



The role of the macroalgae *Ulva lactuca* on the cellular effects of neodymium and mercury in the mussel *Mytilus galloprovincialis*

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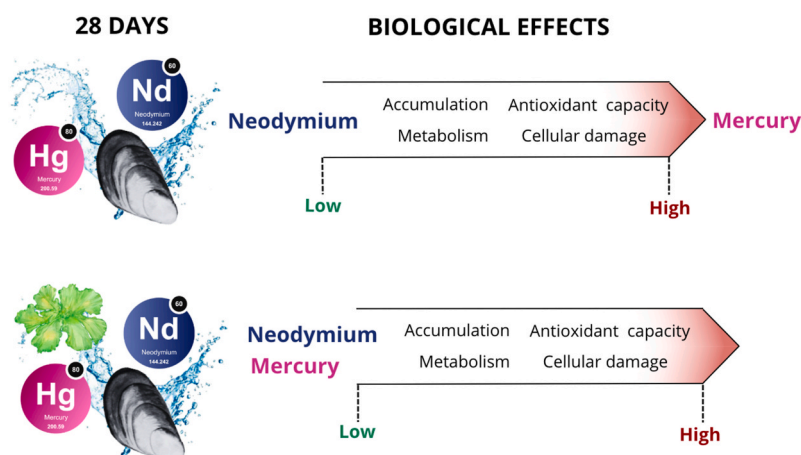
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HIGHLIGHTS

- The presence of *Ulva lactuca* reduced Hg accumulation by mussels.
- The presence of Hg induced mitochondrial activity in mussels without algae.
- Limited cellular damage in the Nd or Hg treatments.
- Mussels co-exposed to Nd and algae showed activation of GPx and CbEs.
- Mussels exposed to Hg with algae exhibited a greater redox imbalance.

GRAPHICAL ABSTRACT



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ABSTRACT

Rare earth elements (REEs) are increasingly being studied mainly due to their economic importance and wide range of applications, but also for their rising environmental concentrations and potential environmental and ecotoxicological impacts. Among REEs, neodymium (Nd) is widely used in lasers, glass additives, and magnets. Currently, NdFeB-based permanent magnets are the most significant components of electronic devices and Nd is used because of its magnetic properties. In addition to REEs, part of the environmental pollution related to electrical and electronic equipment, fluorescent lamps and batteries also comes from mercury (Hg). Since both elements persist in ecosystems and are continuously accumulated by marine organisms, a promising approach for water decontamination has emerged. Through a process known as sorption, live marine macroalgae can be used,

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especially *Ulva lactuca*, to accumulate potential toxic elements from the water. Therefore, the present study aimed to evaluate the cellular toxicity of Nd and Hg in *Mytilus galloprovincialis*, comparing the biochemical effects induced by these elements in the presence or absence of the macroalgae *U. lactuca*. The results confirmed that Hg was more toxic to mussels than Nd, but also showed the good capability of *U. lactuca* in preventing the onset of cellular disturbance and homeostasis disruption in *M. galloprovincialis* by reducing bioavailable Hg levels. Overall, the biochemical parameters evaluated related to metabolism, antioxidant and biotransformation defences, redox balance, and cellular damage, showed that algae could prevent biological effects in mussels exposed to Hg compared to those exposed to Nd. This study contributes to the advancement of knowledge in this field, namely the understanding of the impacts of different elements on bivalves and the crucial role of algae in the protection of other aquatic organisms.

1. Introduction

The contamination of aquatic ecosystems is a continuously increasing challenge as a consequence of population growth and high industrialization of the chemical, agrochemical, and pharmaceutical sectors (Bonanno et al., 2020; Defeo and Elliott, 2021; Okerefor et al., 2020), favoring the release and occurrence of pollutants (Starling et al., 2019). In the last decade, waste from electrical and electronic equipment (EEE), commonly identified as e-waste, has been considered the fastest-growing waste in the world (Abinaya et al., 2021) as a result of a great diversity of electronic devices (Phulwani et al., 2021), the decrease in the lifespan of such equipment (Montalvo et al., 2016), an increase in population (Tansel, 2017), a decrease in prices (Wang et al., 2019), and inadequate recycling processes (Abalansa et al., 2021). According to data provided by Forti et al. (2020), e-waste has increased substantially from 9.2 Mt in 2014 to 53.6 Mt in 2019, and it is expected to reach an alarming amount of 74.7 Mt by 2030 following an annual growth rate of approximately 2 Mt. Concerns with this type of pollution focus on the hazardous components, including rare earth elements (REEs), as they are not biodegradable and can accumulate in the environment, with harmful effects on ecosystems and human health (Coalition, 2019; Jadhao et al., 2016; Wang et al., 2016). Rare earth elements are characterized by strong magnetism, electrical conductivity and high thermal stability, fluorescence, and the ability to emit light in the visible range (Adeel et al., 2019; Dushyantha et al., 2020; Tai et al., 2010). These elements are in constant increasing demand, as they are considered important for a wide range of applications in new technologies that are evolving and depend on the chemical, catalytic, electrical, magnetic, and optical properties of these elements (Xie et al., 2014). Neodymium (Nd), belonging to the group of REEs, was the focus of this study since it is classified as one of the five most critical elements by 2025 according to the United States Department of Energy (Age and Eft, 2011). It has been widely used to produce rare earth alloys, electronic components, optical filters, art glass, steel modifiers, and hydrogen storage (Das and Das, 2013). Neodymium also plays an important role in the production of laser equipment (Palmieri et al., 2000; Xiong et al., 2009). Nowadays, the relevant application of Nd is related to neodymium-iron-boron (NdFeB) based permanent magnets, as these are the most significant component of electronic devices due to their magnetic properties (Liu et al., 2019). Studies indicate that the annual consumption of NdFeB magnets has increased from 20,000 tons in 2006 to 55,000 tons in 2017 (Fröhlich et al., 2017), and the production volume of Nd has also increased by more than 50 % (Menad and Seron, 2017). Permanent magnets can also be found in wind turbines, war tanks, aircraft, missiles, submarines, and warplanes (Padhan et al., 2017). With the increase in the application of Nd in technological processes, the discharge of this element into the environment, mainly into rivers and coastal environments, has also increased and may have significant impacts on organisms inhabiting these ecosystems (Wang et al., 2011). The concentration of Nd in surface water is on the order of a few micrograms per liter, whereas in contaminated environments its concentration increases to hundreds of micrograms per liter (Sultan and Shazili, 2009). According to Wang et al. (2011), the concentration of Nd in rivers in China can reach 103 $\mu\text{g L}^{-1}$, while Adeel et al. (2019) reported that Nd

concentrations in the coastal areas of the Hawaiian coast (Kona) and the Australian coast (Labrador Beach) were 24 and 32 $\mu\text{g L}^{-1}$, respectively. On the other hand, in China, according to Tai et al. (2010) Nd abundance in seawater was estimated at 2.8 ng L^{-1} . Neodymium can also be accumulated in marine species: a study conducted by Briant et al. (2021), aiming to evaluate REEs concentrations in several common bivalves from the French coast, reported Nd concentrations ranging from 0.036 $\mu\text{g g}^{-1}$ dry weight, DW (*Mytilus galloprovincialis*, Littoral, Pômègues est) to 2.088 $\mu\text{g g}^{-1}$ DW (*Crassostrea gigas*, estuary, Bonne Anse Palmyre). In the study of Figueiredo et al. (2022), specimens of *M. galloprovincialis* were collected from six locations along the Portuguese coast to determine REEs concentrations in wild biota: the site of Porto Brandão (located on the south bank of the Tagus estuary) had the highest concentrations of Nd ranging from 0.356 $\mu\text{g g}^{-1}$ DW (Fall) to 0.502 $\mu\text{g g}^{-1}$ DW (Spring), while the lowest concentrations were observed in Alzejur (the southernmost point on the Portuguese coast) ranging from 0.082 $\mu\text{g g}^{-1}$ DW (Fall) to 0.108 $\mu\text{g g}^{-1}$ DW (Spring). There's growing evidence that once accumulated, Nd can provoke biological disturbance: Freitas et al. (2020b) evaluated the biological effects of five different Nd concentrations (2.5; 5; 10; 20 and 40 $\mu\text{g L}^{-1}$) in *M. galloprovincialis*, revealing significant effects on energy metabolism, oxidative stress, and cellular damage. Similarly, Leite et al. (2023) reported histopathological damage in the gills and cellular damage in mussels *M. galloprovincialis* exposed to 10 $\mu\text{g L}^{-1}$ of Nd.

Other well-known trace metals are widely recognized key pollutants in marine environments and pose a threat to ecosystems and human health because of their high toxicity, persistence, and their capability to enter the food chain (Tian et al., 2020; Wang et al., 2018). In marine environments, these metals can have natural and anthropogenic sources, such as geological meteorology, domestic effluents, industrial and agricultural activities, and urban runoff (Garmendia et al., 2019). Among such elements, mercury (Hg) occupies the third position in the priority substances list (Agency for Toxic Substances and Disease Registry (ATSDR) (Walensky et al., 2022): sources of Hg include fluorescent lamps, batteries, and electrical and electronic applications, the latter accounting for most of the Hg pollution associated with REEs (Outridge et al., 2018; Tunsu et al., 2014, 2016; Zhang and Wong, 2007). Nowadays, Hg is mostly used in fluorescent lamps because it plays a crucial role in their energy-efficient lighting technology. According to the Chinese Ministry of Industry and Information Technology, a total of 3.15 billion fluorescent lamps were produced in China in 2017, with 1.68 billion exported. While Hg discharges into the environment have been regulated (Kim and Choi, 2012), this metal has been identified in coastal and bay waters in concentrations ranging from 0.38 to 231 ng L^{-1} (Gworek et al., 2016), whereas in open seawater Hg concentrations range from 0.5 to 3.0 ng L^{-1} (Faganeli et al., 2012). Yu et al. (2020) analysed Hg concentration in edible tissues of aquatic invertebrates in China, reporting concentrations ranging from 0.029 to 0.065 mg kg^{-1} wet weight, WW. Higher concentrations were shown in bivalve mollusks available on the Polish market in the study by Szkoda et al. (2015), with a mean of 0.120 mg kg^{-1} DW, still below the EU limit of 0.50 mg kg^{-1} (EU, 2006). Previous studies showed a plethora of toxic effects in marine biota produced by Hg such as extensive cellular damage in *M. galloprovincialis* exposed to 0.25 mg L^{-1} (Coppola et al., 2017), and

high mortality rates for *Anodonta natine*, a native bivalve species in Europe (Oliveira et al., 2015). Mercury has strong lipophilicity and can thus easily accumulate in the food chain, leading to high exposure to top predators such as humans (Hsu-Kim et al., 2013; Kim et al., 2016) and possible neurotoxic outcomes such as mental retardation, cerebral palsy, deafness, blindness, dysarthria, and even death (Du et al., 2019).

Several methods have been tested to remediate contaminated water, such as liquid-liquid extraction (Xie et al., 2014), chemical precipitation (Zhao et al., 2016), and ion exchange (Anastopoulos et al., 2016). Biosorption is an emerging biological approach that presents advantages compared to conventional methods, namely, it is efficient in removing pollutants from very diluted solutions, it prevents the production of chemical residues, it can be integrated into many systems, biosorbents have low-cost and adsorption and desorption kinetics, and no secondary waste is generated (Chen, 2010; Das and Das, 2013; Kratochvil and Volesky, 1998; Tewari et al., 2005). Macroalgae can be included as biosorbents used in this technology (Diniz and Volesky, 2005; Henriques et al., 2015), since these present large surface area of structurally uniform and physiologically active cells enhancing a higher sorption capacity and they are represented worldwide by many species tolerant to a wide spectrum of temperature and salinity (Ben-Ari et al., 2014; Chen et al., 2015; Henriques et al., 2017). According to previous studies, *Ulva lactuca* is the most widely used algae to remove contaminants from seawater (Ferreira et al., 2020; Henriques et al., 2015, 2017, 2019b; Pinto et al., 2020; Viana et al., 2023). Nonetheless, besides their use in remediation processes, laboratory studies have pointed out the capacity of macroalgae to mitigate the effects caused by REEs and classical metals such as Hg on bivalves (Smii et al., 2023; Trapasso et al., 2021).

While numerous studies have highlighted the toxicity of Nd and, particularly, Hg on marine wildlife, there is still a significant lack of information regarding the ability of algae to mitigate the impacts of these and other contaminants within the aquatic environment. In this regard, recent studies have investigated the application of nature-based solutions (NBS), specifically exploring the use of macroalgae, which play a crucial role in the protection, conservation, restoration, and enhancement of aquatic ecosystems. The present study aimed to bridge this knowledge gap by evaluating the capability of the algae *U. lactuca* to remediate seawater from Nd and Hg and mitigate the cellular toxicity induced on the mussel *M. galloprovincialis*.

2. Materials and methods

2.1. Sampling organisms and experimental conditions

Specimens of *M. galloprovincialis* (width: 3.5 ± 0.2 cm; length: 6.1 ± 0.3 cm) were collected in May 2022 from Ria de Aveiro (Portugal), transported to laboratory facilities, and allowed to acclimate to controlled conditions in aquaria for a period of ten days. This species was selected for this study since it has been considered a good bio-indicator due to its wide geographical distribution, sessile lifestyle, and available biological information (Beyer et al., 2017; Farrington et al., 2016; Goldberg, 1975). Also, this species is abundant in the sampling area, being common on rocky coasts of the Atlantic Ocean and the Mediterranean Sea, where it stands out due to its high frequency and abundant population (Braby and Somero, 2006).

Over the acclimation phase, mussels were kept under constant aeration in artificial seawater (Coral Pro Salt Red Sea) at 17 ± 1 °C, pH 8.1 ± 0.1 , and salinity 28 ± 1 , representative of the conditions at the sampling site during organisms' collection. From the same sampling location, the algae *U. lactuca* were collected and transported to the laboratory where they were gently washed with clean artificial seawater to remove any fragments and epibionts and then transferred into an aquarium with artificial seawater under constant aeration at the same temperature, pH, and salinity condition for ten days acclimation. For mussels and algae, water was renewed every three days.

After the acclimation, mussels and algae were randomly divided in

different aquaria to test the following treatments: i) control (CTL, clean artificial seawater), ii) neodymium (Nd) $70 \mu\text{g L}^{-1}$, and iii) mercury (Hg) $50 \mu\text{g L}^{-1}$. Each treatment was tested with mussels and algae separately, as well as with mussels and algae together. Overall, nine experimental treatments were carried out for 28 days, each in triplicate 4 L aquaria containing five mussels and/or 12 g of algae mass (3 g L^{-1}) per aquarium; algae mass was chosen according to previous studies on water remediation (Ferreira et al., 2020; Henriques et al., 2015).

The selected Nd concentration of $70 \mu\text{g L}^{-1}$ simulates highly polluted aquatic environments with wastewater discharges, based on published literature and a possible future scenario, where REEs concentrations are expected to increase as a result of contamination (Adeel et al., 2019). The exposure Hg concentration, $50 \mu\text{g L}^{-1}$, corresponds to the maximum allowed limit in wastewater discharges from industry (Directive, 2013/39/EU, 2013).

During the experimental period (28 days), water and algae were weekly renewed and the conditions of temperature, pH, salinity and both Nd and Hg concentration re-established. Weekly, before water renewal, the algae were collected from the aquaria and stored at -80 °C until analysed for quantification of adsorbed metals and new algae were added. Throughout the exposure period, aeration was kept constant, the photoperiod was maintained as natural light conditions, and the mussels were fed with Algamac plus protein three times per week (0.75 mL per mussel per day). Organisms' mortality was daily checked. At the end of the exposure, mussels were individually frozen in liquid nitrogen and stored at -80 °C; afterward, the whole soft tissues of each organism were pestled under liquid nitrogen and divided into aliquots (each with 0.5 g of fresh weight, FW) for assessment of biochemical responses and metals quantification. During the exposure period, water samples were collected after spiking from exposure aquaria (with mussels/algae) and from blanks (without mussels/algae) to access Nd and Hg real concentrations and metal stability (one sample per aquarium, 3 aquaria per treatment; $n = 3$).

2.2. Neodymium and mercury quantification in water samples, mussel's soft tissues and macroalgae

The quantification of Nd in water samples was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in a Quadrupole Thermo Scientific X Series spectrometer equipped with a Burhener nebulizer after dilution (20x) in 1 % HNO_3 to reduce their salinity and then analysed directly ($\text{pH} < 2$). Calibration curves were built using 5 standards ranging from 0.02 to $100 \mu\text{g L}^{-1}$, obtained from successive dilution of a certified standard for ICP analysis (Inorganic Ventures™, $998 \pm 1 \mu\text{g Nd mL}^{-1}$). The error associated with each standard did not surpass 10 % and the limit of quantification (LOQ) for water samples was assumed as the lowest calibration standard, $0.02 \mu\text{g L}^{-1}$. See details about methodology validation in Supplementary Material.

The quantification of Hg in water samples was performed by Cold Vapour Atomic Fluorescence Spectrometry (CV-AFS) on a PSA cold vapour generator with a Merlin PSA detector, using SnCl_2 (2 % w v^{-1} in 10 % v v^{-1} HCl) as the reducing agent. Calibration curves were built using 5 standards ranging from 0.1 to $0.5 \mu\text{g L}^{-1}$, obtained from successive dilution of a certified stock solution provided by Merck ($1001 \pm 2 \mu\text{g Hg mL}^{-1}$). Calibration curves were remade every 4 samples. Each sample was analysed at least in triplicate, with an acceptable coefficient of variation ≤ 5 %. The limit of quantification of the method was $0.03 \mu\text{g L}^{-1}$.

For the quantification of Nd in mussels (whole soft tissues), homogenized freeze-dried samples (0.2 g) were digested with 1 mL of HNO_3 (65 %), 2 mL of hydrogen peroxide (H_2O_2 , 30 % v v^{-1}), and 1 mL of H_2O , in Teflon vessels, in a microwave by increasing the temperature to 170 °C (15-min ramp) which was then maintained for 10 min. After cooling, samples were transferred to polyethylene vessels, made to a final volume of 25 mL with H_2O ultrapure, and analysed by ICP-MS. The samples of macroalgae were also acid-digested using a microwave prior

Nd quantification by ICP-MS, first with 2 mL of HNO₃ (65 %) and a temperature ramp up to 150 °C for 15 min (held for 10 min) and afterward with 250 µL of H₂O₂ (30 % v v⁻¹) and a temperature ramp up to 150 °C for 15 min (held for 10 min). Blanks, sample duplicates, and certified reference materials BCR-668 (Mussel Tissue, total Nd 54 ± 4 µg kg⁻¹) and SRM-1515 (Macroalgae, total Nd 17 mg kg⁻¹) were used for quality control purposes. The mean value of Nd in blanks was 0.03 µg L⁻¹ (below the LOQ), the coefficient of variation of duplicates was lower than 5 % and the mean percentage of recovery for BCR-668 was 114 % and for SRM-1515 was 87 %. All the results were corrected considering the percentages of recovery, which remained within the acceptable range of 80–120 %. See details about methodology validation in Supplementary Material.

The quantification of Hg in mussels (whole soft tissues) and macroalgae was performed by Thermal decomposition atomic absorption spectrometry with gold amalgamation (LECO model AMA-254). Freeze-dried samples (1–40 mg) were analysed directly, avoiding the need for digestion or pre-treatment. Blanks were run before and between samples to prevent Hg from being carried between samples. The quality control was assured by using duplicates and certified reference material ERM-CD200 (macroalgae *Fucus vesiculosus*, total Hg 0.0186 ± 0.0016 mg kg⁻¹) and TORT-3 (Lobster Hepatopancreas; total Hg 0.137 ± 0.012 mg kg⁻¹). The mean value of Hg in blanks was 0.03 ng Hg (below the LOQ), the coefficient of variation of duplicates was lower than 10 % and the mean percentage of recovery for BCR-668 was 82 % and for SRM-1515 was 94 %. All the results were corrected considering the percentages of recovery, which remained within the acceptable range of 80–120 %.

2.3. Biochemical parameters

To evaluate the biological effects induced by Nd and Hg in *M. galloprovincialis* at the end of the exposure period, a set of biochemical parameters was selected, considering previous studies that already demonstrated the impacts of Hg and Nd on bivalves metabolic capacity, oxidative and neurotoxic status (Coppola et al., 2017; Freitas et al., 2020a,b; Leite et al., 2023). The biomarkers selected were related to mussels: **metabolic capacity** (electron transport system (ETS) and Acyl-CoA oxidase (ACOX) activity); **energy reserves content** (glycogen (GLY) content); **antioxidant defenses** (catalase (CAT) and glutathione peroxidase (GPx) activity); **biotransformation defenses** (glutathione S-transferases (GSTs) and carboxylesterases (CbEs) activity); **redox balance** (oxidized glutathione content (GSSG) and total oxyradical uptake capacity (TOSC) evaluated toward peroxy radicals ROO·, hydroxyl radicals HO· and peroxyxynitrite ONOO⁻) and **cellular damage** (lipid peroxidation (LPO) and protein carbonylation (PC) levels). The protein content (PROT) was determined and used to express the biomarkers ACOX, TOSC ROO·, TOSC HO·, TOSC ONOO⁻, ETS, CAT, GPx, GSTs, CbEs and PC (Table 1SM, supplementary material). In the Supplementary Material, detailed techniques for the biochemical parameters examined are presented.

2.4. Data analysis

2.4.1. Bioconcentration factor

The bioconcentration factor (BCF) is commonly used to evaluate the potential of a chemical to accumulate in an organism from water (values measured after spiking) (Arnot and Gobas, 2006; McGeer et al., 2003). BCF is defined as the ratio of the chemical concentration in the tissue of the organism to the chemical concentration in the surrounding water (McGeer et al., 2003; Arnot and Gobas, 2006). In the present study, BCF was used to specifically assess the accumulation of Nd and Hg in mussels allowing us to ascertain the potential for accumulation of these elements under different exposure treatments.

2.4.2. Statistical analyses

The biochemical data and the measured Nd and Hg concentrations

Table 1

Quantification of Neodymium (Nd) and Mercury (Hg) in water samples (µg L⁻¹) collected after spiking from exposure aquaria (with mussels/algae) and from blanks (without mussels/algae) and algae/mussel tissues (µg g⁻¹, dry weight) collected along (algae) and after (mussels) 28 days of exposure (2 replicates per aquarium: 6 per treatment) and the respective bioconcentration factor (BCF) for mussels (L kg⁻¹).

Treatment		Water (µg L ⁻¹)	Algae (µg g ⁻¹)	Mussels		
				µg g ⁻¹	BCF (L kg ⁻¹)	
Mussels	Nd	0 µg L ⁻¹	< 0.02	0.26 ± 0.01	–	
		70 µg L ⁻¹	77 ± 7.7	6.1 ± 0.8	83 ± 10.1	
	Hg	0 µg L ⁻¹	< 0.03	0.20 ± 0.03	–	
		50 µg L ⁻¹	44 ± 1.4	65 ± 2.5	1297 ± 49.9	
Algae	Nd	0 µg L ⁻¹	0.31 ± 0.14	–	–	
		70 µg L ⁻¹	99 ± 2.5	47 ± 1.3	–	
	Hg	0 µg L ⁻¹	< 0.03	0.06 ± 0.01	–	
		50 µg L ⁻¹	–	60 ± 1.9	–	
Mussels + Algae	Nd	0 µg L ⁻¹	< 0.02	–	–	
		70 µg L ⁻¹	74 ± 7.2	40 ± 4.4	5.9 ± 1.3	80 ± 17.4
	Hg	0 µg L ⁻¹	< 0.03	–	–	–
		50 µg L ⁻¹	–	31 ± 4.3	33 ± 2.0	661 ± 39.4
Blanks	Nd	70 µg L ⁻¹	83 ± 5.7	–	–	
	Hg	50 µg L ⁻¹	52 ± 0.2	–	–	

were subjected to hypothesis testing using multivariate permutational analysis of variance. In order to investigate whether the presence of the algae reduces the toxicity of Nd and Hg, the results obtained from the biochemical parameters in the mussel soft tissues were submitted to the PERMANOVA routine of the PRIMER v6 software (Anderson et al., 2008), testing the null hypothesis: i) in the absence of the algae, no significant differences exist among treatments (CTL, Nd and Hg). Significant differences are identified in the figures with lower-case letters; ii) in the presence of the algae, no significant differences exist among treatments (CTL, Nd and Hg). Significant differences are identified in the figures with upper-case letters; iii) for each treatment (CTL, Nd and Hg) no significant differences exist between the presence and absence of the algae. Significant differences are identified in the figures with an asterisk. The significance level was set at p < 0.05 and if the main test was significant, pairwise comparisons were performed.

2.4.3. Principal coordinate ordination

The matrix including all biomarker responses was used to generate an Euclidean distance similarity matrix, which was reduced by calculating the distance between centroids based on treatment (CTL, Nd, Hg) with and without algae. This matrix was subsequently subjected to a principal coordinate ordination (PCO) analysis. As supplementary variables, Pearson correlation vectors of biochemical descriptors (correlation >0.75) were provided and placed on the PCO plot.

3. Results

3.1. Chemical concentrations in water

Neodymium concentration in control water was below the limit of quantification, 0.02 µg L⁻¹ (Table 1) while in the treatments with Nd at a

nominal concentration of $70 \mu\text{g Nd L}^{-1}$, the concentration of the element ranged from 74 to $99 \mu\text{g L}^{-1}$.

Mercury was also below the limit of quantification, $0.03 \mu\text{g L}^{-1}$ (Table 1) in control water, while in the treatments with Hg dosed at a nominal concentration of $50 \mu\text{g Hg L}^{-1}$, the mean value was $44 \mu\text{g L}^{-1}$.

3.2. Neodymium and mercury concentrations in mussels' soft tissues and macroalgae

The concentrations of Nd and Hg in the mussel and algae tissues are shown in Table 1. The concentration of Nd in CTL mussels' tissues at the end of the exposure was $0.26 \pm 0.01 \mu\text{g g}^{-1}$ DW, while in those exposed to Nd, without or with the macroalgae, the concentrations were $6.1 \pm$

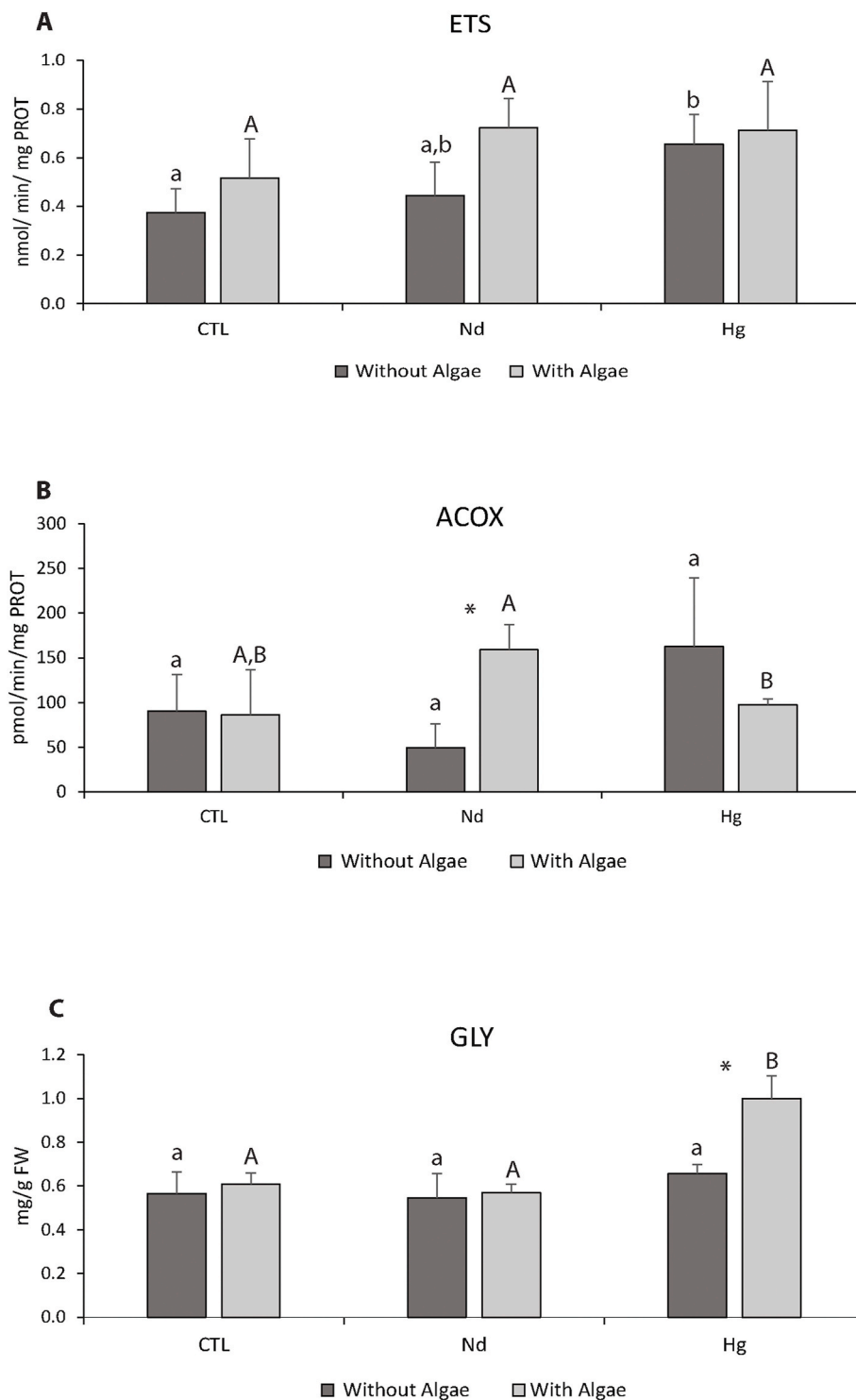


Fig. 1. A: Electron transport system activity (ETS); B: Acyl-CoA oxidase activity (ACOX) and C: Glycogen content (GLY), in *Mytilus galloprovincialis* exposed to different treatments (CTL, Nd, Hg) with and without algae. Results are mean + standard deviation ($n = 3$, wherein one test unit was regarded as an aquarium). Significant differences ($p < 0.05$) among treatments are shown with different lowercase (without algae) and uppercase (with algae) letters. For CTL, Nd and Hg, asterisks represent significant differences between with and without algae treatments.

0.8 $\mu\text{g g}^{-1}$ DW and $5.9 \pm 1.3 \mu\text{g g}^{-1}$ DW, corresponding to bio-concentration factors (BCF) of 83 and 80, respectively. Concerning algae tissues, the concentration of Nd in CTL algae (without mussels and metals) was $0.31 \pm 0.14 \mu\text{g g}^{-1}$ DW, while in those exposed to Nd, without and with mussels, the concentrations were $47 \pm 1.2 \mu\text{g g}^{-1}$ DW and $40 \pm 4.4 \mu\text{g g}^{-1}$ DW, respectively (Table 1).

The concentration of Hg in CTL mussels was $0.32 \pm 0.04 \mu\text{g g}^{-1}$ DW, while in organisms exposed to this element, without and with macro-algae, Hg concentrations were $65 \pm 2.5 \mu\text{g g}^{-1}$ DW and $33 \pm 2.0 \mu\text{g g}^{-1}$ DW, respectively, corresponding to a BCF of 1297 and 661, respectively (Table 1). For the algae tissues, the concentration of Hg in CTL algae (without mussels and metals) was $0.06 \pm 0.01 \mu\text{g g}^{-1}$ DW, while in algae exposed to Hg, without and with mussels, the concentrations were $60 \pm 1.9 \mu\text{g g}^{-1}$ DW and $31 \pm 4.3 \mu\text{g g}^{-1}$ DW, respectively (Table 1).

3.3. Biochemical responses

3.3.1. Metabolic capacity and energy reserves content

No significant variations in electron transport system (ETS) activity were observed in mussels exposed to Nd, neither with nor without algae. A significantly higher ETS activity was observed in mussels exposed to Hg without algae in comparison to CTL, but no significant effects were observed when mussels were co-exposed with algae (Fig. 1A).

No significant differences were found in the Acyl-CoA oxidase (ACOX) activity among treatments without algae. When co-exposed to algae, a significantly lower ACOX activity was observed in mussels treated with Hg in comparison to those treated with Nd, which also showed significantly higher activity in comparison to mussels exposed to

Nd without the algae (Fig. 1B).

No significant differences were found in terms of glycogen (GLY) content among treatments without algae, while significantly higher levels were observed in Hg-contaminated mussels with algae in comparison to the counterpart without algae or the remaining treatments with the algae (Fig. 1C).

3.3.2. Antioxidant defenses

Regardless of the presence or the absence of the algae, catalase (CAT) activity was significantly higher in mussels exposed to Hg in comparison to Nd-exposed ones (Fig. 2A).

No significant differences were found in the glutathione peroxidase (GPx) activity among treatments without algae. In the presence of algae, the activity of GPx activity was significantly higher in mussels exposed to Nd in comparison to CTL and Hg-exposed ones. In mussels exposed to Nd, GPx activity was significantly higher in organisms co-exposed with algae, while the opposite response was observed in Hg treatments, with significantly higher GPx activity without algae (Fig. 2B).

3.3.3. Biotransformation defenses

Among the treatments without algae, glutathione S-transferases (GSTs) activity was significantly lower in mussels exposed to Hg in comparison to Nd-exposed ones, while in the presence of the algae, GSTs activity was significantly higher in mussels exposed to Nd in comparison to CTL and Hg-exposed ones. Between Nd treatments (with and without algae), GSTs activity was significantly higher in organisms co-exposed with algae (Fig. 3A).

Regardless of the presence or absence of the algae, no significant

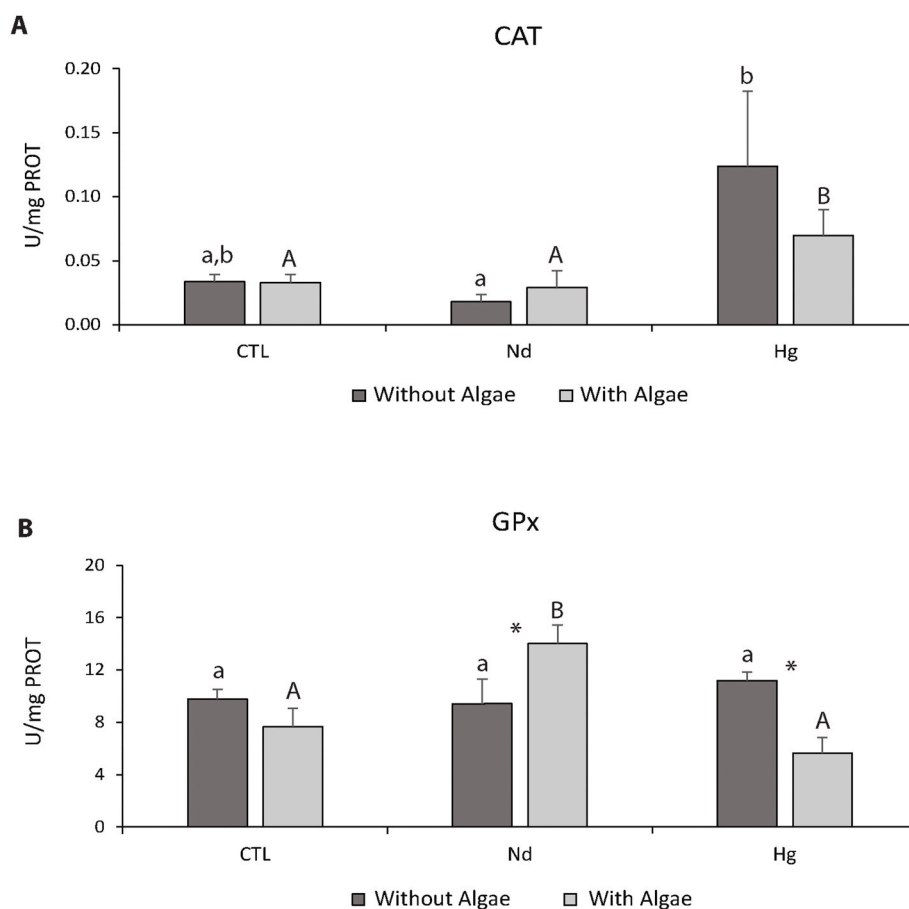


Fig. 2. A: Catalase activity (CAT) and B: Glutathione Peroxidase activity (GPx), in *Mytilus galloprovincialis* exposed to different treatments (CTL, Nd, Hg) with and without algae. Results are mean + standard deviation ($n = 3$, wherein one test unit was regarded as an aquarium). Significant differences ($p < 0.05$) among treatments are shown with different lowercase (without algae) and uppercase (with algae) letters. For CTL, Nd and Hg, asterisks represent significant differences between with and without algae treatments.

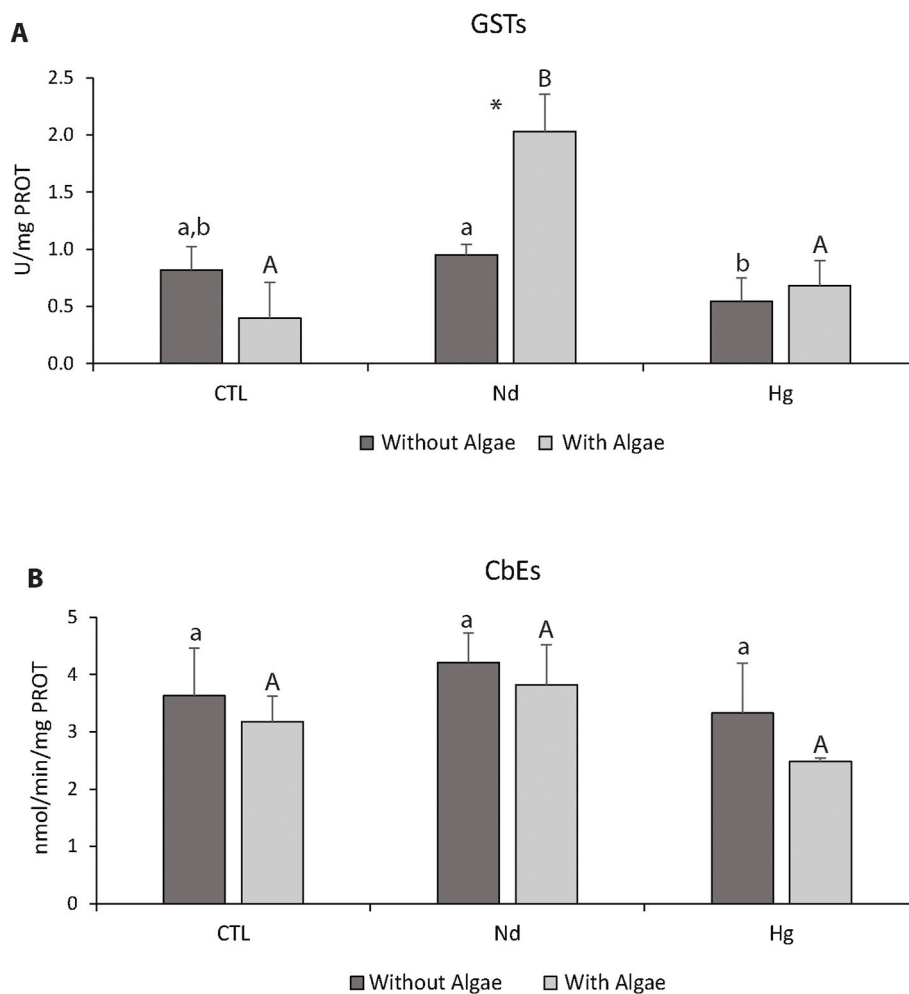


Fig. 3. A: Glutathione S-transferases activity (GSTs) and B: Carboxylesterases activity (CbEs), in *Mytilus galloprovincialis* exposed to different treatments (CTL, Nd, Hg) with and without algae. Results are mean + standard deviation (n = 3, wherein one test unit was regarded as an aquarium). Significant differences ($p < 0.05$) among treatments are shown with different lowercase (without algae) and uppercase (with algae) letters. For CTL, Nd and Hg, asterisks represent significant differences between with and without algae treatments.

differences were observed in carboxylesterases (CbEs) activity among treatments (Fig. 3B).

3.3.4. Redox balance

In the absence of the algae, a lower total oxyradical uptake capacity (TOSC) towards peroxy (ROO•) and hydroxyl (HO•) radicals was observed in mussels exposed to Nd and Hg in comparison to CTL organisms, while when co-exposed with algae this response was observed only in mussels exposed to Hg (Fig. 4A and B).

Regarding TOSC towards peroxynitrite (ONOO⁻), significantly lower values were observed in mussels exposed to Nd and Hg in comparison to CTL ones in the treatments without algae, with the lowest value measured in Hg-exposed organisms. Conversely, no significant differences were found among treatments with algae (Fig. 4C).

The oxidized glutathione (GSSG) content did not show any significant difference among treatments without algae, while in the treatments with algae GSSG levels were significantly higher in mussels exposed to Hg in comparison to CTL and Nd-exposed ones. Mussels exposed to Hg in the presence of algae showed significantly higher GSSG levels than mussels contaminated with the same element but without algae (Fig. 4D).

3.3.5. Cellular damage

No significant differences were found in the lipid peroxidation (LPO) levels among treatments without algae, while LPO levels were

significantly lower in mussels exposed to Nd and Hg in comparison to CTL considering treatments with algae (Fig. 5A).

Treatments without algae showed significantly higher protein carbonylation (PC) levels in mussels exposed to Hg in comparison to Nd-exposed ones, while no significant differences were found on the PC levels among treatments with algae. In Hg-contaminated mussels, PC levels were significantly lower in the presence of the algae (Fig. 5B).

3.4. Principal coordinate ordination

According to the principal coordinate ordination (PCO, Fig. 6), there is a clear distinction between the three different main exposure treatments: CTL, Nd and Hg. In particular, mussels exposed to Nd and Hg tend to group separately and apart from the CTL as a result of a distinct response induced by each metal on mussels' biochemical response. The PCO1 axis explained 54.3% of the total variation, while the PCO2 axis explained 23.1% of the total variation; according to the PCO1, Hg treatments, in the positive side of the axis, were separated from Nd and CTL treatments (without and with algae) in the negative side of the axis. This separation is associated with ACOX activity, ETS activity and GSSG content that presented the highest values in mussels exposed to Hg. Regarding the PCO2 axis, CTL (without and with algae) and Hg + Algae treatments on the negative side were separated from the remaining treatments on the positive side of the axis. This separation was supported by higher total oxyradical scavenging capacity (TOSC ONOO⁻,

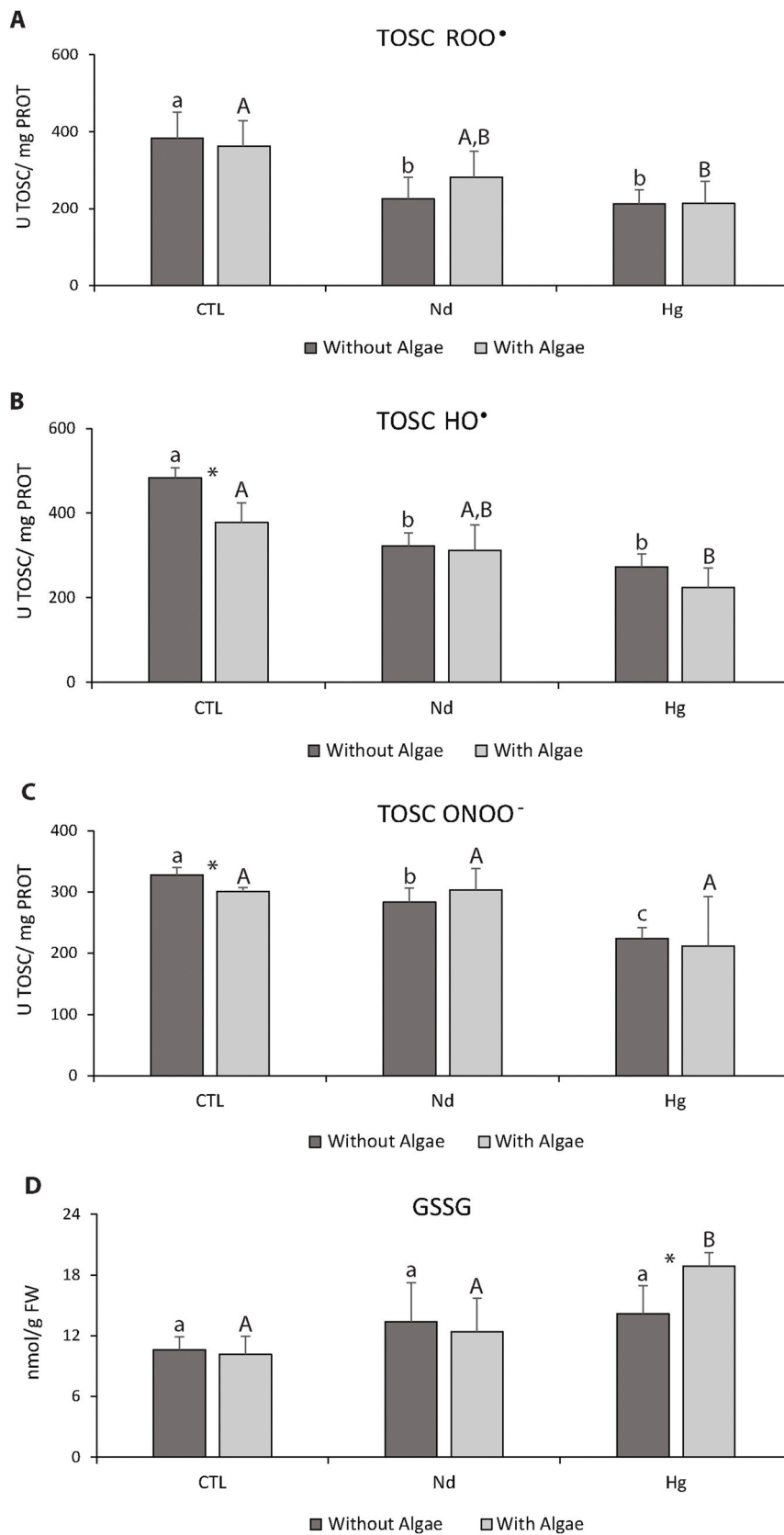


Fig. 4. A: Total oxyradical scavenging capacity toward peroxy radical (TOSC ROO[•]); B: Total oxyradical scavenging capacity toward hydroxyl radical (TOSC HO[•]); C: Total oxyradical scavenging capacity toward peroxynitrite (TOSC ONOO⁻) and D: Oxidized glutathione content (GSSG), in *Mytilus galloprovincialis* exposed to different treatments (CTL, Nd, Hg) with and without algae. Results are mean + standard deviation (n = 3, wherein one test unit was regarded as an aquarium). Significant differences (p < 0.05) among treatments are shown with different lowercase (without algae) and uppercase (with algae) letters. For CTL, Nd and Hg, asterisks represent significant differences between with and without algae treatments.

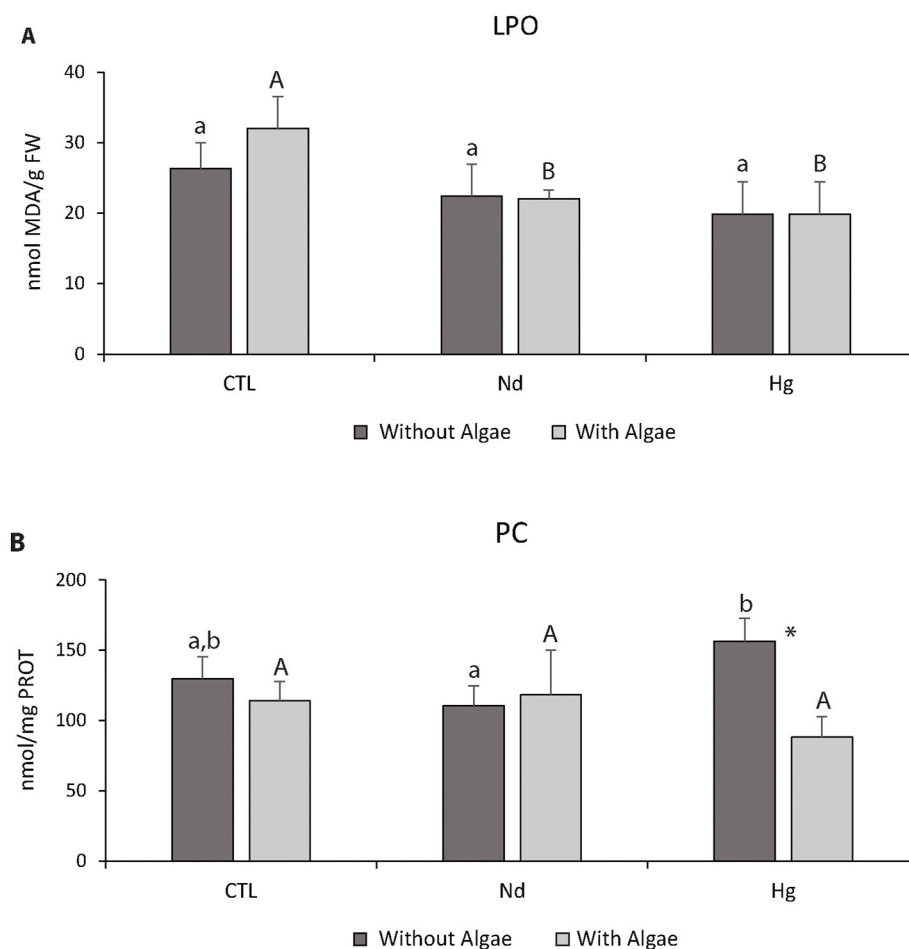


Fig. 5. A: Lipid peroxidation levels (LPO) and B: Protein Carbonylation levels (PC), in *Mytilus galloprovincialis* exposed to different treatments (CTL, Nd, Hg) with and without algae. Results are mean + standard deviation ($n = 3$, wherein one test unit was regarded as an aquarium). Significant differences ($p < 0.05$) among treatments are shown with different lowercase (without algae) and uppercase (with algae) letters. For CTL, Nd and Hg, asterisks represent significant differences between with and without algae treatments.

TOSC HO· and TOSC ROO·) and LPO levels.

4. Discussion

Macroalgae specimens have been commonly used as bioindicators of metal environmental pollution owing to their wide geographical dispersion, as well as their capacity to endure, accumulate, and concentrate these elements compared to the surrounding seawater (Alkan et al., 2020; Bryan and Langston, 1992; Conti and Cecchetti, 2003; García-Seoane et al., 2018; Volterra and Conti, 2000). However, recently, the use of living algae as biosorbents to remove pollutants from seawater has received substantial attention. Several studies demonstrated that, under laboratory conditions, macroalgae possess notable capabilities for this bioremediation due to their efficient uptake of metals. In this regard, previous studies documented the role of green macroalgae (Henriques et al., 2017) and brown macroalgae (Bibi et al., 2023; Cho et al., 2013) in seawater remediation from metals. Furthermore, studies demonstrated that macroalgae are a promising alternative for removing REEs from contaminated waters (Ferreira et al., 2020; Jacinto et al., 2018). Additionally, recent studies have also reported not only the capacity of *Ulva lactuca* to bioremediate seawater contaminated with the REE gadolinium (Gd) but also their role in limiting the toxic effects induced in *Mytilus galloprovincialis* (Trapasso et al., 2021). Smii et al. (2023) also demonstrated the protective role of *U. lactuca* when *M. galloprovincialis* was exposed to titanium (Ti) or Ti nanoparticles (nTi). Although the use of macroalgae to mitigate the impacts of

pollutants in marine ecosystems is mostly related to laboratory-controlled conditions (references above mentioned), their use as nature-based solutions has been recently investigated, especially in what concerns climate mitigation (Edworthy et al., 2023). As an example, the ability of macroalgae to mitigate the negative effects of ocean acidification on different species of bivalves in the North Atlantic was reported (Young and Gobler, 2018). These authors demonstrated that the presence of macroalgae in acidified environments can serve as a refuge for calcified animals that would otherwise be negatively impacted by higher levels of carbon dioxide.

Considering the gap in information regarding the protective role of macroalgae toward marine bivalves under contaminated scenarios, the present study aimed to evaluate the potential toxic effects of Nd and Hg on *M. galloprovincialis*, comparing mussels' biochemical responses in the presence and absence of the macroalgae *U. lactuca*. Previous studies have already highlighted the consequences of exposure to classical metals (namely Hg) and REEs in *M. galloprovincialis* (Coppola et al., 2020; Henriques et al., 2019a; Morais et al., 2023; Pinto et al., 2019), but there is no information on the capacity of *U. lactuca* to mitigate the impacts induced by Hg and Nd on this mussel species. To our knowledge, only two studies reported the ability of *U. lactuca* to protect bivalves against metals by reducing their available concentration in the seawater.

The present study revealed that after 28 days of exposure to Nd (with and without algae) mussels accumulated roughly the same concentration of this element, with similar BCF values. On the other hand, organisms exposed to Hg showed a significantly lower accumulation when

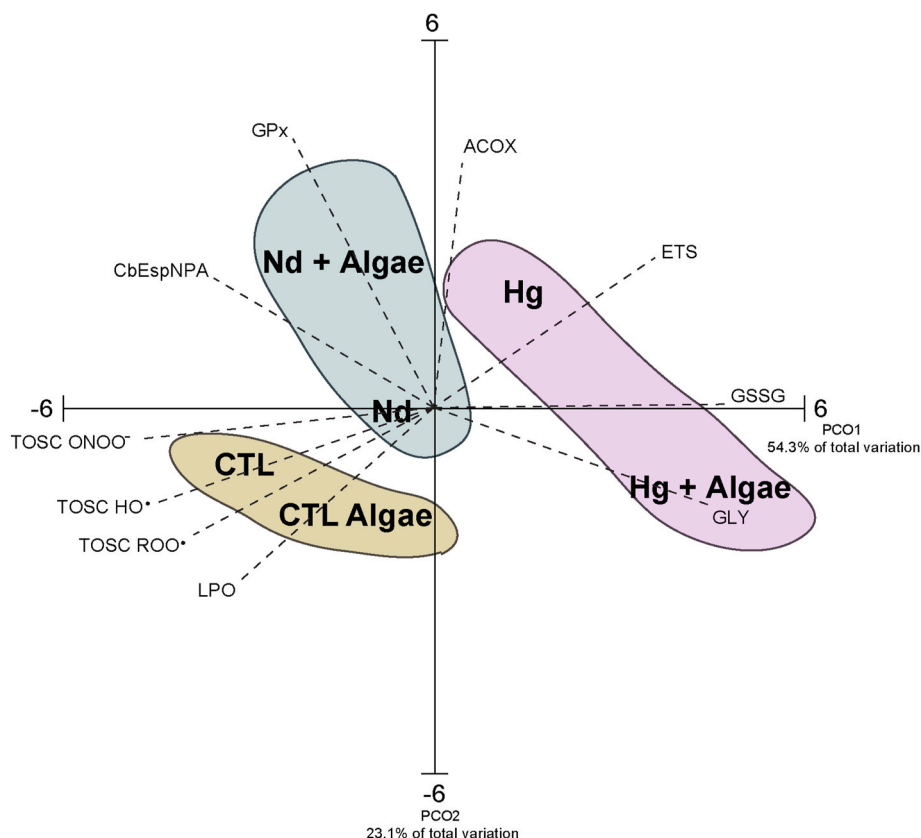


Fig. 6. Centroids ordination diagram (PCO) based on the tested conditions and biochemical markers measured in *Mytilus galloprovincialis*. The experimental conditions are presented as: CTL, Nd, Hg with and without algae. Pearson correlation vectors are superimposed as supplementary variables, namely biochemical data ($r > 0.85$): ETS = Electron transport system activity; ACOX = Activity of the enzyme Acyl-CoA oxidase; GLY = Glycogen concentration; GPx = Glutathione Peroxidase activity; TOSC ROO[•] = Peroxyl radicals; TOSC HO[•] = Hydroxyl radicals; TOSC ONOO⁻ = Peroxynitrite; CbEspNPA = Carboxylesterases activity with substrate *p*-nitrophenyl acetate and LPO = Lipid peroxidation levels.

algae were present, with a BCF value of approximately 50 % lower compared to mussels exposed to Hg without algae. These results were further supported by the accumulation of Hg in algae, confirming the efficient role of *U. lactuca* in the removal of metals from water (Henriques et al., 2017; Rahhou et al., 2023). In Nador lagoon (Morocco) *U. lactuca* was shown to accumulate trace metals, thus attenuating eutrophication processes (Rahhou et al., 2023). Of all the species of macroalgae, available studies show that *U. lactuca* has the greatest capability to remove these elements (Bonanno et al., 2020; Henriques et al., 2017, 2019b; Rahhou et al., 2023). This species is one of the most suitable algae for the uptake of contaminants, due to its large surface area of structurally uniform and physiologically active cells, wide geographic distribution, and elevated tolerance to salinity and temperature variations (Ben-Ari et al., 2014; Chen et al., 2015; Ferreira et al., 2020; Henriques et al., 2015). On the other hand, a limited capability of *U. lactuca* to bioremediate water from Nd was demonstrated by our results. According to Henriques et al. (2017), *U. lactuca* capacity to accumulate metals may depend on the element, with a higher affinity for Hg removal compared to other metals such as cadmium (Cd) and lead (Pb). Pinto et al. (2020) demonstrated that after 72 h of exposure to 10 and 500 $\mu\text{g L}^{-1}$ Nd, *U. lactuca* removed between 70 and 90 % of the element, respectively. Additionally, considering that Henriques et al. (2019b) obtained a great Hg removal after a shorter period (98 % after 12 h) we can assume that the affinity of *U. lactuca* towards Hg was greater than for Nd. Viana et al. (2023) further demonstrated that Hg was mostly accumulated inside algae cells, while REEs were retained on the algae surface. Therefore, all these findings indicate that removal capacity and efficiency may depend on the element, which will influence the final induced toxicity. Thus, in the present study higher removal of

Hg from water explains the greater reduction of the effects caused by this element when in the presence of *U. lactuca*.

Energy availability is crucial to an organism's physiological and biochemical performance under stressful conditions, and it is a vital ability to balance energy reserves with the activity of the mitochondrial electron transport system (ETS) (Smolders et al., 2004). Under exposure to xenobiotics (such as metals), the internal mitochondrial membrane of organisms can be adversely influenced (Guppy and Withers, 1999). The ETS is present in this membrane where organisms use this group of protein complexes for the process of aerobic respiration (Guppy and Withers, 1999). Therefore, the ETS activity allows the quantification of the amount of oxygen consumed if all the enzymes were working at full capacity and has been evaluated to assess the metabolic capacity of aerobic organisms by estimating mitochondrial energy consumption (de Coen and Janssen, 1997). In the present study, an increased metabolic capacity, represented by higher ETS activity, was observed in mussels exposed to Hg (without the algae) in comparison to non-contaminated and Nd-exposed mussels. This response may be related to a higher level of stress caused by Hg in comparison to Nd and the need to activate defense mechanisms. It has already been documented that the presence of metals can induce ETS activity as a defense strategy in bivalves, as is the case in the study of Freitas et al. (2020a) in which *M. galloprovincialis* showed an increased metabolism upon exposure to 40 $\mu\text{g L}^{-1}$ dysprosium. Furthermore, the results demonstrated that when the algae were present, similar metabolic capacity was observed in mussels exposed to CTL treatments, Nd or Hg. As stated above, these results may corroborate the remediation capacity of *U. lactuca*, leading to a lower impact on exposed organisms.

Mitochondrial activity is directly related to energy homeostasis, in

which the enzyme acyl-CoA oxidase (ACOX) plays a key role in lipid metabolism (Reddy and Mannaerts, 1994). This enzyme is located in the peroxisomes and it is involved in the beta-oxidation of fatty acids, being the rate-limiting enzyme in the peroxisomal conversion of long-chain acyl-CoA molecules into *trans*-2-enoyl-CoA, with generation of hydrogen peroxide (H₂O₂) as byproduct (Cajaraville et al., 2003; Cajaraville and Ortiz-Zarragoitia, 2006; Cancio et al., 1998). The most consistent changes that we observed in terms of ACOX activity were related to differences among Nd-exposed organisms with and without algae: we hypothesize that this response is related to a stimulated fatty acids metabolism in mussels exposed to Nd and the algae, probably due to exudates produced by *U. lactuca* (Johnson and Welsh, 1985). A different trend was observed in mussels exposed to Hg, in which the toxicity of Hg limited the activation of peroxisomal fatty acid metabolism, probably in favor of other metabolic pathways. Indeed, previous studies reported reduced ACOX activity in *M. galloprovincialis* exposed to pollution gradient in the NW Mediterranean Sea (Zorita et al., 2007).

The balance between energy production and consumption is crucial to determine how an organism could respond to stressful situations (Sokolova et al., 2012). Energy reserves are essential to rapidly compensate for high energetic demand deriving from stressful conditions (Ansaldò et al., 2006; Sokolova et al., 2012). In the present study, the glycogen (GLY) content was measured to evaluate the organism response when exposed to Nd and Hg. According to our results the maintenance of ETS and the lowered lipid metabolism observed in organisms co-exposed to Hg and algae was compensated by the significant increase of their GLY content, allowing them to cope with the greater energy expenditure during the stress-response phase. As highlighted before (Possik et al., 2015), the present results may indicate that mussels could adjust their metabolism towards the accumulation of GLY in response to stressful conditions. Accordingly, similar results were obtained in previous studies on *M. galloprovincialis* exposed to lanthanum (La), Gd and Hg, showing an increase in energy reserves to maintain normal metabolism (Coppola et al., 2017; Cunha et al., 2022). Trapasso et al. (2021) observed an increased GLY content in mussels exposed to Gd in the presence of *U. lactuca* which also did not exhibit any variation in the electron transport system.

The need to maintain energy homeostasis to support stress-response mechanisms derive from the mode of action of certain contaminants and their cellular targets: indeed, exposure to chemicals, including metals, often leads to increased formation of reactive oxygen species (ROS) in the form of hydrogen peroxide, singlet oxygen, hydroxyl radicals, and superoxide anion (Rahman et al., 2019; Regoli and Winston, 1999; Vlahogianni et al., 2007). With an excessive increase of these ROS the cells can enter a pro-oxidant state causing a progressive loss of their cellular functions or even death (Regoli and Giuliani, 2014). For this reason, cells are provided with a network of enzymes and low molecular scavengers, necessary to counteract oxygen toxicity, such as catalase (CAT) and glutathione peroxidase (GPx) among others (Bojarski et al., 2020; Regoli and Giuliani, 2014). In the present study, Hg exposure with and without algae led to an increased CAT activity, suggesting an increased demand for hydrogen peroxide detoxification mediated by Hg prooxidant effects. These results are in accordance with increased CAT activity observed in *M. galloprovincialis* exposed to 50 µg L⁻¹ of Hg (Coppola et al., 2020). When mussels were co-exposed to Hg or Nd with algae, we observed an increased activity of CAT and GPx, respectively, indicating that despite the presence of algae, the accumulation of metals was not sufficiently limited and there was a need for the activation of antioxidant defense mechanisms. Trapasso et al. (2021) also observed an increase in antioxidant enzyme activity when *M. galloprovincialis* specimens were co-exposed to Gd and algae. Overall, the present findings highlighted that, despite the presence of the algae, Nd and Hg were able to cause disturbance in the oxidative balance of mussels and triggered the activation of ROS-defense mechanisms.

The evaluation of individual antioxidants, such as CAT and GPx, is important for understanding their specific role in neutralizing ROS, but

these responses can be influenced by different prooxidant challenges (Gorbi and Regoli, 2003; Regoli, 2000). For this reason, a more comprehensive and general picture of the antioxidant defenses efficiency was evaluated by measuring the total capacity to neutralize different types of ROS, allowing a more in-depth comprehension of the cellular redox status (Camus et al., 2002; Moraes et al., 2006; Regoli, 2000; Regoli and Winston, 1999). In the present study, despite the limited variations of individual antioxidants, total oxyradical scavenging capacity towards peroxy (ROO•), hydroxyl (HO•) and peroxynitrite (ONOO⁻) generally decreased in all experimental treatments compared to the control, revealing an overall lowered efficiency of antioxidants caused by metals exposure. This was confirmed by the levels of oxidized glutathione (GSSG), which increased in Hg-exposed mussels with algae, addressing the consumption of the reduced form of glutathione (GSH) in response to a greater pro-oxidative challenge due to Hg rather than Nd. Similar changes in GSSG content in Hg treatments may reflect a certain perturbation in the glutathione cycle, leading to an imbalance in the cellular redox status (Regoli and Giuliani, 2014). High levels of GSSG may result from direct oxidation of GSH, indicating potential oxidative stress attributable to the presence of Hg.

In addition to antioxidant enzymes, organisms also have the ability to activate biotransformation enzymes as a form of defense. The conjugation (glutathione S-transferases, GSTs) and hydrolytic (carboxylesterases, CbEs) enzymes catalyze chemical conversion to facilitate excretion of xenobiotics from the cells, or to repair damages from peroxidation mechanisms (Regoli and Giuliani, 2014). In the present study, although Nd accumulation was similar in mussels contaminated with and without algae, a significant increase in the activity of GSTs was observed in Nd-exposed mussels with algae. This could be explained by the increased trend of mitochondrial activity in these organisms, as already observed by Freitas et al. (2020a,b) on *M. galloprovincialis* exposed to 5 µg L⁻¹ of Nd, in which an increased activity of GSTs was associated with a higher metabolism. On the other hand, there was no significant increase in both enzymes GSTs and CbEs for Hg-exposed mussels: this evidence may reflect that other mechanisms, such as metallothioneins, are involved in the detoxification process (Velez et al., 2016), or that the cellular disturbance was overwhelming for the activation of detoxification processes. Morais et al. (2023) and Morosetti et al. (2020) also found that there was no induction of detoxification enzymes (GSTs and CbEs) in *M. galloprovincialis* exposed to 25 µg L⁻¹ and 10 µg L⁻¹ of Hg, respectively, which is consistent with the present results.

When the exposure to pollutants results in excessive production of ROS not properly counteracted by the cellular antioxidant system, these can react with macromolecules as membrane lipids causing oxidation of lipids and proteins (Regoli and Giuliani, 2014). In the present study, lipid peroxidation (LPO) was not increased in any of the treatments, despite the lowered total oxyradical scavenging capacity. As previously reported, antioxidant enzymes were activated with and without algae by both contaminants (Nd and Hg), thus allowing us to hypothesize that with longer exposure periods the magnitude of stress could have targeted lipids integrity, especially in the absence of the algae. Regarding PC levels, there was a significant increase in this typology of damage in organisms exposed to Hg without algae, while in those exposed to algae, the PC decreased highlighting the role of *U. lactuca* in preventing protein degradation. According to the study by Pinto et al. (2019), a decrease in LPO and PC levels was also seen in *M. galloprovincialis* exposed to La. Also, in the study of Smii et al. (2023), the potential of *U. lactuca* in preventing protein damage was verified showing that in *M. galloprovincialis* exposed to Ti and nTi the PC levels increased, while a significant decrease was obtained in the presence of *U. lactuca*.

Overall, in terms of biological responses independently on the presence or absence of the algae, the biochemical alterations observed in organisms exposed to Nd and Hg were distinct, as revealed by the PCO analysis, being also isolated from the CTL. With the results obtained from the PCO analysis, it was clear that 3 groups were distinguished

(CTL, Nd and Hg). The positive axis of PCO1 is characterized by the group of Hg closely associated with higher GSSG content. Mussels exposed to Hg in the presence of algae are characterized by higher GLY content while the ones contaminated with Hg but without the algae were characterized by higher ETS activity. The other two groups (Nd, CTL) are on the negative axis of PCO1 with the Nd group on the positive axis of PCO2 and the CTL group on the negative axis of PCO2. Nd-exposed mussels are closely associated with higher values of GPx and CbEs activity while uncontaminated mussels were characterized by higher total oxyradical scavenging capacity (TOSC) and LPO levels.

5. Conclusion

Overall, the present study revealed that Hg induced higher toxicity compared to Nd, although Hg impacts were limited by the presence of *U. lactuca* which contributed to lower Hg accumulation by mussels. Conversely, *U. lactuca* did not appear to protect mussels from the Nd-contaminated seawater, as the presence of the macroalgae did not prevent Nd-accumulation by the mussels. Nevertheless, compared to Hg, Nd showed limited cellular toxicity for *M. galloprovincialis*. The results obtained in this study contribute to the advancement of knowledge in this field, namely to the understanding of the impacts of metals on bivalves and the crucial role of algae in the bioremediation of water bodies. Furthermore, the knowledge gathered with this study will contribute to achieving the objectives of the Sustainable Development Goals (SDGs), including SDG14 ("Conserving Marine Biodiversity"), by reporting the impacts caused by pollutants on marine wildlife, and SDG 9 ("Industry, Innovation and Infrastructure") by demonstrating the potential use of macroalgae as biosorbents to minimize the impacts of pollutants. However, the present findings should be considered with caution since the results may differ when considering other ecosystem components, such as microbial communities or other aquatic organisms. Therefore, further research should be conducted using other species to evaluate the role of macroalgae in the protection against metal pollution.

CRedit authorship contribution statement

Marta Cunha: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Alessandro Nardi:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Bruno Henriques:** Methodology, Writing – review & editing. **Amadeu M.V.M. Soares:** Funding acquisition, Resources. **Eduarda Pereira:** Funding acquisition, Resources, Writing – review & editing. **Francesco Regoli:** Funding acquisition, Resources, Writing – review & editing. **Rosa Freitas:** Conceptualization, Funding acquisition, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2024.141908>.

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