

Research Article

Metabolic Effects in the Bivalve *Perna perna* and *Mytilus galloprovincialis*: Impact on the Environment due to Contamination by Copper

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This work presents the study of the effects of acute exposure to various concentrations of copper from 0.03 to 0.59 $\mu\text{mol}\cdot\text{L}^{-1}$ on the metabolic activities (nitrogen and phosphorus), protein levels, catalase (CAT) enzyme activities, and the biological response malondialdehyde (MDA) in the mollusks *Perna perna* and *Mytilus galloprovincialis*. The concentrations above 0.88 $\mu\text{mol}\cdot\text{L}^{-1}$ have proven to be lethal to *P. perna*. The results show slight disturbances of metabolism as a result of pollutant and a significant correlation between metal contamination and ammonia nitrogen levels, resulting in an increase in the latter after 48 h of *M. galloprovincialis* exposure to 0.59 $\mu\text{mol}\cdot\text{L}^{-1}$ of copper concentrations. CAT was rapidly induced even by low concentrations of copper; MDA was increased only with low concentrations of copper in *P. perna*.

1. Introduction

Coastal and estuarine ecosystems are subjected to many stressors, including salinity fluctuations and anthropogenic disturbance [1]; the new environmental development problems are associated with multiple contaminant discharges that may threaten the whole ecosystems [2]. The impact of chemical contaminants on organisms in the marine environment has always been difficult to quantify which led to new ways of researches such as ecotoxicological bioassays on sentinel species of shellfish, fish, and plants [3–7].

Seafood, such as oysters and mussels that are consumed raw or cooked, had often been reported as sources of food poisoning [8]. The Food and Agriculture Organization (FAO) classifies shellfish as food of a high risk [9]. Mussels can be a vector of contamination in the presence of pollution [10, 11]; they have been widely used as indicators of this pollution due to their capacity to bioaccumulate and concentrate organic and metallic pollutants [12].

Highly soluble molecules such as nitrate, which are generated from animal-derived nitrogenous compounds (e.g., urea),

could prove to be toxic to fish and invertebrates [13]. Similarly, levels of ammonia, nitrite, and nitrate, derived from human activities [14], can impair the ability of aquatic animals to survive, grow, and reproduce [15].

In addition to the chemical analyses, the use of different biomarkers has been introduced in monitoring programs in order to evaluate the effects of pollutants on the organisms [16, 17]. They can be used to measure the interaction between a biological system and a chemical physical or biological environmental agent [18, 19].

Mytilidae are considered to be good bioindicators resulting in their use in marine water biomonitoring programs [6, 7, 20]. These pollutants generate oxidative damage in various pathological and toxicological processes through reactive oxygen species (ROS) formation [21, 22]. The oxidative/reductive role of some metals (Fe, Mn, Cu, and Mo) in the intercellular reactions has already been emphasized [23]. Copper is one of the agents involved in the oxidative stress [2, 24] by the formation of ROS [25, 26]; the exposure of marine mollusks to this metal induces lipid peroxidation [27].

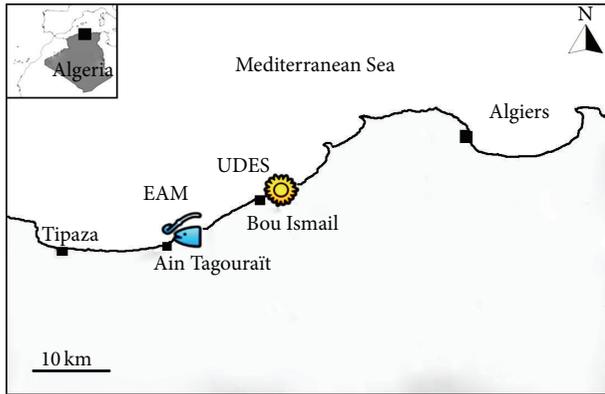


FIGURE 1: Geographical area of the study.

Against these hazards, mussels, particularly the species *Perna perna* and *Mytilus galloprovincialis*, have developed enzymatic antioxidant defense mechanisms such as super oxide dismutase (SOD, EC 1.15.1.1) or CAT (EC 1.11.1.6) [7, 28–31].

CAT is an important antioxidant enzyme of the defense system protecting organisms against oxidative stress by catalysing the dismutation of hydrogen peroxide H_2O_2 [32].

The malondialdehyde (MDA), considered as a byproduct of lipid peroxidation, has been widely used to assess the effects of many pollutants on aquatic ecosystems [26, 33–35].

The aims of this work consisted in monitoring metabolic activities (nitrogen and phosphorus excretion) and biological responses of CAT, MDA, and proteins after induced stress by acute copper exposure in the mussels *Perna perna* and *Mytilus galloprovincialis*.

2. Materials and Methods

2.1. Place and Study Period. This work was carried out under the Aquatic Ecosystem Management Program and the Environmental Monitoring adopted by the National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA) of Bou Ismail about 36 km west of Algiers (Figure 1).

The experiments were conducted during March-June 2010 and May-July 2011 on *Mytilus galloprovincialis* in CNRDPA Laboratory and on *Perna perna* in the Development Unit Laboratory of Solar Equipment of Bou Ismail (UDES), respectively.

2.2. Mussel Collection and Acclimatization. Mussels *M. galloprovincialis* were collected at the EAM site by divers about 1 km from the coast at a depth of 7 m and delivered within 24 h to the CNRDPA laboratory in isothermal box at 4°C.

P. perna mussels were purchased from trade and have undergone a purification phase of three weeks in physico-chemical and bacteriological quality seawater. Selected specimens were of the same size class (40–55 mm), cleaned thoroughly with water, freed of their epibionts by hand scraping, and measured with a calliper.

A period of two weeks of acclimatization preceded each copper contamination test. All the mussels were held under identical environmental conditions; the use of air pumps ensured aeration of the rearing water trays, daily renewed for nutrients supply collected at the geographical point (N36°36'41.30" E2°37'25.06", Garmin GPS 12).

To ensure the only effect of copper as stressor, the bacteriological quality of mussels and that of seawater taken from the same site have been verified; the nutrient contents were measured in each tray at different copper concentrations for each period of experiments.

2.3. Experimental Design. Sterilized glass and polyethylene bottles of 500 mL were used for seawater physicochemical analyses.

Temperature and salinity were daily monitored, before and after the change of water, with a Cond 330i WTW thermometer.

The metal solutions were prepared from copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$; purity 97%) at $0.059 \text{ mmol} \cdot L^{-1}$ in distilled water. The mussels were divided into rectangular polystyrene trays $36 \times 24 \times 17 \text{ cm}$ (11 mussels per tray, Figure 2) in a total volume of 10 L of seawater/tray.

After 2 weeks of adaptation, the specimens were exposed to copper at different concentrations from 0.03 to $0.59 \mu\text{mol} \cdot L^{-1}$ during 3 days. A tray with pure seawater had served as control. After the exposure, mussels were decontaminated in clean seawater.

2.4. Determination of Metabolites. Every day, about 0.5 L was taken from each tray and filtered. The different nutrients were measured according to the method described by Aminot and Chaussepied [36].

Ammonia nitrogen ($NH_3\text{-N} + NH_4^+\text{-N}$) was determined by the indophenol blue method of Koroleff [37]. In a slightly basic medium, in the presence of hypochlorite, ammonia forms monochloramine which reacts with phenol to form blue-indophenol; the absorbance was measured at 630 nm.

Nitrite ions react with sulfanilamide [38], in acid medium ($pH < 2$), to form diazonium compound which reacts with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a pink dye; the absorbance was measured at 543 nm.

The method retained for nitrate nitrogen deduction is based on the measurement of NO_2^- ions obtained by quantitative reduction of NO_3^- ions (>95%). The level of nitrate ions is obtained by deducting that of the nitrite ions from the overall measure of NO_2^- and NO_3^- ions.

The reduction is done by passing through a column of copper-treated cadmium. The phosphate ions react with ammonium molybdate in the presence of antimony (III), forming a complex which is reduced by ascorbic acid. The blue color reduced form contains phosphorus; the absorbance was measured at 885 nm.

Polyphosphates and organic phosphorus were not determined by this method.

2.5. Biochemical Determinations. At the end of each period (after 24 h, after 72 h, and in decontamination), 3 to 4



FIGURE 2: (a) The experimental device of the study. (b) The mussels in their tray.

mussels were collected from each tray in order to determine proteins levels, CAT, and MDA. To ensure the stability of organelles and pH, mussel's gills initially underwent a homogenization by mixing at a rate of 1/10 w/v in tris(hydroxymethyl)aminomethane 20 mM buffer, pH 7-8, and then were centrifuged at 10,000 g for 10 min at 10°C. The obtained supernatant (S9 fraction) was used to determine the proteins levels, the CAT activity, and the MDA.

Proteins. Proteins were assayed by the method of Lowry et al. [39] that combines a biuret reaction and a reaction with Folin-Ciocalteu reagent. Samples of the supernatant obtained above (S9 fraction) were diluted to 1/5, 1/8, and 1/10. After addition of 5 mL Lowry reagent, the mixture was homogenized for 10 min and then completed by 0.5 mL Folin-Ciocalteu reagent freshly diluted to 1/2. After stirring and temporary resting in the dark for at least 30 min, the absorbance was read on spectrophotometer at 660 nm. The calibration range was established from bovine serum albumin (BSA) solution, stock standard 7.6 g%.

CAT Activity. The CAT activity was determined by the method of Lartilot described by Atli et al. [40]. The kinetic approach is to track the amount of active enzyme (appearing or disappearing) per unit time. Fifty μL of the S9 fraction was added to 100 μL of 30% H_2O_2 solution and 2.4 mL of 75 mM phosphate buffer at pH 7 and then placed in a cuvette of the spectrophotometer in kinetic mode. The decomposition of hydrogen peroxide was monitored in kinetic mode at 280 nm for 2 min.

MDA Quantitation. The lipid peroxidation was determined by quantifying the MDA levels in the gills of the mussels using the method of Draper and Hadley [41]. Levels of MDA were deducted by estimating the optical density of the colored chromogene issued from the reaction of the MDA with thiobarbituric acid (TBA). Trichloroacetic acid (TCA 2.5 mL) was added to 0.5 mL of S9 fraction and then placed in a boiling water bath for 15 min. After water cooling, the mixture was centrifuged at 1000 g for 10 min; then 2 mL of the supernatant was added to 1 mL of thiobarbituric acid (TBA)

and placed once more in boiling water bath for 15 min. After water cooling, the absorbance of the supernatant was read at 532 nm. The MDA level was obtained using the extinction coefficient of the MDA-TBA complex ($1.56 \cdot 10^5 \text{ cm}^{-1} \cdot \text{M}^{-1}$).

2.6. Statistical Analysis. The normality of samples and the homogeneity of data variances were verified using Shapiro Wilk and Levene tests, respectively [42]. Samples were assayed at 0.03, 0.06, 0.15, 0.29, and $0.59 \mu\text{mol} \cdot \text{L}^{-1}$ of copper concentrations. The changes in total proteins, CAT, and MDA were monitored for three periods: a phase of contamination at 24 h and 72 h followed by a decontamination phase. For each series of measures (after 24 h, after 72 h, and in decontamination), 3 to 4 mussels were collected from each tray (control, 0.03 to $0.59 \mu\text{mol} \cdot \text{L}^{-1}$ of copper concentration).

Comparisons of means were determined by one-way ANOVA. The Kruskal-Wallis ANOVA on ranks was used when the assumptions of normality or homogeneity of variance were severely violated. Groups with significant differences ($P < 0.05$) were highlighted by Mann-Whitney tests.

Pearson's correlation coefficients r , between physico-chemical variables and copper levels, were measured. The results are expressed as mean \pm SE (SE: standard error). The analysis was performed using Statistica 7.0, Statsoft Inc., Tulsa, USA, and SPSS 15.0 for Windows, SPSS Inc., Chicago, Illinois, USA.

3. Results and Discussion

Preliminary bacteriologic analyses of the rearing water showed no thermotolerant coliforms, fecal streptococci, or salmonella but a low rate of 16/(100 mL) of total coliforms ($\ll 500/(100 \text{ mL})$, European standard of 76/160/CEE), what denotes a good microbiological breeding water quality that is not going to interact with CAT (a variable which increases during microbial stress) [43, 44]. Thus, any exogenous stress due to bad microbial quality of the seawater was eliminated. The microbiological control of mussels showed a rate of total aerobic mesophilic bacteria (TAMB) of approximately

TABLE 1: Comparison of nutrients levels in seawater at different periods.

Period	Nitrates			Nitrites		
	Mean (mg·L ⁻¹)	RSD (%)	Range (mg·L ⁻¹)	Mean (mg·L ⁻¹)	RSD (%)	Range (mg·L ⁻¹)
0 h	1.11 ± 0.17	30.6	0.64–1.44	0.025 ± 0.011	92.0	0.005–0.049
24 h	1.75 ± 0.09	17.6	1.27–2.30	0.061 ± 0.008	41.0	0.022–0.092
48 h	1.83 ± 0.23	48.1	0.31–3.26	0.058 ± 0.008	54.9	0.012–0.104
72 h	1.84 ± 0.22	45.8	0.87–3.90	0.063 ± 0.008	51.5	0.005–0.111
ANOVA	$F = 1.16$			$F = 1.79$		
P	0.34			0.17		

Period	Phosphorus			Ammonia nitrogen		
	Mean (mg·L ⁻¹)	RSD (%)	Range (mg·L ⁻¹)	Mean (mg·L ⁻¹)	RSD (%)	Range (mg·L ⁻¹)
0 h	0.055 ± 0.039	143.6	0.009–0.172	0.026 ± 0.009 ^a	57.7	0.015–0.043
24 h	0.100 ± 0.027	89.2	0.016–0.285	0.146 ± 0.018 ^a	40.2	0.052–0.234
48 h	0.095 ± 0.035	137.3	0.016–0.538	0.214 ± 0.051 ^a	82.2	0.099–0.705
72 h	0.215 ± 0.069	123.5	0.005–0.865	0.641 ± 0.170 ^b	99.3	0.057–1.770
ANOVA	$F = 1.61$			$F = 4.61$		
P	0.20			0.008		

Different letters show means with significant differences ($P < 0.05$).

110 mL⁻¹ and a total absence of microbial contamination for both species of mussels.

3.1. Physicochemical Parameters. During the experimental period, the average water temperature was $19.4 \pm 0.1^\circ\text{C}$ (range 15.3–22.0) and the average salinity was $36.39 \pm 0.03\text{‰}$. The effect of copper exposure on the nitrogen and phosphorus excretion of the mussels was investigated by calculating the changes of nutrients contents (nitrate, nitrite, ammonia nitrogen, and phosphorus excretion) in the rearing water after 24 h, 48 h, and 72 h (Table 1).

The used copper concentrations were ranging from 0.03 to $0.59 \mu\text{mol}\cdot\text{L}^{-1}$. Such high copper levels could only be found near polluted rivers deltas, or in case of contamination by copper sulfate, or after significant discharge wastes containing copper after treatment in silts, or other cases of severe contamination. The average level of copper in seawater free of pollutants, with salinity of 35‰ at 25°C, is mostly $<0.02 \mu\text{mol}\cdot\text{L}^{-1}$. The nutrient contents of seawater remained very low, what excludes their toxic effect on the test organisms; moreover, aquatic animals have adapted to low levels of nutrients in the aquatic environment [15]; for instance, the nitrate levels should not exceed $20 \text{ mg } (\text{NO}_3\text{-N})\cdot\text{L}^{-1}$ in marine environment to protect aquatic animals [13].

The values observed at the beginning of experiment (0 h) for various nutrients were below acceptable values. Thus, exogenous stress by nutrients sourced from anthropogenic contamination remained unlikely. In the literature, findings suggested that increased nitrate concentrations may be associated with areas of enhanced human activities on the riverine watersheds [14]. Thus, the present results confirmed the good physicochemical and bacteriological quality of seawater; EAM site could be used as a reference site.

The only positive, but highly significant, correlation was observed after 48 h between copper concentrations and levels

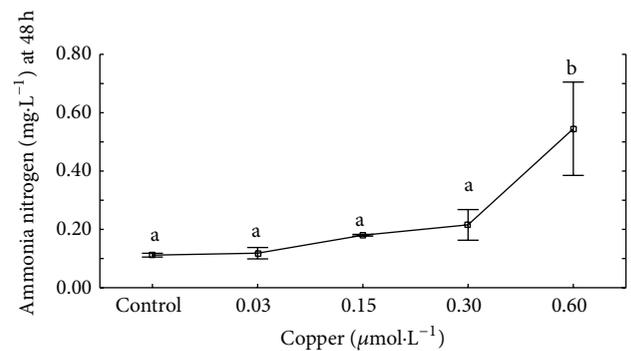


FIGURE 3: Ammonia levels in seawater after 48 h of copper exposure at different concentrations. Different letters show means with significant differences ($P < 0.05$).

of ammonia nitrogen ($r = 0.88$, $P < 0.001$). The average content of ammonia was $0.55 \pm 0.16 \text{ mg}\cdot\text{L}^{-1}$, for $0.59 \mu\text{mol}\cdot\text{L}^{-1}$ copper concentration, with level significantly higher than that of the other groups (Figure 3). Comparisons of mean nutrients levels for different periods by ANOVA (Table 1) have confirmed the results of Pearson's correlations. The fluctuations of nitrates, nitrites, and phosphorus excretions remained low throughout the experiment ($P > 0.05$), except for ammonia nitrogen which presented a very significant increase; the mean levels are ranging from $0.03 \text{ mg}\cdot\text{L}^{-1}$ earlier in the experiment to $0.64 \text{ mg}\cdot\text{L}^{-1}$ after 72 h ($P = 0.008$, Figure 4).

The variability was highlighted through the relative standard deviation $\text{RSD} = (\sigma/\bar{x}) * 100$. The nitrates had the lowest variability (18–48%, Table 1), while phosphorus excretions had the highest (89–144%).

3.2. Proteins. The mean values of proteins in the gills of *P. perna* mussels to 0.03, 0.15, 0.29, and $0.59 \mu\text{mol}\cdot\text{L}^{-1}$ copper

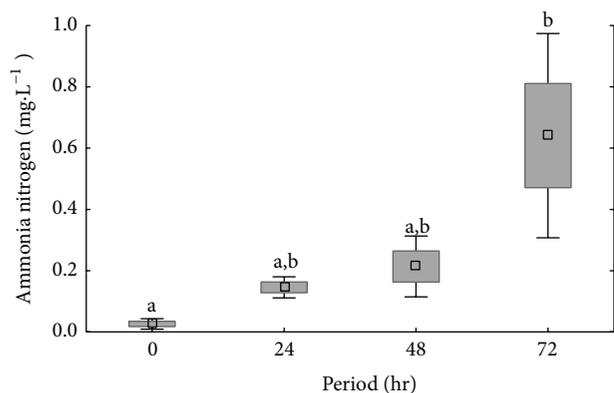


FIGURE 4: Ammonia levels in seawater at different periods of the experiment. Different letters show means with significant differences ($P < 0.05$).

exposure showed no significant difference after 24 h, 72 h, and 4 days of decontamination (Kruskal-Wallis test: $P = 0.07, 0.23, \text{ and } 0.46$, resp.). Protein reserves did not appear to be affected by the chemical pollutant. At low copper concentration and physiological concentrations of H_2O_2 , protein damage is restricted to amino acid modification at specific metal binding sites [24]. No significant difference in protein contents was reported by Borković et al. [45] in mussels *M. galloprovincialis* collected in a place which is known for intensive industrial pollution during both winter and spring seasons. However, according to Mosleh et al. [46], depletion of proteins is a response to early defense against chemical stress. Thus, depletion of protein levels can be attributed to protein catabolism in response to energy demand. To overcome stress, the organisms need a lot of energy, and this demand can induce protein catabolism; in addition, the decrease of protein levels may be due to the lipoproteins formation which will be used to repair damage in cells, tissues, and organs [32].

3.3. Catalase. After 24 h, the CAT levels in *P. perna* remained similar to that of the control group ($12.1 \pm 1.8 \text{ IU}\cdot\text{mg}^{-1}$ proteins, Figure 5) except that of $0.15 \mu\text{mol}\cdot\text{L}^{-1}$ copper group, where the average value was slightly higher ($24.8 \pm 5.6 \text{ IU}\cdot\text{mg}^{-1}$ proteins).

After 72 hours of exposure, a very significant increase in activity was observed for 0.15 and $0.59 \mu\text{mol}\cdot\text{L}^{-1}$ copper groups (179.8 ± 23.5 and $68.7 \pm 14.0 \text{ IU}\cdot\text{mg}^{-1}$ proteins, resp.), compared to the control group.

Higher concentrations at 0.88 and $1.17 \mu\text{mol}\cdot\text{L}^{-1}$ were lethal since no mussels survived.

The organisms exposed to 0.03 and $0.29 \mu\text{mol}\cdot\text{L}^{-1}$ copper concentrations showed CAT activity similar to that of control organisms. The enzymatic activity decreased for all the groups after the transfer of mussels in a pollutant-free water (decontamination period). Meanwhile, this activity remained significantly higher for 0.15 and $0.59 \mu\text{mol}\cdot\text{L}^{-1}$ copper groups (11.0 ± 2.2 and $11.3 \pm 0.6 \text{ IU}\cdot\text{mL}^{-1}$ proteins, resp.) compared to that of the control group ($5.8 \pm 0.7 \text{ IU}\cdot\text{mL}^{-1}$ proteins).

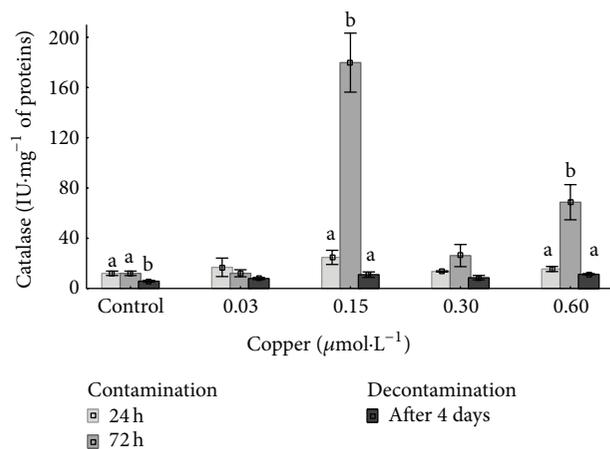


FIGURE 5: CAT activity at 24 h, at 72 h, and after 4 days in the gills of the mussel *Perna perna* under copper exposure. In each group, the means with different letters have significant differences ($P < 0.05$).

These results are in agreement with those reported by Rajalakshmi and Mohandas [47] who observed a significant induction of antioxidant defenses in *Lamellidens corrianus* after 24 h of copper exposure at different concentrations. Guecheva et al. [28] observed a significant induction of CAT in freshwater planarian *Dugesia (Girardia) schubarti* after 24 h of copper exposure at $40, 80, \text{ and } 160 \mu\text{g}\cdot\text{L}^{-1}$. An increase of the enzyme activity with copper concentrations was reported by Roméo et al. [48] in transplanted mussels from clean water to stations of Nice and Cannes bays. Jebali et al. [49] noted in clam *Ruditapes decussatus* a higher CAT activity in the gills and the digestive glands, in relation to pollutants presence.

The exposed organisms to 0.03 and $0.29 \mu\text{mol}\cdot\text{L}^{-1}$ copper concentrations showed CAT activity similar to that of control group. These concentrations did not appear to affect the enzymatic activity of the specimens.

The results are sometimes contradictory *in vivo* since some authors show induction of activity [28, 31, 50, 51], while other ones show inhibition [52]. One retained hypothesis is that enzymatic activity is very sensitive to anthropogenic or natural environmental factors [53] and this can result from a delicate balance between induction and inhibition by redox compounds cycle. Inhibitions of CAT activity, following a marine environment disturbance by chemical contaminants such as copper and cadmium, have been reported by several authors in different indicator species [54–56].

Company et al. [54] showed these inhibitions in *Bathymodiolus azoricus* under laboratory conditions in the Adriatic Sea. Varanka et al. [57] reported similar observations in *Cyprinus caprio* L. after copper or tannic acid exposure (or their mixture) under laboratory conditions. Gharbi-Bouroufi et al. [56] found an inhibited CAT activity in *Murex trunculus* during the summer season, inhibition that may be due to microbial contamination. A possible explanation can be the noninduction of the enzyme after a short exposure time (24 h) or the low copper concentration ($0.03 \mu\text{mol}\cdot\text{L}^{-1}$). Another phenomenon, the complexation, is occurring in the consumption of free metal ions by the seawater complex

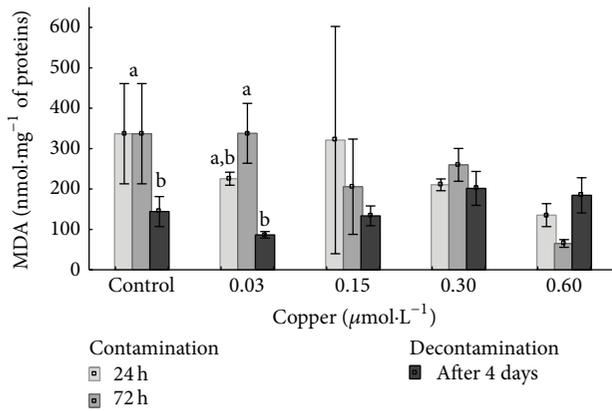


FIGURE 6: Mean levels of MDA at 24 h, at 72 h, and after 4 days in the gills of the mussel *Perna perna* under copper exposure. In each group, the means with different letters have significant differences ($P < 0.05$).

(HCO_3^- , CO_3^{2-} , $\text{OH}^- \dots$), which induce a decrease in the bioavailable metal fraction and so a decrease in the toxicity of xenobiotics versus the contaminated specimens, knowing that the other complex forms are less toxic and that the majority is copper carbonate CuCO_3 [58]. There also may be complex formation on the surface of organic fecal particles [59], reducing the bioavailability of copper ion versus the contaminated organisms. The precipitation may reduce the free fraction of the metal ion and, in natural waters with $\text{pH} > 6$, the precipitation complex $\text{Cu}_2(\text{OH})_2\text{CO}_3$, $\text{Cu}_3(\text{OH})_2(\text{CO}_3)_2$, $\text{Cu}(\text{OH})_2$ leads to a decrease in copper toxicity [58]. A final major cause in the decrease of bioavailable forms of trace elements can be adsorption; copper can be adsorbed on the trays walls [59].

The CAT mechanism could also be better understood by controlling the speciation of copper which plays a fundamental role in the interactions between metal ions and living organisms [60]. The experimental research showed that aquatic organisms are essentially affected by the free metal ions concentrations in solution [59, 61, 62]. CAT has proved capable of reflecting the state of the surrounding environment in a short time (3 to 4 days in decontamination). This biomarker seems to be able to reflect the curability of the mussels health status and the reversibility of the physiological mechanism. The same observations were reported by Company et al. [26] after six days of depuration cycle with the mussels *Bathymodiolus azoricus* that had followed 24 days of exposure to $25 \mu\text{g}\cdot\text{L}^{-1}$ copper; CAT activities recorded in gills were found below those of contamination phase.

3.4. MDA. After 24 h, the mean levels of MDA in *P. perna* were similar for all the samples, including the control group, ($P = 0.68 \gg 0.05$). These mean levels remained comparable after 72 h ($P \approx 1.00$) and after 4 days in decontamination ($P = 0.18$). That does not mean there were no changes in the mean levels between the different periods, because MDA levels in different groups can change simultaneously (increasing or decreasing) while remaining comparable (Figure 6). Thus,

a significant decrease was observed for the control group after 4 days in decontamination, with MDA levels decreasing from 449 to $144 \text{ nmol}\cdot\text{mg}^{-1}$ proteins ($P = 0.02$). The $0.03 \mu\text{mol}\cdot\text{L}^{-1}$ sample showed a significant increase in MDA contents after 72 h, followed by a sharp decline after 4 days in decontamination period from 338 to $87 \text{ nmol}\cdot\text{mg}^{-1}$ proteins. For other concentrations, mean levels remained comparable over time.

These results showed that the MDA levels were only influenced at low copper concentration ($0.03 \mu\text{mol}\cdot\text{L}^{-1}$) after 72 h of exposure; the increased levels were probably due to copper action which is known for its ability to initiate and propagate lipid peroxidation by reactive oxygen species [63]. After 4 days, brutal and significant decreases were observed for the control group and that at $0.03 \mu\text{mol}\cdot\text{L}^{-1}$. These results seem to be in agreement with those found by Franco et al. [64] who reported no significant difference in MDA levels in *P. perna* exposed to $30 \mu\text{g}\cdot\text{L}^{-1}$ zinc concentration. Similarly, Pytharopoulou et al. [65] found that copper did not affect significantly the MDA in *M. galloprovincialis* exposed for 15 days in laboratory conditions. However, Company et al. [54] have noted a significant decrease of lipid peroxidation in the mussel *Bathymodiolus azoricus* after 0.4 mM copper exposure.

4. Conclusion

This work aimed to study metabolic disturbances in *Perna perna* and *Mytilus galloprovincialis* as a result of metal contamination and the analysis of the reliability and effectiveness of biomarkers CAT and MDA in the prevention of risks related to heavy metal (copper) contamination. Preliminary microbiological analyses of seawater and mussels showed a complete lack of fecal contamination control germs ruling out immediately the influence of an exogenous source of pollution on biomarker responses. A significant increase in ammonia nitrogen was observed in *M. galloprovincialis* after 48 h of metal exposure. Any time, the nutrient contents were very low, reflecting the good physicochemical quality of seawater in EAM site. The effect of *P. perna* acute exposure to 0.15 and $0.59 \mu\text{mol}\cdot\text{L}^{-1}$ copper concentrations on enzymatic and metabolic activities showed a significant induction of CAT after a short term of 24 h, followed by a significant depletion after 72 h (decontamination period). This reactivity, at certain copper concentrations, could be indicative as a response to metal contamination. However, these effects of induction and depletion were not apparent for *P. perna* exposure to 0.03 and $0.29 \mu\text{mol}\cdot\text{L}^{-1}$ copper concentrations. This lack of responses in the intermediate copper concentrations is one of the limits of this study which highlight the necessity of the multimarker approach for a better understanding of results and therefore, a better prevention against pollutants. The CAT activity of the mussel *M. galloprovincialis* did not seem to be influenced by the chosen copper concentrations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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