic Cd (except Cu_{part} which was not correlated with Cd_{tot} and Cd_{diss}), and with the total and particulate fractions of Pb, indicating that they are affected by the same factor.

To better understand the effect of the Po River on the metal species distribution, a multivariate analysis (PCA) was performed to reduce the dimensionality of the dataset to few components that summarize the information contained in the overall dataset.

The PCA applied to the standardized variables, led to the identification of three significant, cross-validated principal components (PCs) counting for about 68% of the total variation. [Figure 9](#) shows the results of the PCA in terms of loading plot ([Fig. 9a](#)) and score plot ([Fig. 9c](#)) of PC1 vs. PC2 and in terms of loading plot ([Fig. 9b](#)) and score plot ([Fig. 9d](#)) of PC2 vs. PC3.

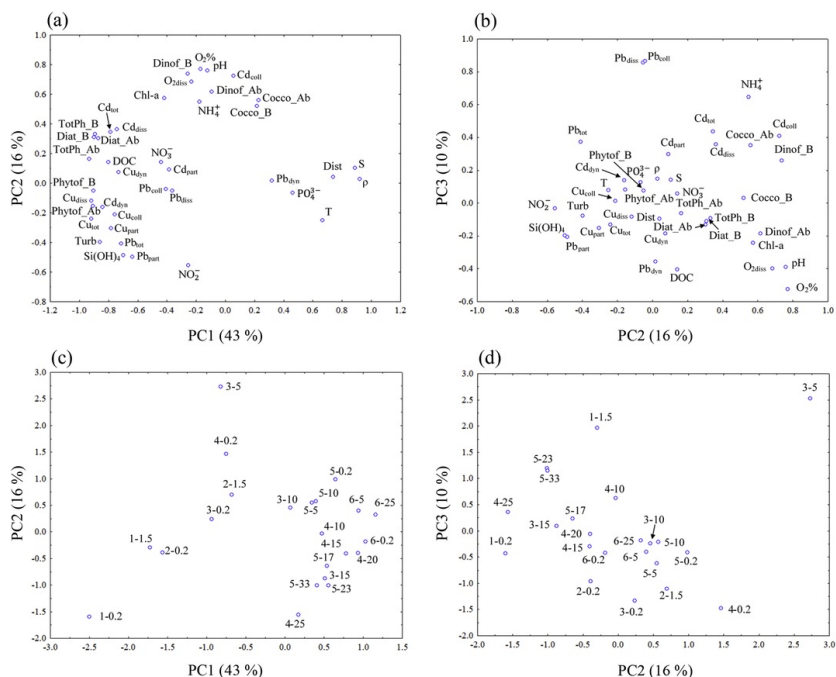


Fig. 9 PCA plots: (a) plot of the loadings of PC1 vs. PC2; (b) plot of the loadings of PC2 vs. PC3; (c) plot of the scores of PC1 vs. PC2; (d) plot of the scores of PC2 vs. PC3. The following abridgements were used for the variables: temperature (T), salinity (S), density (ρ), dissolved oxygen (O_{2diss}), oxygen saturation percentage ($O_2\%$), turbidity (Turb), chlorophyll-a (Chl-a), Coccolithophorid abundances (Cocco_Ab), Coccolithophorid biomass (Cocco_B), Diatom abundances (Diat_Ab), Diatom biomass (Diat_B), Dinoflagellate abundances (Dinof_Ab), Dinoflagellate biomass (Dinof_B), Phytoflagellate abundances (Phytof_Ab), Phytoflagellate biomass (Phytof_B), total phytoplankton abundance (TotPh_Ab), total phytoplankton biomass (TotPh_B). For metal fraction abridgements see the text in [Section 2.3](#). The objects code refers to the sampling station and to the depth.

alt-text: Fig. 9

The first principal component (explained variance 42%, [Fig. 9a](#), [9c](#)) is strongly associated to the main hydrographic parameters (temperature, salinity, density) and to the distance from the Po mouth (positive loadings), which are in contrast with NO_3^- , $Si(OH)_4$, turbidity, DOC and the main phytoplanktonic groups (diatoms and phytoflagellates). This group of variables (nutrients and phytoplankton community) also includes all the metals and almost all the

metal fractions (even if not all have high loads on PC1), with the exception of Pb_{dyn} and Cd_{coll} , that showed positive loadings on PC1. Thus, PC1 expresses the effect of the river outflow on the distribution of metal species, nutrients and main phytoplankton communities. Quite surprisingly, phosphates were associated to salinity gradient and in contrast with the general trend of other nutrients and metals. PC2 (explained variance 16%, Fig. 9a) is associated to O_2 , pH, dinoflagellates, Cd_{coll} , coccolithophorids, Chl-a and NH_4^+ (positive loadings). All these variables are in contrast with NO_2^- and, to a lesser extent with silicates, Pb_{part} and turbidity. Hence, the second principal component expresses the biological activity and regeneration processes (see the score plots, Fig. 9c). The third component (explained variance 10%, Fig. 9b, 9d) is dominated by the speciation of dissolved Pb with the contrast between Pb_{diss} and Pb_{coll} , on one side, and Pb_{dyn} (together with DOC) on the other side. The third principal component expresses the role of the organic matter on the dissolved Pb speciation.

4.4 Discussion

4.1.4.1 Environment

The oceanographic cruise along the Po plume (October–November 2002) was carried out after a particularly rainy period. The mean daily discharge measured at the hydrometric station of Pontelagoscuro (located at 80-km before the Po River mouth) was around $1300 \text{ m}^3 \text{ s}^{-1}$ (ARPA Emilia-Romagna, 2002), similar to the long-term average for the period of $1828 \text{ m}^3 \text{ s}^{-1}$ (Boldrin et al., 2005; Campanelli et al., 2012). However, the cruise was preceded by a particularly rainy period, where the Po river discharge reached values up to $3000 \text{ m}^3 \text{ s}^{-1}$ (ARPA Emilia-Romagna, 2002). Consequently, the influence of the Po River, in terms of reduced salinity at the surface, was evident about 50-km offshore from the mouth. This resulted in a marked horizontal stratification of the water column, highlighted by the presence of thermocline, strong halocline and pycnocline ($N_2(z) \sim 400 \times 10^{-4} \text{ s}^{-2}$) throughout most part of the transect. In the open sea, the effect of the Po outflow disappeared and the stratification of the water column sharply reduced ($N_2(z)$ up to $15 \times 10^{-4} \text{ s}^{-2}$, at 50-m depth of Stn. 6).

Principal component analysis applied to the whole dataset revealed that surface waters of the frontal area were affected by other factors than the Po river runoff (Fig. 9). These stations were associated with oxygen supersaturation levels (up to ~140%), high value of pH and NH_4^+ , corresponding to phytoplankton abundance and biomass peaks. Although diatoms were mainly responsible for total phytoplankton abundance and biomass distribution (see below), a dramatic increase (~80%) of both dinoflagellates and coccolithophorids occurred in the frontal area, while in the other stations these groups were practically absent (especially coccolithophorids). We hypothesize that saltier, well-oxygenated and high productive waters originated by the northern part of the Adriatic Sea (and maybe moved by winds) intrude in the frontal area and proceeded southward. Unfortunately, wind data of that period are not available, hence we are not able to corroborate this hypothesis.

DOC concentrations were similar to those typical of the region during the autumn. They were positively correlated with both phytoplankton abundance and biomass, highlighting that phytoplankton significantly contributed to DOC rather than the Po River. In the water column, DOC sharply decreased within the stratified layer, then below the pycnocline it showed a quite uniform profile, reaching values very close to the background levels ($76 \pm 10 \mu\text{mol L}^{-1}$) found by Pettine et al. (2001) in this area and typical of the surface oceanic waters (Guo et al., 1995).

Nutrient distributions along the transect highlighted the role of the Po River discharge and that of the biological processes in controlling the nutrient levels in the Northern Adriatic Sea as already observed in previous surveys (Boldrin et al., 2005; Grilli et al., 2005; Campanelli et al., 2012). Nutrient concentrations are typical of the autumn season for the Northern Adriatic Sea (Boldrin et al., 2005; Campanelli et al., 2012) and showed a clearly seaward decreasing gradient. The nutrient vertical profiles were generally characterized by high surface concentrations (especially in the pro-delta and frontal areas), decreasing down to a depth of about 5–10 m (within the stratified layer), a trend opposite to that of the salinity, highlighting the influence of the Po river input. Below the 10-m depth concentrations were vertically uniform or increased with depth.

4.2.4.2 Metal speciation along the Po River plume

Several processes, both internal (i.e. trace metal removal from the dissolved phase by adsorption, precipitation and co-precipitation of solutes, or by flocculation and net sedimentation with suspended matter, or by biological uptake; production of trace metals in the dissolved phase by desorption or solubilisation of particulate matter; transformation and migration of trace metals at the sediment/water interface) and external (mainly anthropogenic activities, such as, pollution, discharging of pollutants or cooling waters) control the speciation and the distribution patterns of metals in the mixing zone between salt and fresh waters. These processes are affected, in turn, by the strong longitudinal variations in physical, chemical and biological parameters that develop within a salinity gradient.

Principal component analysis reveals a strong influence of the Po River outflow on the spatial and vertical distribution of Cd, Pb and Cu species. Almost all the metal fractions linearly decreased with salinity gradient, consequently to the dilution effect (i.e. all the metals showed near-conservative behaviours). Although all the metal fraction contents globally decreased with salinity, speciation distribution patterns significantly differed from one metal to another.

Cd was mainly present in its dissolved form (particulate fraction up to 20% of the total). The contribution of Cd particulate fraction increased within the stratified layer, following the maximum phytoplankton abundance. Below the pycnocline, dissolved/particulate fractionation changed again, due to the Cd release by dead phytoplankton cells that sedimented down to the bottom (Bruland and Lohan, 2003).

Concerning Pb and Cu, the particulate fractions of the two metals were dominant within the pro-delta area (accounting for ~70% of the Pb_{tot} and for ~55% of the Cu_{tot}), while the dissolved fractions were mainly present in the open sea. Nevertheless (with the exception of the pro-delta area), Cu partitioning was dominated by the dissolved fraction throughout the study transect. Contrary to its oceanic “hybrid” distribution in the water column (e. g. surface depletion, linear increase with depth and deep scavenging by particles) (Bruland and Lohan, 2003; Jacquot and Moffett, 2015), dissolved Cu remained elevated all the way down to the bottom. Only at the seafloor of the frontal area, an increase in Cu content occurred, suggesting additional inputs from sediments (such as re-suspended sediments), as observed in open ocean waters (Tankere et al., 2000; Waeles et al., 2008; Jacquot and Moffett, 2015).

Depth profile of Pb confirmed its nature of particle-reactive metal. Because of desorption processes from the particulate matter, dissolved Pb concentrations increased along the water column, while the particulate fraction became again significant in proximity of the seafloor. In fact, sediment can be considered as an additional source of particulate Pb due to several biogeochemical processes within the sediments themselves and at the sediment/water interface (Tankere et al., 2000; Cobelo-Garcia and Prego, 2004).

Differences between Cd, on one hand, and Pb-Cu on the other hand, were observed also for the dissolved-fraction speciation. Cd was mainly present as dynamic fraction in the pro-delta area. In the remaining part of the transect, the contribution of the colloidal fraction to the dissolved Cd became more and more important within the stratified layer, up to 40–50% of Cd_{diss} in the marine area. As observed by Baeyens et al. (1998) in the Scheldt estuary (Netherlands), it seems likely that the production of dissolved Cd contributed to the dynamic fraction (the maximum bio-available fraction) near the Po mouth, afterwards it decreased in favour of less dynamic species, probably colloidal species. Martin et al. (1995) in the Venice Lagoon also found a similar colloidal contribution (30–40%) to the dissolved Cd, as well as in the Ochlockonne estuary (Powell et al., 1996) and in the Scheldt estuary (Baeyens et al., 1998). As reported by Comans and Van Dijk (1988), the speciation of Cd is generally dominated in estuaries by stable and soluble chlorocomplexes, but in some systems, organic complexation could be important too. As shown in the PCA (Fig. 9), Cd_{coll} is significantly associated with small algal cells, the dinoflagellates and the coccolithophorids, which increased in the frontal area by a percentage much higher (80–98%, respectively) than diatoms (~40%) or phytoflagellates (see in Section 4.1). Thus, the increase in colloidal Cd seawards could also be ascribed to complexation with algal cells or with exudates produced by phytoplankton. Below the pycnocline and down to the bottom, Cd_{diss} was once again constituted by the dynamic fraction (90–100% of the Cd_{diss}), following the release of this fraction from phytoplankton dead cells that sedimented in the water column.

Dissolved Pb and Cu speciation was dominated by the non-dynamic fraction (40–95% of the dissolved fraction for both metals). The low proportion of Pb_{dyn} and the consequently strong association of Pb with colloidal material is in agreement with previous studies (Martin et al., 1995; Waeles et al., 2008; Braungardt et al., 2011). For example, Martin et al. (1995) reported that colloidal Pb accounted for up to ~90% of the total dissolved Pb in the Venice Lagoon (northern Adriatic Sea). Scarponi et al. (1995) during speciation studies carried out through voltammetric titration procedures in seawater of different areas (e.g. Adriatic Sea, Antarctic Ocean, and North Eastern Pacific) found that the ASV-labile fraction (similar to our dynamic fraction) accounted for about 15% of the total Pb at the surface.

When considering the contribution of colloidal copper in various estuarine or delta systems, disparate results were obtained. As example, in the Danube and in the Loire estuaries (Waeles et al., 2004, 2009), Cu_{coll} accounted for only 10–40% of the total dissolved metal. In the Penzé estuary (Waeles et al., 2008) or in the Restronguet Creek (Braungardt et al., 2011) the colloidal fraction showed percentages similar to our results. The high presence of non-dynamic Cu confirmed the results obtained in previous speciation studies carried out by Zago et al. (2002) in the Northern Adriatic Sea. The authors did not detect labile metal concentrations of the two studied metals (Cu and Zn), but, on computing the ligand concentrations, they concluded that the total dissolved metal concentration of Cu was exclusively present as organically complexed metals. Several speciation studies worldwide (Capodaglio et al., 1994; L'Her Roux et al., 1998; Waeles et al., 2004, 2008, 2009; Buck et al., 2007) have suggested a model with two classes of ligands for both dissolved Pb and Cu, the strongest consisting of detrital matter (e.g. fulvic and humic acids) and living biogenic material (living organisms, such as algae, bacteria, virus, or organic exudates).

Since the phytoplankton highly contributed to the DOC pool (see Section 4.1), it is plausible to ~~hypethesise~~[hypothesize](#) an organic origin for Pb and Cu ligands in the northern Adriatic Sea. Further studies, including a better spatial resolution and dissolved organic matter speciation are therefore necessary to gain more insight into the speciation of the two dissolved metals and the nature of Pb-Cu ligands in the Adriatic Sea.

The impact of the different processes controlling trace metal specie distributions along the Po plume can be established also by investigating metal-nutrient relationships. In open ocean water, bioactive trace metals (Cd, Cu, Fe, Ni, Zn) have positive relationships with limiting nutrients (PO_4^{3-} and $Si(OH)_4$), following the assumption that the metal:P slopes in seawater should be equal to the ratios in which these elements are present in the cells; the so-called “extended Redfield ratio” (Morel and Hudson, 1985). Deviations from this linear metal-nutrient relationship may be indicative of additional inputs not observed in open ocean environments (Martin et al., 1980). In the present work, all the metal fractions did not show significant positive correlations with PO_4 (Tab. [1e](#) S5). Similar ratios were observed also if surface values (which are more affected by river discharge) were excluded (data not shown). The opposite gradients observed in the PCA between metals and P (Fig. 9) cause the negative Me:P slopes recorded in these waters (Tab. [1e](#) S5), highlighting the influence of other factors than biological cycles on metal distribution, i.e. the effect of the Po River discharge that masked, for example, the typical nutrient-like profile of Cd and Cu.

~~4.3.4.3~~ Significance of metal speciation

The dynamic metal concentrations here measured could offer some interesting insights on the toxic or limiting effects of specific trace metals, since its peculiar characteristic to approximate the bio-available fraction (Buffle and

[Tercier-Waeber, 2005](#)) more than the dissolved fraction. Several studies have been carried out on assessing the toxicity of trace metals on marine organisms, most of them dealing with the toxicity of the free metal ions. Very little is known about the toxicity effect of the dynamic fraction. Studies based on laboratory cultures ([Verweij et al., 1992](#); [Perez et al., 2010](#)) reported that adverse effects on cyanobacteria and many marine phytoplankton species occurred for concentrations in the range 0.001 – 0.1 nmol L^{-1} for Cu^{2+} , 1 – 5 nmol L^{-1} for Cd^{2+} and $<0.1 \text{ nmol L}^{-1}$ for Pb^{2+} . Our dynamic Cd and Pb concentrations did not represent a risk to marine organisms in the Northern Adriatic Sea, even if Pb showed concentrations very close or slightly below the thresholds previously reported. However, the Pb^{2+} concentrations measured by the PLM technique (no data available from CGIME sensor) were few tens of pico-molar per L (from 1 to $\sim 15 \text{ pmol L}^{-1}$), far below the toxic levels measured in laboratory cultures. On the contrary, the dynamic fraction of Cu showed values of the pro-delta ($\sim 4.0 \text{ nmol L}^{-1}$) being potentially toxic to 21 marine phytoplankton species examined in laboratory cultures. Moreover, studies ([Verweij et al., 1992](#); [Beiras and Albertosa, 2004](#); [Rivera-Duarte et al., 2005](#)) on the embryogenesis success of bivalves (*Mytilus galloprovincialis*, *Ruditapes decussatus*), sand dollar (*Dendraster excentricus*), and sea urchin (*Strongylocentrotus purpuratus*) showed sensitivity towards Cu^{2+} at values of EC_{50} (effective concentration of metal causing a 50% inhibition of embryos development with respect to the control) ranged from $\sim 0.06 \text{ nmol L}^{-1}$ to $\sim 0.16 \text{ nmol L}^{-1}$. Therefore, Cu dynamic fraction along Po plume showed concentrations likely to be toxic to sensitive phytoplankton community and to have negative effects on larva development of coastal macroinvertebrate species. Also, Cu^{2+} concentrations ($\sim 0.3 \text{ nmol L}^{-1}$) at the Po mouth were higher than the EC_{50} values provided for coastal macroinvertebrate larvae sensitive to copper ([Beiras and Albertosa, 2004](#)), and the same order of magnitude of the toxic threshold range reported for plankton was noted ([Brand et al., 1986](#)). In the case of copper, hydroxy complexes are also believed to be toxic, with toxicity decreasing in the order, Cu^+ and Cu^{2+} > inorganic copper > organic copper ([Allen and Hansen, 1996](#)).

Moreover, culture studies have demonstrated that several metals may act synergistically or antagonistically to influence growth limitation or toxicity to marine organisms ([Annett et al., 2008](#); [Semeniuk et al., 2009](#)). In force of these metal:metal antagonisms, elevated levels of free ions of one metal (e.g., Mn^{2+}) may alleviate the toxic effects of Cu^{2+} ([Sunda and Huntsman, 1983](#)) or Zn^{2+} ([Twining et al., 2011](#)). Therefore, further studies including other metals in addition to those of the present work are necessary in order to gain more insights into metal speciation and the potentially harmful effects of metal species to marine organisms in the Northern Adriatic Sea.

5.5 Conclusions

The speciation of Cd, Pb and Cu was studied for the first time along the Po River plume. The oceanographic cruise (Autumn 2002) was carried out after a particularly rainy period. Consequently, the influence of the river, in terms of reduced salinity at the surface, was evident about 50-km offshore from the mouth. The distribution and speciation of Cd, Pb and Cu in the Po River plume is mainly controlled by the river outflow.

Almost all the metal fractions linearly decreased with the salinity gradient, consequent to the dilution effect (i.e. all the metals showed conservative behaviours). Cadmium was mainly present as the dynamic fraction in the pro-delta area, while in the remaining part of the transect, the contribution of the colloidal fraction to the dissolved Cd became more and more important. On the contrary, the Pb and Cu partitioning was dominated by the particulate fraction in the stations closer to the Po mouth and by the dissolved fraction in the open sea. The colloidal fraction of these two metals constituted a significant proportion of the dissolved concentration and it seems to be related to a biogenic origin. On the basis of speciation studies on the dissolved organic matter in the Northern Adriatic Sea, we hypothesize a biogenic origin (small algae, or phytoplankton exudates) of these colloidal fractions.

Metal concentrations measured in the present work are far below (at least one order of magnitude lower) the EQS established by the Italian law (Italian DPR 172/15, 2015) ([Link to the reference Italian DPR Reference below](#)) in the enactment of the revised Priority Substances Daughter Directive of the European Union, [2013/39/EU \(2013\)](#), even if these Directives do not take copper into account at all (the only national legal limit existing for Cu dates back to the Italia ([Link to the Italian DPR 2006 in the reference list below](#))n DPR 152/06 (2006), that established a threshold concentration of $16 \text{ } \mu\text{mol L}^{-1}$ referring to waters suitable to the life of salmonid and cyprinid organisms). Our values are also far below the Acute Water Quality Criteria (WQC) developed through both probabilistic and deterministic approaches and to which political authorities referred to enforce water quality standards for each pollutant ([Durán and Beiras, 2013](#)). However, our Cu dynamic fraction along the Po plume showed concentrations likely to be toxic to sensitive phytoplankton community and to have negative effects on larva development of coastal macroinvertebrate species. Furthermore, Cu^{2+} concentrations at the Po mouth were higher than the EC_{50} values provided for coastal macroinvertebrate larvae sensitive to copper.

The present work allows a better comprehension of the processes regulating marine biogeochemical cycles of trace metals. It also improves our insights on transport and fate of contaminants in the marine environment, giving valuable tools for more reliable predictions of future changes and actions to protect it, as recommended by the Marine Strategy Framework Directive (descriptor 8th), ([European Directive 2008/56/EC, 2008](#)).

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~~Appendix A.~~Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marchem.2019.04.001>.

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~~Appendix A.~~ **Appendix A. Supplementary data**

[Multimedia Component 1](#)

Highlights

- In-situ trace metal speciation in the Adriatic Sea by submersible voltammetric sensors
 - Metal species distribution mainly affected by Po River outflow
 - Metal dynamic concentrations below legal limits
 - Cu dynamic concentrations toxic to sensitive phytoplankton
-

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