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# Remediation of Arsenic from contaminated seawater using manganese spinel ferrite nanoparticles: ecotoxicological evaluation in *Mytilus galloprovincialis*

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## ABSTRACT

In the last decade different approaches have been applied for water remediation purposes, including the use of nanoparticles(NPs) to remove metals and metalloids from water. Although studies have been done on the toxic impacts of such NPs, very scarce information is available on the impacts of water after decontamination when discharged into aquatic environments. In this way, the present study we aimed to evaluate the ecotoxicological safety of seawater previously contaminated with arsenic (As) and remediated by using manganese-ferrite (MnFe2O4) nanoparticles (NPs). For this, mussels *Mytilus galloprovincialis* were exposed for 28 days to different conditions, including clean seawater (control), As (1000 µg $L^{-1}$) contaminated and remediated (As 70 µg $L^{-1}$) seawater, water containing manganese-ferrite (MnFe2O4) nanoparticles (NPs) (50 mg $L^{-1}$) with and with the presence of As. At the end of exposure, concentrations of As in mussels tissues were quantified and biomarkers related to mussels' metabolism and oxidative stress status were evaluated. Results revealed that mussels exposed to water contaminated with As and to As+NPs accumulated significantly more As (between 62% to 76% more) than those exposed to remediated seawater. Regarding biomarkers, our findings demonstrated that in comparison to remediated seawater (conditions a, b, c) mussels exposed to contaminated seawater (conditions A, B, C) presented significantly lower metabolic activity, lower expenditure of energy reserves, activation of antioxidant and biotransformation defences, higher lipids and protein damages and greater AChE inhibition. Furthermore, organisms exposed to As, NPs or As+NPs revealed similar biochemical effects, both before and after water decontamination. In conclusion, the present study suggests that seawater previously contaminated with As and remediated by manganese-ferrite (MnFe2O4) NPs presented significantly lower toxicity than As contaminated water, evidencing the potential use of these NPs to remediate seawater contaminated with As and its safety towards marine systems after discharges to these environments.



**Keywords:**

Oxidative stress; Toxicity; Mussels; Magnetic spinel ferrite nanoparticles; nanosorbents; Metalloids; Bioaccumulation.

## 1. INTRODUCTION

The increment of pollutants in aquatic environments is closely related with the growth of the world population (Zhang et al., 2015). Studies demonstrated that intense urbanization and industrial activities, with the associated effluents, result in an increase of pollution in the aquatic systems, especially in marine environments (Nardi et al., 2017, Belivermiş et al., 2016; Oliveira, 2015; Ventura-Lima et al., 2011). Often, the final destination of pollutants are lagoons and estuaries (Zhang et al., 2015), with tendency to be accumulated not only in sediments but also by organisms inhabiting these areas (Buffet et al., 2014; Ventura-Lima et al., 2009, 2011). Among the most common pollutants in aquatic environments is arsenic (As), a naturally occurring element (ATSDR, 2015; Saxe et al., 2006) released by natural activities, such as volcanism, dissolution of minerals (particularly into groundwater), but also by human activities, such as mining, metal smelting, combustion of fossil fuels, agricultural pesticide production and use, remobilization of historic sources, including mine drainage water (WHO, 2010; Mandal and Suzuki, 2002; Bhattacharya et al., 2007; Matschullat, 2000; Jang et al., 2016). As a results of its high toxicity, even at trace levels, As presents environmental concerns (IARC, 2012; Quasimeme, 2003; Fattorini et al., 2006). For this reason, currently As is considered the most priority hazardous substance in the environment based on the combination of substance frequency, toxicity and human exposure potential (ATSDR, 2015; Khan et al., 2010). In particular, the presence of As in aquatic systems has already proven to induce toxic impacts in a diversity of species, namely in bivalves, including physiological and biochemical impairments in clams (Freitas et al., 2018) and mussels (Coppola et al., 2018).

Because of aquatic pollution and associated concerns, nowadays an important research topic is the development of new technologies for wastewater decontamination (Gehrke et al., 2015; Davidescu et al., 2015). Different methodologies have been developed to remove pollutants from waters, including oxidation/precipitation (Leupin et. al, 2005; Dutta et. al, 2005; Lee et al., 2002), coagulation/co-precipitation (Hansen et al., 2006; Kumar et al., 2004), sorption, ion-exchange (Baciocchi et al., 2005; Kim and Benjamin, 2004), membrane technologies (Kim et al., 2006; Ballinas et al., 2004), solvent extraction and bioremediation (Kordmostafapour et al., 2006; Iberhan et al., 2003; Katsoyiannis et al., 2002). Some of these

techniques have shown a great potential for removing inorganic pollutants from water (Gehrke et al., 2017; Mohan et al., 2006). Among the innovative techniques, one of the most promising approaches to decontaminate water is based on the use of nanoparticles (NPs), with some laboratory studies evidencing their high effectiveness in the removal of metal(loid)s (Tavares et al., 2013; Zhang et al., 2010; Mohan and Pittman, 2007). In particular, manganese-ferrite ($MnFe_2O_4$) nanoparticles (NPs), a common spinel ferrite material has shown to be very effective in decreasing inorganic pollution (including metals and metalloids) in freshwater and seawater (Zhang et al., 2010; Tavares et al., 2013; Jang et al., 2016; Santhosh et al., 2014). However, althought the use of $MnFe_2O_4$-NPs for water decontamination is undoubtedly one of the most challenging research areas, important aspects are still missing, such as the potential toxicity of these NPs and the ecotoxicological evaluation of the remediated water (Bhatt and Tripathi, 2011; Lovern and Klaper, 2006; Lovern et al., 2007; Smith et al., 2007; Warheit et al., 2007). Together with decontaminated water or resulting from leaching of chemical elements, after application these NPs can end up in aquatic environments, making crucial the assessment of decontaminated water potential impacts towards inhabiting organisms. Until now, different studies have already demonstrated the impacts induced directly by magnetic $MnFe_2O_4$ spinel ferrite NPs in algae, crustaceans and fish, revealing their potential hazard potential to different aquatic species (Bahadar et al., 2016; Beji et al., 2010; Aslibeiki et al., 2016; Federici et al., 2007). Nevertheless, no studies have been carried out to evaluate the toxicity of water decontaminated by these NPs.

To evaluate the impacts of the presence of pollutants, including NPs, in the aquatic environment, benthic species are a good biological model as they accumulate and reflect the impacts of different substances (Velez et al., 2015; Attig et al., 2014; Banni et al., 2014a; Hu et al., 2015; Nardi et al., 2017; Coppola et al., 2018; Freitas et al., 2018). Among these species is the mussel *Mytilus galloprovincialis*, identified by several authors as a good bioindicator with the capacity to respond to environmental disturbances, presenting a wide spatial distribution and economic relevance (Coppola et al., 2017; Richir and Gobert, 2014; Freitas et al., 2017; Kristan et al., 2015; Mejdoub et al., 2017). This bivalve is a sedentary filter-feeder and has a large capacity to accumulate pollutants (Coppola et al., 2018; Livingstone et al., 2000; Selvin et al., 2000).

Thus, by the above-mentioned, an important topic of research is to understand if the application of NPs to decontaminate seawater still constitutes a threat to aquatic environment, affecting negatively the inhabiting organisms. For this reason, the present study aimed to evaluate the toxicity induced in the mussel *M. galloprovincialis* exposed to seawater previously contaminated with As and decontaminated with $MnFe_2O_4$, NPs. After exposure to decontaminated seawater, biomarkers related to mussels' metabolic, oxidative stress and neurotoxic status were evaluated.

## 2. MATERIALS AND METHODS

### 2.1 Experimental conditions

The Mediterranean mussel *Mytilus galloprovincialis* was selected as biological model for this study (e.g. Coppola et al., 2018; Della Torre, 2015; Gomes et al., 2011). Organisms were collected in November 2017, at the Ria de Aveiro lagoon (Portugal), with a mean body weight of 21.3 ± 6.6 g, fresh weight (FW).

Bivalves were transported from the field to the laboratory in plastic containers, where they were placed in aquaria for depuration and acclimation to laboratory conditions for 2 weeks. To simulate field conditions, in the laboratory organisms were exposed to: temperature 18.0 ± 1.0 ºC; pH 8.0 ± 0.1, photoperiod 12 h light and 12 h dark, and continuous aeration, in artificial seawater (salinity 30 ± 1) (Tropic Marin® SEA SALT from Tropic Marine Center). Seawater was renewed daily during the first week and then every three days until the end of the acclimation period.

After the acclimation period organisms were distributed in different aquaria according to the conditions described in Table 1. Seven different conditions were evaluated, with 3 aquaria (containing 3 L of seawater each) per condition and 4 individuals per aquarium/replicate (12 individuals per condition).

Decontaminated seawater was obtained by adding 50 mg $L^{-1}$ of $MnFe_2O_4$ NPs to water previously contaminated with 1000 µg $L^{-1}$ of As. The NPs were removed from seawater after 24 hours by applying a magnetic field (although a non-quantifiable residual amount of NPs may hypothetically remain in water) as described by Mohmood et al. (2016).

During the experimental period (28 days), water medium was changed weekly and exposure conditions completely re-established, including contaminants concentrations and seawater characteristics (salinity, pH, temperature). Every week, immediately after medium renewal, samples of seawater were collected from each aquarium for As quantification.

The concentration of As, 1000 µg $L^{-1}$, was selected according to the emission limit value for this element in wastewater discharges (Decree-Law No. 236/98, in Portuguese), while 70 µg $L^{-1}$ is the residual concentration of As reached in seawater after decontamination with $MnFe_2O_4$, NPs (data from preliminary experiments, not shown).

During the entire experimental period (28 days) aquaria were continuously aerated, with a 12 light: 12 dark photoperiod. As for the acclimation, temperature (17 ± 1.0 ºC), pH (8.0± 0.1) and salinity (30 ± 1) values were selected considering measurements done at the sampling site (data not provided), and were daily checked and adjusted if necessary.

During the experimental period organisms were fed with Algamac protein plus (150,000 cells/animal) twice a week. Mortality was also daily checked, with 100% of survival recorded during the experimental period.

At the end of the exposure period, organisms were frozen individually with liquid nitrogen and stored at -80ºC, until homogenization of each individual soft tissue using a mortar and a pestle under liquid nitrogen. Each homogenized organism was divided into aliquots (0.5 g each) for biomarkers analyses and As quantification.

## 2.2 Synthesis and characterization of $MnFe_2O_4$ nanoparticles

$MnFe_2O_4$ nanoparticles were prepared by the chemical oxidative hydrolysis of a mixture of $FeSO_4.7H_2O$ and $MnSO_4.H_2O$ in alkaline conditions. Different techniques were applied to perform the chemical, physical and structural characterization of NPs. The morphology and particle size of the NPs were confirmed by transmission electron microscopy (TEM) using the Hitachi H-9000 TEM microscope operating at 300 kV. For TEM analysis, one drop of sample dispersed in ethanol was placed onto carbon-coated copper grid and then let the solvent evaporate. The surface area of the NPs was determined by $N_2$ adsorption/desorption on a Gemini V2.0 Micromeritics instrument. The crystalline phase of the NPs was identified by x-ray powder diffraction of the powders using a Philips Analytical PW 3050/60 X'Pert PRO (θ/2θ) diffractometer equipped with an X'Celerator detector and with automatic data acquisition (X'Pert Data Collector v2.0b software) by a monochromatized Cu Kα radiation (λ = 1,54056 Å) at 45 Kv/40 Ma. The NPs Fourier-Transform Infrared (FT-IR) spectrum was recorded on a Mattson 7000 spectrometer, at 4 $cm^{-1}$ resolution, using a horizontal attenuated total reflectance (ATR) cell.

The average size distribution of $MnFe_2O_4$ NPs in water at salinity 30 were measured by Dynamic Light Scattering (DLS) at T0 (immediately injected into seawater media), T1 (after 1 hour) and T24 (after 24 hours) (Table 2). These time periods were selected based on previous

studies (Yao et al., 2014, Yang et al., 2012; Aubery et al., 2011) that showed aggregation and precipitation of different Fe-NPs within 24 h. DLS measurements were performed on a Delsa Nano C from Beckman Coulter, Inc. (Fullerton, CA) equipped with a laser diode operating at 658 nm. Scattered light was detected at 165° angle and analysed by using a log correlator over 120 accumulations, for 1.0 mL of sample in a UV cuvette semi-micro. Each sample was reproducibly shaken before analysis and exposed to the minimum of DLS measurements needed to obtain at least three valid data. The calculation of particle size distribution and distribution averages was performed by using CONTIN particle size distribution analysis routines through Delsa Nano 3.73 software. The hydrodynamic radius and polydispersity index of the analysed dispersions were calculated on three replicates of each sample by using the cumulant method. Undetected colloidal material at the end of each measurement is indicated as Invalid data (I.d.).

## 2.3 Arsenic quantification

The quantification of As in water samples collected from each condition (Table 3) was performed by inductively coupled plasma mass spectrometry (ICP-MS), on a Thermo ICP-MS X Series equipped with a Burgener nebulizer. The quantification limit of the method was 1 µg/L (n = 12), with an acceptable relative standard deviation among replicates (n≥2) < 5% (Henriques et al., 2019).

Total As concentrations in *M. galloprovincialis* whole soft tissues (Table 4) were quantified by ICP-MS, after microwave assisted acid digestion. Samples with 100–200 mg (freeze-dried) were digested in a CEM MARS 5 microwave, firstly with 2 mL of $HNO_3$ (70%) at 170 ℃ for 15 min, followed by a second identical m icrowave cycle with 0.5 mL of $H_2O_2$ (30%). After addition of $H_2O_2$, the mixture was allowed to stand for 15 min so that the microwave reaction was not as violent. The obtained digests were transferred into 25 mL polyethylene vessels and the volume made up with ultrapure water. The quality control was assured by running procedural blanks (reaction vessels with only $HNO_3$ and $H_2O_2$) and certified reference material TORT-2 (Lobster Hepatopancreas; 21.6 ± 1.8 mg $Kg^{-1}$ As) in parallel with samples. Blanks were always below the quantification limit and mean percentage of recovery for As was 110 ± 4% (n = 4) (Coppola et al., 2018).

## 2.4 Biomarkers evaluation

The whole tissue of mussels was used for biomarkers determination (see section 2.1). For each parameter, 0.5 g of tissue per organism was used, with 2 individuals per replicate (6 per condition). For each condition, metabolic capacity (electron transport system activity, ETS), energy-related (glycogen content, GLY; total protein content, PROT), antioxidant defence (superoxide dismutase activity, SOD; glutathione peroxidase activity, GPx; glutathione S-transferases activity, GSTs), oxidative damage (lipid peroxidation levels, LPO; protein carbonyl levels, PC; glutathione content ratio, GSH/GSSG) and neurotoxicity (Acetylcholinesterase activity, AChE) biomarkers were assessed. Each sample was performed at least in duplicate. All measurements were done using a microplate reader (BioTek, Synergy HT). The extraction for each biomarker was performed with specific buffers: phosphate buffer for SOD, GPx, GSTs, PROT, GLY, CP and AChE; magnesium sulphate buffer for ETS; trichloroacetic acid buffer for LPO and KPE buffer for GSH/GSSG. Each sample was sonicated for 15 s at 4 ºC and centrifuged for 25 min (or 15 min for GSH/GSSG) at 10 000 *g* (or 3 000 *g* for ETS) (Coppola et al., 2018; De Marchi et al., 2018; Freitas et al., 2018). Supernatants were stored at -20 ºC and used within a maximum period of 3 weeks.

### *Metabolic capacity and energy-related biomarkers*

ETS activity was measured based on King and Packard (1975) and the modifications performed by De Coen and Janssen (1997). The absorbance was measured at 490 nm during 10 min with intervals of 25 s. The amount of formazan formed was calculated using ε = 15,900 $M^{-1}cm^{-1}$ and the results expressed in nmol $min^{-1}$ per g of fresh weight (FW).

For GLY quantification the sulphuric acid method was used, as described by (Dubois et al., 1956). A calibration curve was obtained using glucose standards prepared in concentrations between 0 and10 mg $mL^{-1}$. Absorbance was measured at 492 nm and the results were expressed in mg per g FW.

The PROT content was determined following the spectrophotometric method of Biuret (Robinson and Hogden, 1940), and bovine serum albumin (BSA) was used as standard (0–40 mg $mL^{-1}$) to obtain a calibration curve. Absorbance was measured at 540 nm. Concentrations of PROT were expressed in mg per g FW.

*Antioxidant defences biomarkers*

The activity of SOD was quantify following the method of Beauchamp and Fridovich (1971) and was performed with a calibration curve using SOD standards between 0.25 and 60 U $mL^{-1}$. The absorbance was measured at 560 nm and the results were expressed in U per g of FW, where U represents the quantity of the enzyme that catalyses the conversion of 50% of nitroblue tetrazolium (NBT).

The activity of GPx was determined following the method of Paglia and Valentine (1967). Absorbance measurements were performed at 340 nm during 5 min in 10 s intervals and the activity was determined using the extinction coefficient of $\varepsilon$ = 6.22 $mM^{-1}$ $cm^{-1}$. Results were expressed in U/g FW, where U corresponds to the quantity of enzyme which catalyzes the conversion of 1 µmol nicotinamide adenine dinucleotide phosphate (NADPH) per min.

GSTs activity was determined according to Habig et al. (1976). The absorbance was measured at 340 nm. The activity of GSTs was determined using $\varepsilon$=9.6 $mM^{-1}$ $cm^{-1}$. The enzymatic activity was expressed in U per g of FW where U is defined as the amount of enzyme that catalysis the formation of 1 µmol of dinitrophenyl thioether per min.

*Oxidative damage biomarkers*

LPO was determined following the method described by Ohkawa et al. (1979). LPO levels were measured trough the quantification of malondialdehyde (MDA), a by-product of lipid peroxidation. Absorbance was measured at 532 nm ($\varepsilon$=156 $mM^{-1}$ $cm^{-1}$). LPO levels were expressed in nmol of MDA per g FW.

PC content was obtained following Levine et al. (1990). Absorbance of samples was measured at 370 nm and the carbonyl content was calculated using an absorption coefficient ε= 0.022 $mM^{-1}$ $cm^{-1}$. Results were expressed in nmol of PC groups formed per g FW

GSH and GSSG glutathione contents were measured at 412 nm (Rahman et al., 2014) and used as standards (0–60 µmol $L^{-1}$) to obtain a calibration curve. Absorbance was measured at 412 nm, for both assays. The results were expressed as nmol per g of FW. The ratio GSH/GSSG was determined taking in account the number of thiol equivalents (GSH/ 2 ∗ GSSG).

*Neurotoxicity biomarker*

Acetylthiocholine iodide (ATChI, 470 µmol $L^{-1}$) substrates were used for the determination of Acetylcholinesterase (AChE) following the methods of Ellman et al. (1961) and modification by Mennillo et al. (2017). Enzyme activity was recorded continuously for 5 min at 412 nm and expressed in nmol per g FW.

## 2.5 Integrated biomarker response (IBR)

The integrated biomarker response (IBR) was calculated according to Beliaeff and Burgeot (2002) aiming to evaluate the general mussel's biochemical response among 6 conditions. All biomarkers determined were used in the calculation of the IBR and they were arranged clockwise in the following order: ETS, GLY, PROT, SOD, GPx, LPO, CP, GSH/GSSG, GST, AChE. Values were discussed in terms of a general response given by the final IBR value, where higher values correspond to higher mussels' response.

## 2.6 Statistical analyses

All the biochemical results (ETS, GLY, PROT, SOD, GPx, GSTs, LPO, PC, GSH/GSSG and AChE) and As concentrations in mussels tissues, obtained from each condition, were submitted to statistical hypothesis testing using permutational analysis of variance, employing the PERMANOVA+add-on in PRIMER v6 (Anderson et al., 2008). The pseudo-F *p*-values in the

PERMANOVA main tests were evaluated in terms of significance. When significant differences were observed in the main test, pairwise comparisons were performed. Values lower than 0.05 ($p \leq 0.05$) were considered as significantly different. For each biomarker, *p*-value obtained for pair-wise comparisons are represented with *p*-value in Table 5.

For As concentrations and each biomarker, the null hypotheses (H0) tested were: i) no significant differences exist among CTL and all the contaminated conditions (CTL, A, B and C). *p*-values are presented in Table 5, with significant differences highlighted in bold; ii) no significant differences exist among decontaminated conditions (CTL, a, b, and c). *p*-values are presented in Table 5, with significant differences highlighted in bold; iii) no significant differences exist between A vs a, B vs b, C vs c conditions. Significant differences between each pair of conditions are represented with an asterisk in figures.

# 3. RESULTS

## 3.1 Characterization of $MnFe_2O_4$ nanoparticles

$MnFe_2O_4$ NPs showed a spheroidal morphology (Figure 1) with a mean diameter and standard deviation of 75 ± 15 nm. The infrared spectrum of the NPs displayed a characteristic band at 537 $cm^{-1}$ related to metal-O stretching vibration of the $MnFe_2O_4$ NPs (Bellusci et al., 2009; Mehran et al., 2016; Tavares et al., 2013). The band at 1107 $cm^{-1}$ was attributed to metal-OH and to metal-$OH_2$ stretching vibrations, which correspond to water sorption on oxide, while 1635 $cm^{-1}$ band is due to H-O-H bending and corresponds to molecular water adsorbed or incorporated into the crystalline lattice (Bellusci et al., 2009). The broad band at 3309 $cm^{-1}$ corresponds to symmetric and asymmetric stretching of O-H bond (Margabandhu et al., 2016). Powder X-ray diffraction (XRD) pattern show peaks that are characteristics of the presence of $MnFe_2O_4$ with the spinel structure (JCPDS–International centre diffraction data, PDF card 01-071-4919). In seawater, an aggregation of the NPs was observed by DLS, reaching an average size of approximately 60000 nm, after 24 hours. The presence of As in water did not influence NPs aggregation since sizes in conditions A, B, a and b, after 24 hours, the average sizes were statistically indistinguishable.

## 3.2 Arsenic quantification in seawater and mussels' tissues

Concentrations of As in water samples revealed that real and nominal concentrations were similar, both for A and a conditions. In water samples from conditions without As (B and b) the concentrations of this metalloid were lower than the quantification limit (1.5 µg $L^{-1}$). Concentration of As in water after decontamination was 55 ± 13 µg $L^{-1}$. Because sorption of As by the NPs is extremely rapid, As was not possible to quantify in water from condition C (Table 3).

The results obtained from As quantification in mussels showed a significant difference between organisms exposed to CTL and those exposed to A and C conditions (Table 4).

No significant differences were found between *M. galloprovincialis* submitted to CTL and the organisms exposed to conditions a, b and c (Table 4).

Significant differences were observed in terms of As concentrations between mussels exposed to initial (before decontamination) and final (after decontamination) conditions (A vs a

and C vs c) (Table 4). Organisms exposed to condition A accumulated more 76% of As than those exposed to condition a, while the contents of As in the mussels exposed to condition C were 62% higher than those in condition c.

## 3.3 Biochemical markers

*Metabolic capacity and energy-related biomarkers*

The ETS activity was significantly higher at control (CTL) in comparison to the values obtained in mussels exposed to As contaminated seawater (conditions A, B, C; resembling initial concentrations, measured before decontamination), with the lowest values at condition A (Figure 2A, Table 5). ETS activity was significantly higher at control (CTL) in comparison to the values obtained in mussels exposed to decontaminated seawater (conditions a, b, c) (Figure 2A, Table 5).

The ETS activity was significantly higher in organisms exposed decontaminated seawater (conditions a, b, c) in comparison to organisms exposed to As contaminated seawater (conditions A, B, C) (Figure 2A).

The GLY content was significantly lower in mussels exposed to control (CTL) in comparison to the values observed in mussels exposed to As contaminated seawater (conditions A, B, C) (Figure 2B, Table 5).

Significantly lower GLY content was obtained in organisms exposed to decontaminated seawater (conditions a, b, c) in comparison to organisms exposed to As contaminated seawater (conditions A, B, C) (Figure 2B).

The PROT content was significantly lower in mussels exposed to control (CTL) in comparison to values observed in mussels exposed to As contaminated seawater (conditions A, B), while no significant differences were observed between CTL and C conditions (Figure 2C, Table 5).

The PROT content was significantly lower in organisms exposed to decontaminated seawater (conditions a, b, c) in comparison to organisms exposed to contaminated seawater (conditions A, B, C) (Figure 2C).

*Antioxidant defence biomarkers*

The SOD activity was significantly lower at CTL in comparison to values obtained in mussels exposed to As contaminated seawater (A, B, C) (Figure 3A, Table 5). Significantly higher values were obtained in mussels exposed to condition A in comparison to organisms exposed to conditions B and C (Figure 3A, Table 5).

The SOD activity was significantly lower in organisms exposed to decontaminated seawater (conditions a, b, c) in comparison to organisms exposed to contaminated seawater (conditions A, B, C) (Figure 3A).

The activity of GPx was significantly lower at CTL in comparison to values obtained in mussels exposed to contaminated seawater (A, B, C) (Figure 3B, Table 4). Significant differences were observed between organisms exposed to A and C conditions (Figure 3B, Table 5).

Regarding to organisms exposed to decontaminated seawater, significantly higher GPx activity was observed at a, b and c conditions in comparison to control (CTL) (Figure 3B and Table 5). No significant differences were observed between organisms exposed to conditions a and b (Figure 3B, Table 5).

The GPx activity values were significantly lower in organisms exposed decontaminated seawater (conditions a, b, c) in comparison to organisms exposed to contaminated seawater (conditions A, B, C) (Figure 3B).

The GSTs activity was significantly lower at CTL in comparison to values obtained in mussels exposed to contaminated seawater (A, B, C) (Figure 3C, Table 5). No significant differences were observed between organisms exposed to A and C conditions (Figure 3C, Table 5).

Organisms under control (CTL) conditions showed significantly lower GSTs activity than those exposed to decontaminated seawater (condition c) (Figure 3C and Table 4). No significant differences were observed between organisms exposed to a and c conditions (Figure 3C, Table 5).

The GSTs activity values were significantly lower in organisms exposed to decontaminated seawater (conditions a, b, c) comparatively to organisms exposed to contaminated seawater (conditions A, B, C) (Figure 3C).

*Cellular damage biomarkers*

The LPO levels were significantly lower at control (CTL) in comparison to values obtained in mussels exposed to contaminated seawater (conditions A, B, C) (Figure 4A, Table 4). Significantly lower LPO values were found in organisms exposed to condition B in comparison to organisms exposed to conditions A and C (Figure 4A, Table 5).

Significantly lower LPO levels were observed in organisms exposed to CTL compared to organisms exposed to condition a, b and c (Figure 4A and Table 5). No significant differences were observed among organisms exposed to a, b and c conditions (Figure 4A, Table 5).

The LPO levels were significantly lower in organisms exposed to decontaminated seawater (conditions a, b, c) in comparison to organisms exposed to contaminated seawater (conditions A, B, C) (Figure 4A).

The PC levels were significantly lower in mussels exposed to control (CTL) in comparison to values observed in mussels exposed to contaminated seawater (conditions A, B, C) (Figure 4B, Table 5).

The PC levels in mussels exposed to control (CTL) were significantly lower than those observed in mussels exposed to conditions a and b (Figure 4B, Table 4). No significant differences were observed among organisms exposed to a, b and c conditions (Figure 4B, Table 5).

The PC levels were significantly lower in organisms exposed to decontaminated (conditions a, b and c) seawater comparatively to organisms exposed to contaminated seawater (conditions A, B, C) (Figure 4B).

The GSH/GSSG values were significantly higher in mussels exposed to control (CTL) in comparison to values observed in mussels exposed to contaminated seawater (conditions A, B, C) (Figure 4C, Table 5).

Significantly higher GSH/GSSG values were observed in mussels exposed to control (CTL) in relation to the values observed in mussels exposed to decontaminated seawater (conditions a, b, c) (Figure 4C, Table 5). No significant differences were observed between organisms exposed to a and c conditions (Figure 4C, Table 5).

The GSH/GSSG ratio was significantly higher in organisms exposed to decontaminated seawater (conditions a, b, c) than in organisms exposed to contaminated seawater (conditions A, B, C) (Figure 4C).

*Neurotoxicity biomarker*

The AChE activity was significantly higher in mussels exposed to control (CTL) in comparison to the values observed in mussels exposed to contaminated seawater (conditions A, B, C) (Figure 5, Table 5).

Significantly higher AChE values were observed in mussels exposed to control (CTL) in comparison to those observed in mussels exposed to decontaminated seawater (conditions a, b, c) (Figure 5, Table 5).

Significantly higher AChE values were observed in organisms exposed to decontaminated seawater (conditions a, b, c) than in organisms exposed to contaminated seawater (conditions A, B, C) (Figure 5).

## 3.4. Integrated Biomarker Response (IBR)

IBR values showed the highest score (16.7) for the mussels exposed to condition B, which indicates higher impacts in organisms under MnFe2O4-NPs (50 mg $L^{-1}$). Moreover, organism exposed to condition c showed the lowest IBR values (1.18), with values for conditions A, a, B, b and C (10.9, 2.31, 16.7, 1.45, 7.34 respectively).

# 4. DISCUSSION

The wide proliferation of nanoparticles for different uses may result in their release and dispersion in the environment with potential harmful effects (Katuli et al., 2014; Keller et al., 2010; Guan et al., 2018; Hanna et al., 2013). Moreover, when in the environment, the capacity of nanoparticles to interact with other pollutants may influence their toxicity (Moore et al., 2006; Fabrega et al., 2011). Studies focused on the toxicity of NPs in aquatic environment showed that NPs toxicity depends on their nature, exposure concentration, shape, size, and surface charge (Sun et al. 2016; Jastrzębska and Olszyna 2015) as well as on the time of exposure, medium composition, route of particle administration and target species (Khosravi-Katuli et al. 2017). Regarding the use of NPs for water decontamination, the available information is very scarce, in particular the one devoted to the possible toxic impacts of remediated water, resulting from incomplete removal of contaminants, prevalence of NPs residues, or other changes in water physicochemical properties induced by the remediation condition. Although the adsorption capacity for both organic and inorganic toxicants of various nanoparticles was evaluated and summarized by several authors (Davidescu et al., 2015; Gehrke et al., 2017; Mohan et al., 2007; Aslibeiki et al., 2015), no knowledge on the possible toxicity of the decontaminated water is available. In the present study we assessed the toxicity of magnetic manganese spinel ferrite nanoparticles, $MnFe_2O_4$, which have high capacity to adsorb As from seawater, as well as the efficiency of the treatment from an ecotoxicological point of view, assessing the toxicity of the remediated seawater towards the mussels *Mytilus galloprovincialis*.

## 4.1 Impact of As single exposure, before and after decontamination (conditions A and a)

Regarding As bioaccumulation in the whole soft tissues, as it was expected, the present study demonstrated that the higher contents of As were found in the mussels exposed to condition A (1000 µg/L). Previous studies also demonstrated a direct relationship between As exposure and element accumulation (Velez et al., 2015; Hsiung and Huang, 2004; Celia et al., 2009).

As a consequence of As exposure and bioaccumulation, higher cellular alterations were observed in mussels exposed to the highest As concentration. In particular, the present findings

clearly evidenced that mussels exposed to As at a concentration equal to the maximum permissible value for wastewater discharges (1000 µg/L of As, condition A, previous to 24 h decontamination process) strongly decreased their metabolic capacity (preventing energy expenditure), while increasing their antioxidant defences, cellular damages and neurotoxicity. Furthermore, at a smaller scale, the results also demonstrated that mussels exposed to As in a concentration of 70 µg/L (which corresponds to the value achieved by the NPs-based decontamination methodology – condition a), still presented an oxidative stress and neurotoxic status, with inefficient antioxidant capacity that led to observable cellular damages. In particular, the present study demonstrated that seawater contaminated with As at initial (condition A) and final (condition a) concentration levels (1000 and 70 µg/L, respectively) induced biochemical alterations in mussels that resulted in a general oxidative and neurotoxic status, with higher impacts when organisms were exposed to the highest As concentration (condition A). Mussels exposed to As initial concentration (1000 µg/L) clearly reduced their metabolism, preventing the use of energy reserves. However, when exposed to seawater at As concentration equal to that of decontaminated seawater (70 µg/L), albeit minor, the organisms had a metabolic capacity close to those of control indicating that higher impacts on mussels metabolism result from the exposure to the highest As concentration. The decrease of mussels' metabolism may be related to the capacity of bivalves to close their valves and reduce their filtration and respiration rates when exposed to contaminants (Gosling, 2003; Ortmann and Grieshaber, 2003). Previous studies also demonstrated that metals and metalloids even at lower exposure concentrations induced similar metabolic depression in bivalves (Errahmani et al., 2014; Liu et al., 2012; Velez et al., 2017). The present results also demonstrated that mussels exposed to As 1000 µg/L contaminated seawater strongly increased their antioxidant defences, which may result from the overproduction of reactive oxygen species due to the stress induced by As, which were efficient in limiting the occurrence of LPO. Nevertheless, at this condition, mussels clearly revealed oxidative damages with lower GSH/GSSG ratio compared to control organisms and damages in proteins with higher protein carbonylation values compared to control organisms. Under decontaminated seawater (condition a) no cellular damages were observed, evidenced by lower LPO levels in comparison to control values, which may be explained by higher antioxidant (GPx activity) and biotransformation (GSTs activity) defence capacities at this condition.

Nevertheless, still oxidative stress was observed in decontaminated seawater, identified by lower GSH/GSSG values in organisms exposed to condition a in comparison to control. These findings are in accordance with previous studies that demonstrated induced of oxidative stress and metabolic depression in bivalves exposed to pollutants (Freitas et al., 2016; Velez et al., 2016; Moreira et al., 2016; Mejdoub et al., 2017; Coppola et al., 2018; Jaishankar et al., 2014; Mandal and Suzuki 2002). In what regards to the neurotoxic impacts, both conditions A and a inhibited AChE activity, with higher injuries when organisms were exposed to the highest As concentration (contaminated seawater). Rajkumar (2013) also showed that As concentrations (80 µg/L) induced neurotoxicity in mussels. A similar pattern was shown by other authors with clams (e.g. *Ruditapes decussatus* and *R. philippinarum*) and oysters (e.g. *Crassostrea gigas* and *C. angulata*) exposed to As contamination (Velez et al., 2015; Freitas et al., 2012; Moreira et al., 2016a; b).

## 4.2 Impact of $MnFe_2O_4$ NPs single exposure, before and after decontamination (conditions B and b)

In what regards to $MnFe_2O_4$ NPs exposure conditions, the present study demonstrated that seawater contaminated with NPs at initial (condition B, 50 mg $L^{-1}$, previous to decontamination process) and final (condition b, NPs residuals in non-quantifiable concentration, after decontamination process) concentrations induced biochemical alterations in mussels that resulted in metabolism depression and a general oxidative and neurotoxic status, with higher impacts when organisms were exposed to the highest NPs concentration (condition B). In particular, the present findings demonstrated that mussels decreased their metabolic capacity and reduced energy expenditure when exposed to NPs concentration of 50 mg $L^{-1}$, probably because of valves closure to prevent bioaccumulation of NPs and higher injuries, a behaviour observed in bivalves when exposed to stressful conditions (Anestis et al., 2007; Gosling, 2003). Nevertheless, when mussels were exposed to NPs at final concentration ETS activity and energy reserves concentrations were closer to control condition evidencing the capacity of organisms to maintain their metabolism at lower NPs concentrations. No previous studies evaluated the metabolic impacts derived from exposure to $MnFe_2O_4$ NPs, although some works already demonstrated that other NPs (titanium ($TiO_2$), gold (Au) and copper (CuO))

decrease bivalves' metabolism (Xia et al., 2017; Cid et al., 2015; Teles et al., 2016; Gomes et al., 2011). Our results also demonstrated that mussels exposed to NPs increased their antioxidant enzymes activity, a response to higher ROS production due to the presence of NPs. It is known that the presence of NPs (TiO2, Au and CuO NPs) increases the production of ROS, which leads to the activation of antioxidant enzymes in bivalves (Xia et al., 2017; Cid et al., 2015; Gomes et al., 2012; Pan et al., 2012). As a result of increased antioxidant defences in mussels exposed to NPs at concentration of 50 mg $L^{-1}$ damages of the cellular membrane were prevented. Nevertheless, at this condition, mussels clearly revealed oxidative damages with lower GSH/GSSG ratio compared to control organisms and damages in proteins revealed by higher PC values compared to control organisms. When organisms were exposed to residual levels of NPs (condition b) still oxidative damages were observed, with mussels revealing a limited capacity to eliminate the excess of ROS that originated peroxidation of membrane lipids. Such limited antioxidant capacity may result from lower toxicity induced by condition b in comparison to NPs at initial concentration (condition B). These results agree with studies conducted by Tedesco (2010), which also showed that AuNPs (20 mg/L) induced lipid damage in mussels. Regarding the neurotoxic impacts, both NPs conditions (B and b) led to the inhibition of AChE activity, with higher injuries when organisms were exposed to higher NPs concentration (condition B). These results are in line with different studies conducted with diverse NPs: $TiO_2$, 0.4-10 mg $L^{-1}$, AuNPs 80 µg $L^{-1}$ -100 mg $L^{-1}$ (Guan et al., 2018, Pan et al, 2012; Teles et al., 2016; Gomes et al., 2011).

### 4.3 Impact of As and $MnFe_2O_4$ NPs combined exposure before decontamination

Concerning the impacts derived from the combined exposure to As and NPs (condition C), the present study demonstrated that initial concentrations of As and NPs (1000 µg $L^{-1}$ and 50 mg $L^{-1}$, respectively) reduced mussels' metabolism, increased oxidative stress and neurotoxicity compared to control organisms. In particular, organisms exposed to condition C decreased their metabolic capacity while increasing their energy reserves and increased their antioxidant defences, which were not enough to prevent cellular damages, with lower GSH/GSSG ratio and higher PC values in comparison to control values. This response pattern

was similar to those observed in organisms at single exposures (conditions A and B), revealing that the combination of contaminant and nanoparticles did not induce an additive or synergetic response. Although no previous studies showed biochemical stress induced by the combination of As and $MnFe_2O_4$ NPs in bivalves, former works demonstrated that NPs and metal(loid)s (such as As) had similar impacts, including metabolism alteration and increased antioxidant defences when bivalves were exposed to combination of both pollutants (De Marchi et al., 2017; Velez et al., 2016a; Monteiro et al., 2018; Della Torre et al., 2015). Nevertheless, the present results are in agreement with studies conducted by Freitas et al. (2018), which showed that functionalized NPs (MWCNTs, 0.1 mg $L^{-1}$) in combination with As (1000 µg $L^{-1}$) induced reduction of metabolic capacity, increase of oxidative stress and lipid damage in mussels, with a similar effect when organisms were exposed to As and NPs separately. Conversely, results obtained from a study conducted with AuNP and cadmium chloride ($CdCl_2$) on *M. edulis* by Tedesco et al. (2010) showed the highest oxidative stress and cellular damage in organism when exposed to these NPs and $CdCl_2$ contamination. Regarding the neurotoxicity activity, As+NPs (condition C) induced reduction of AChE activity, which is in accordance with former studies that analysed this biomarker in different invertebrates' species after exposure to different pollutants such as metals and NPs (Monteiro et al. 2018; Fan et al., 2018; Freitas et al., 2018; Xia et al., 2017; Xiong et al., 2011).

### 4.4 Impact of As and $MnFe_2O_4$ NPs acting in combination after seawater decontamination

The present study demonstrated that organisms exposed to the decontaminated water (condition c, As 70 µg/L and non-quantifiable concentration of NPs) changed their biochemical performance in comparison to control organisms, namely reducing their metabolism, increasing their oxidative stress and neurotoxic status. In comparison to organisms exposed to conditions a and b, where each contaminant was acting individually, the impacts induced were similar, with no significant differences for most of the biomarkers analysed among conditions (a, b, c). Nevertheless, the impacts induced in organisms exposed to decontaminated seawater (condition c) were significantly lower than the impacts observed in organisms exposed to both contaminants at initial concentrations (condition C). In fact, organisms exposed to the

decontaminated seawater presented higher metabolism than organisms exposed to the water enriched with As+NPs (condition C). Higher metabolic capacity did not result into higher antioxidant capacity, which probably was not activated due to low stress induced at this condition, originating in turn higher LPO levels and lower GSH/GSSG values at this condition. Furthermore, greater inhibition of AChE was observed when organisms were exposed to condition C compared to condition c, indicating the highest neurotoxic potential of As+NPs initial conditions.

## 5. CONCLUSION

The present study demonstrated that As decontaminated seawater (condition c) still generates oxidative stress in mussels, with increased cellular damage and oxidative stress in comparison with the control conditions (CTL), but contaminated conditions A, B and C clearly caused higher oxidative stress than the decontaminated seawater (conditions a, b and c) with higher increase in antioxidant defences, neurotoxicity and reduction in metabolism followed by increase of energy reserves. Overall, these results are innovative since, up to our knowledge, no published information is available on the ecotoxic effects induced in mussels when exposed to As contaminated seawater remedied by $MnFe_2O_4$, NPs.

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**Figure 1.** Transmission Electronic Microscopy image of $MnFe_2O_4$ nanoparticles.

**Figure 2.** A: Electron transport system activity (ETS); B: Glycogen content (GLY); C: Total protein content (PROT) in *Mytilus galloprovincialis* exposed to different conditions (CTL, a, A, b, B, c and C) at the end of the experiment. Results are mean + standard deviation. Significant differences between conditions A vs a, B vs b, C vs c are presented with asterisks.

**Figure 3**. A: Superoxide dismutase activity (SOD); B: glutathione peroxidase activity (GPx); C: Glutathione S-transferases activity (GSTs), in *Mytilus galloprovincialis* exposed to different conditions (CTL, a, A, b, B, c and C) at the end of the experiment. Results are mean + standard deviation. Significant differences between conditions A vs a, B vs b, C vs c are presented with asterisks.

**Figure 4.** A: Lipid peroxidation levels (LPO); B: protein carbonyl levels (PC); C: ratio between reduced and oxidized glutathione (GSH/GSSG), in *Mytilus galloprovincialis* exposed to different conditions (CTL, a, A, b, B, c and C) at the end of the experiment. Results are mean + standard deviation. Significant differences between conditions A vs a, B vs b, C vs c are presented with asterisks.

**Figure 5.** Acetylcholinesterase activity (AChE), in *Mytilus galloprovincialis* exposed to different conditions (CTL, a, A, b, B, c and C) at the end of the experiment. Results are mean + standard deviation. Significant differences between conditions A vs a, B vs b, C vs c are presented with asterisks.

**Table 1.** Experimental conditions.

| CONDITIONS | | DESCRIPTION |
|---|---|---|
| CTL | | Seawater with As 0 µg $L^{-1}$ + NPs 0 mg $L^{-1}$ |
| Water before As decontamination | A | Seawater with As 1000 µg $L^{-1}$ |
| | B | Seawater with NPs 50 mg $L^{-1}$ |
| | C | Seawater with As 1000 µg $L^{-1}$ and NPs 50 mg $L^{-1}$ |
| Water after As decontamiantion | a | Seawater with As 70 µg $L^{-1}$ |
| | b | Seawater after 24h in contact with NPs (50 mg $L^{-1}$), which were afterwards separated from seawater |
| | c | Seawater previously contaminated with As (1000 µg $L^{-1}$), then remediation using NPs (50 mg $L^{-1}$) during 24 h (which were afterwards separated from seawater). |

**Table 2.** Aggregation of NPs MnFe2O4 in seawater (nm), at different time (T0, T1, T24) after the beginning of the experiment.

| Time (hours) | MEAN (nm) | SD | CV% |
|---|---|---|---|
| **T0** | 3987 | 614 | 15 |
| **T1** | 14045 | 498 | 35 |
| **T24** | 67013 | 152 | 23 |

**Table 3.** Arsenic concentration (µg $L^{-1}$) measured in water samples collected immediately after the weekly water renewal. Results correspond to the mean value and standard deviation of the four weeks.

| | | [As] water µg $L^{-1}$ |
|---|---|---|
| CTL | | <1.5 |
| As | **A** | 947 ± 17 |
| | **a** | 82 ± 15 |
| NP | **B** | <1.5 |
| | **b** | <1.5 |
| As + NP | **C** | * |
| | **c** | 55 ± 13 |

*Because sorption of As by the NPs is extremely rapid, its quantification in this condition was not performed.

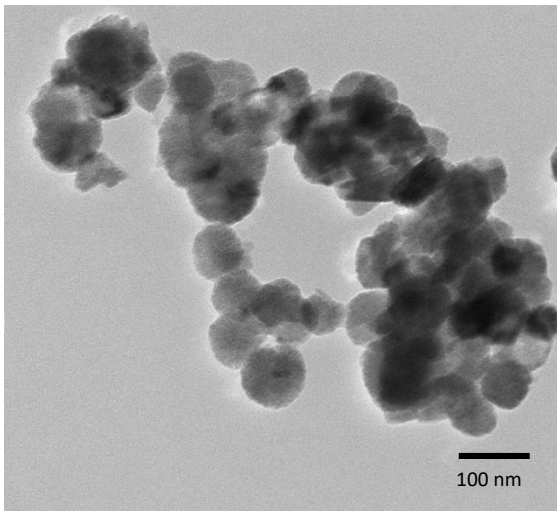
**Table 4.** Arsenic concentration in mussels (mg $Kg^{-1}$), 28 days after the beginning of the experiment. Concentrations were measured in organisms from different conditions: (CTL, a, A, b, B, c and C). Asterisks represent differences between A vs a, B vs b and C vs c conditions, while different lowercase letters represent differences between CTL vs a, CTL vs b, CTL vs c and uppercase CTL vs A, CTL vs B, CTL vs C conditions.

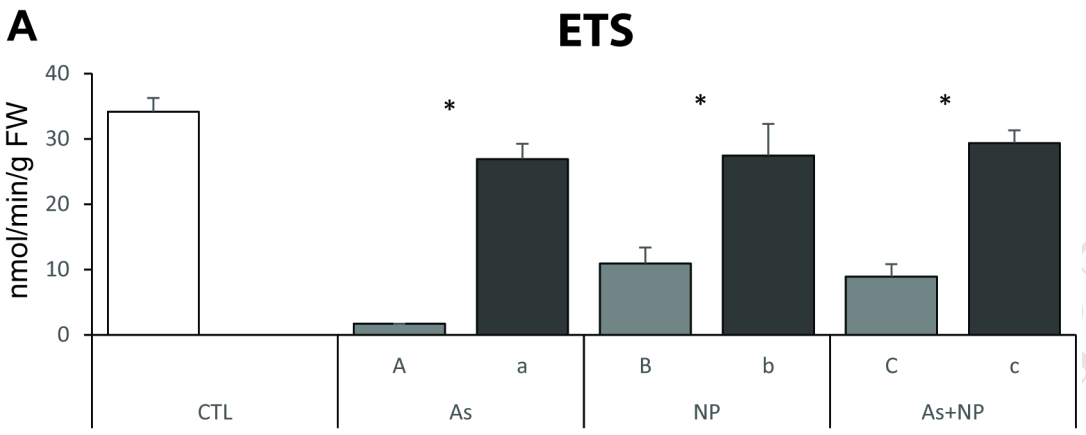
| | | As concentration (mg $Kg^{-1}$) | |
|---|---|---|---|
| **CTL** | | $7.4 \pm 1.5^{A,a}$ | |
| **As** | A | $12 \pm 2.6^{B}$ | * |
| | a | $6.8 \pm 2.2^{a}$ | |
| **NP** | B | $5.2 \pm 0.9^{A}$ | |
| | b | $4.4 \pm 0.2^{a}$ | |
| **As+NP** | C | $11 \pm 2.7^{B}$ | * |
| | c | $6.8 \pm 2.2^{a}$ | |

**Table 5.** *p*-values obtained by pair-wise comparisons between conditions (CTL vs A, CTL vs B, CTL vs C CTL vs a, CTL vs b, CTL vs c, A vs B, A vs C, B vs C a vs b, a vs c and b vs c) for each biomarker: ETS, electron transport system activity; GLY, glycogen content; PROT, total protein content; SOD, superoxide dismutase activity, GPx, glutathione peroxidase activit; GSTs, glutathione S-transferases activity; LPO, lipid peroxidation levels; PC, protein carbonyl levels; glutathione ratio, GSH/GSSG; acetylcholinesterase activity, AChE. Significant differences ($p \leq 0.05$) are highlighted in bold.

| | ETS | GLY | PROT | SOD | GPx | GSTs | LPO | CP | GSH/GSSG | AChE |
|---|---|---|---|---|---|---|---|---|---|---|
| **CTL vs A** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **CTL vs B** | **0.0001** | **0.0001** | 0.2061 | **0.0001** | **0.0001** | **0.0006** | **0.0001** | **0.0001** | **0.0001** | **0.0001** |
| **CTL vs C** | **0.0001** | **0.0001** | **0.0003** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** |
| **CTL vs a** | **0.0001** | 0.0550 | 0.2100 | 0.1983 | **0.0003** | 0.0707 | **0.0003** | **0.0293** | **0.0045** | **0.0093** |
| **CTL vs b** | **0.0001** | 0.9800 | 0.0770 | 0.2334 | **0.0168** | 0.8774 | **0.0020** | **0.0448** | **0.0003** | **0.0018** |
| **CTL vs c** | **0.0001** | 0.1936 | 0.0609 | 0.1310 | **0.0001** | **0.0054** | **0.0021** | 0.2694 | **0.0026** | **0.0043** |
| **A vs B** | **0.0076** | **0.0001** | 0.9018 | **0.0002** | 0.1127 | **0.0001** | **0.0001** | **0.0001** | 0.2629 | 0.8423 |
| **A vs C** | **0.0438** | **0.0017** | **0.0032** | **0.0009** | **0.0055** | 0.3656 | 0.7131 | 0.8632 | 0.0868 | 0.9424 |
| **B vs C** | 0.5399 | 0.9497 | **0.0331** | 0.4324 | 0.1733 | **0.0001** | **0.0001** | **0.0001** | 0.9478 | 0.7912 |
| **a vs b** | 0.1146 | 0.8939 | 0.3760 | 0.759 | 0.1757 | **0.0013** | 0.7522 | 0.8243 | **0.0298** | 0.3500 |
| **a vs c** | 0.1934 | 0.6053 | 0.1222 | 0.4063 | **0.0384** | 0.0694 | 0.066 | 0.1524 | 0.9786 | 0.4114 |
| **b vs c** | 0.3265 | 0.4136 | 0.4084 | 0.2369 | **0.0006** | **0.0001** | 0.1131 | 0.2086 | **0.0188** | 0.9893 |

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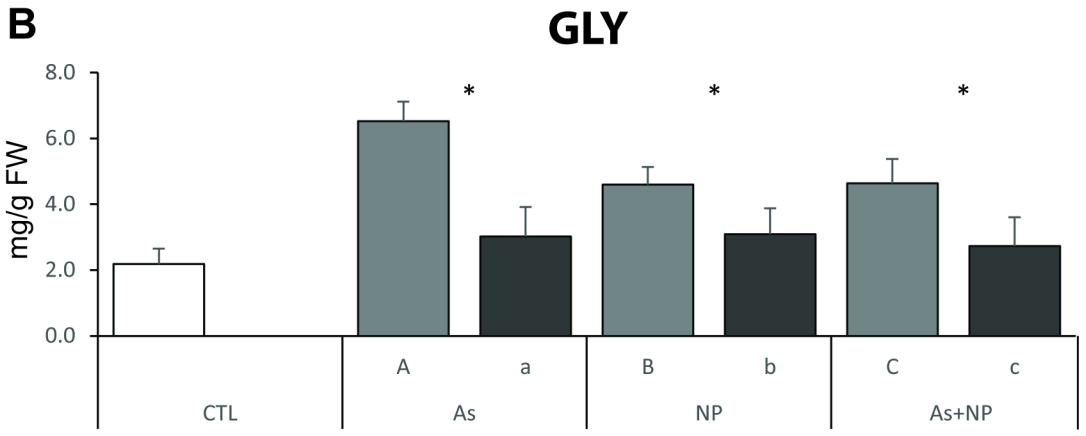
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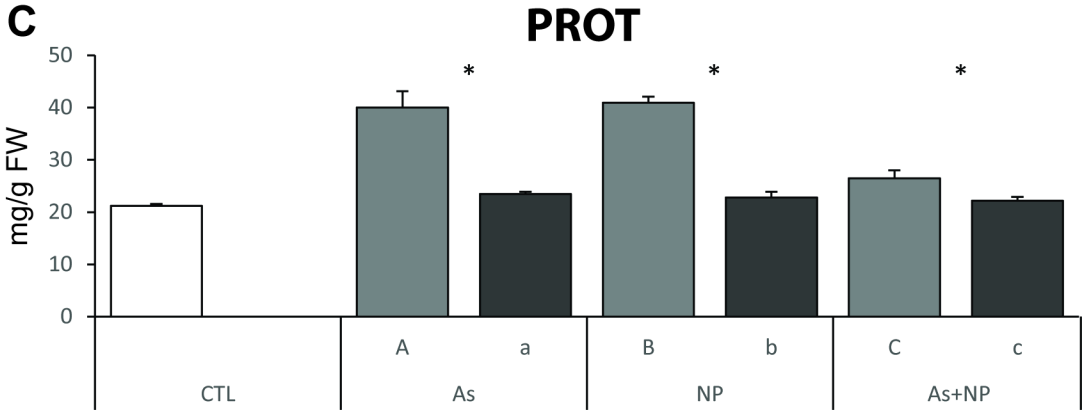
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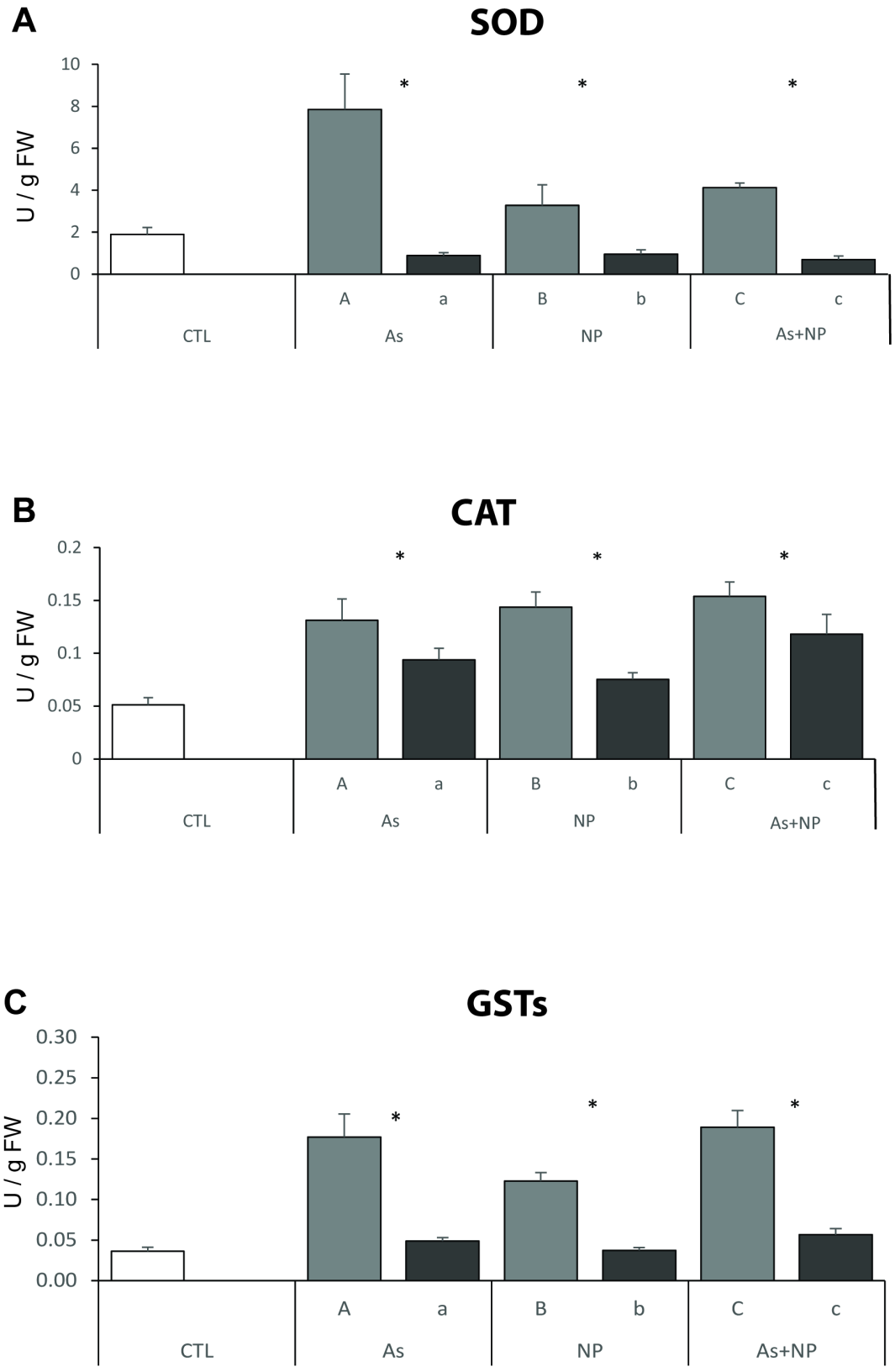
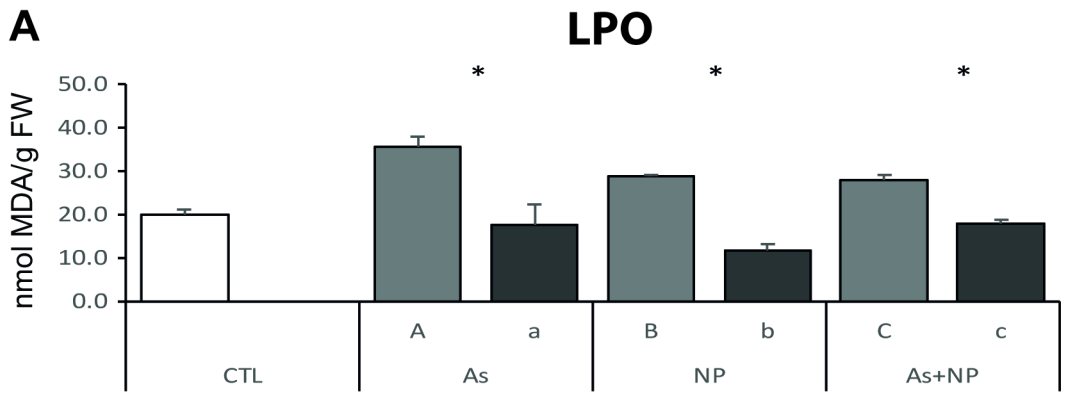
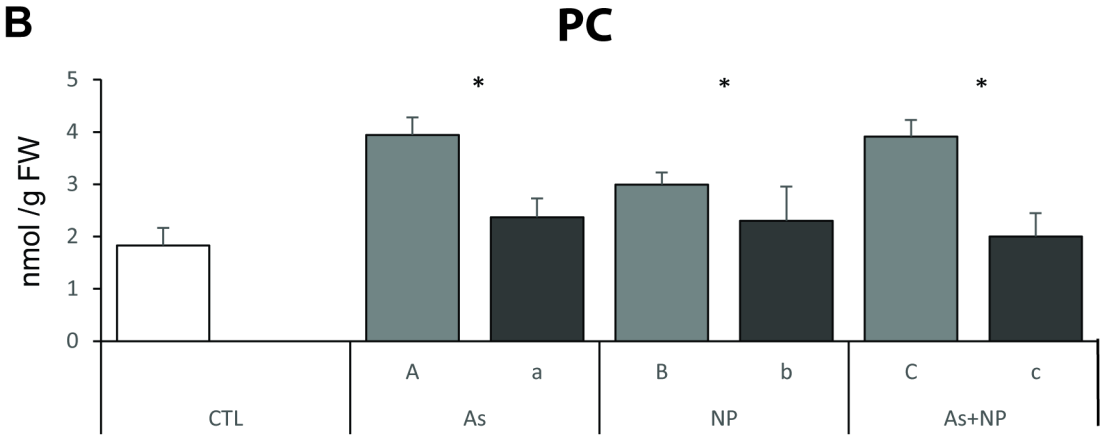
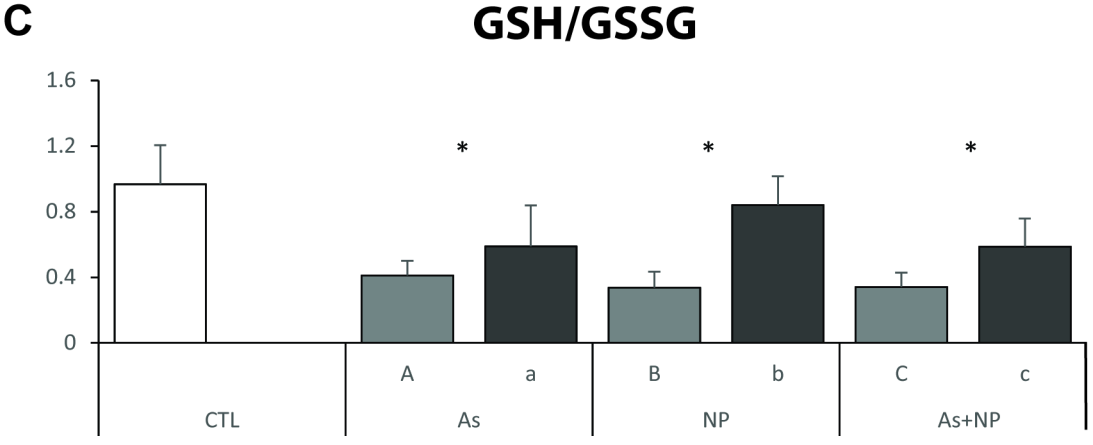
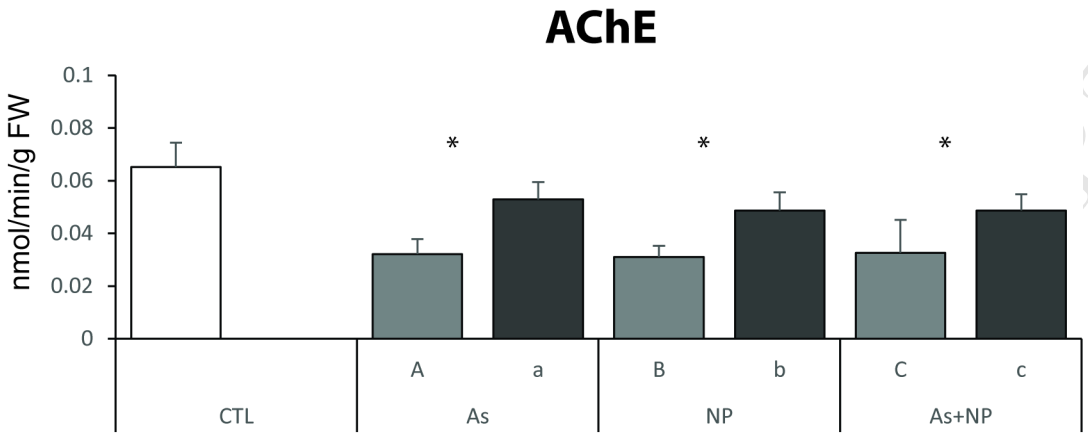
NP
As+NP


A
SOD
U / g FW
10
8
6
4
2
0
*
*
*
A
a
B
b
C
c
CTL
As
NP
As+NP
B
CAT
U / g FW
0.2
0.15
0.1
0.05
0
*
*
*
A
a
B
b
C
c
CTL
As
NP
As+NP
C
GSTs
U / g FW
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
*
*
A
a
B
b
C
c
CTL
As
NP
As+NP


A
LPO
nmol MDA/g FW
50.0
40.0
30.0
20.0
10.0
0.0
*
*
*
A
a
B
b
C
c
CTL
As
NP
As+NP


B
PC
nmol /g FW
5
4
3
2
1
0
*
*
*
A
a
B
b
C
c
CTL
As
NP
As+NP


C
GSH/GSSG
1.6
1.2
0.8
0.4
0
*
*
*
A
a
B
b
C
c
CTL
As
NP
As+NP



- Decontaminated seawater did not affect mussels metabolic capacity
- Contaminated mussels enhanced their antioxidant and biotransformation enzymes activities
- No cellular damages were observed in mussels exposed to decontaminated seawater
- Neurotoxicity was induced in contaminated mussels