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Research Article

Catalase Activity in Brown Mussels (*Perna perna*) under Acute Cadmium, Lead, and Copper Exposure and Depuration Tests

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Brown mussels (*Perna perna*) were exposed to cadmium (Cd), lead (Pb), and copper (Cu) concentrations under acute exposure and exposure-depuration tests for the estimation of biochemical biomarker catalase (CAT). The acute tests showed accumulated Cd, Pb, and Cu in *Perna perna* correlated linearly with the exposure concentrations ($R^2 = 0.794$, $R^2 = 0.891$, and $R^2 = 0.985$ for Cd, Pb, and Cu, resp.). The results of CAT increased significantly in tissues of treatment mussels after 72 h exposure when compared to control. The values of total protein were disturbed in exposed groups when compared with control. These results suggest that metabolites and catalase activity were affected by heavy metal exposures. Analysis using the Spearman rank correlation coefficient showed that the CAT activity appeared to have a significant positive correlativity ($R_s = 0.921$, $R_s = 0.949$, and $R_s = 0.949$ for Cd, Pb, and Cu, resp.) with the accumulated Cd, Pb, and Cu concentrations, respectively. The result of exposure-depuration tests showed that there is a general tendency for CAT to decrease in depuration phase, suggesting that the induction of catalase is metal and/or mixture of metals dependent.

1. Introduction

The coastal zone receives a large amount of metal pollution from agricultural and industrial activity [1]. In Algeria, metals traces, in surface and seawaters and sediments harbours, originate from industrial activities, namely, tanneries and paper mills (Cd, Zn, Cu, Hg, Cr, and Ni), wastewater (Zn, Cu, Cd, Pb, and Ni), and agriculture by improper use of mineral pesticides [2–9].

Moreover, pollution by heavy metals is a serious problem due to their toxicity and ability to accumulate in the biota [10]. At the cellular level, heavy metals are often involved in oxidative stress, which results in the production of reactive oxygen species (ROS) [11]. ROS includes the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical, all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids [11].

Furthermore, the importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stresses by scavenging of ROS. The antioxidant system comprises several enzymes such as superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPx) [11, 12]. The catalase (EC 1.11.1.6), although not responding to a group of contaminants, has been shown to be a part of the antioxidant defence. Catalase removes hydrogen peroxide from cells during basal aerobic metabolism or after a pollution-enhanced oxyradical generation that can be within the limit of tolerance of the organism [13]. Besides, it has been demonstrated that the activity of CAT is induced upon metal exposure to animal species by some environmental parameters and chemicals pollution [11, 14].

Among all kinds of aquatic organisms, brown mussels (*Perna perna*) have been widely used in the evaluation of

the quality of aquatic environments [15–17]. This study chose *Perna perna* as the test animal and aimed to investigate the influence of cadmium, lead and copper on catalase activity, total proteins and bioaccumulation, and their correlation during 72 h of exposure. In the other part, we investigate the reversibility of catalase in exposure-depuration test (72 h/10 days) and if this induction of catalase is metal and/or mixture of metals dependent.

2. Materials and Methods

Mussels *Perna perna* (4.5–5.5 cm shell length) were collected from natural beds at Figuier, Boumerdes, Algeria (36°46′05.38′N, 3°28′37.92′E). After collection, mussels were immediately transported to the laboratory and acclimatized in tanks with aerated natural filtered seawater for a period of 15 days. Stock solutions of cadmium, lead, and copper were freshly prepared by dissolving the proper metal salts of cadmium sulfate, lead (II) nitrate, and copper (II) chloride dihydrate in deionized (double distilled) water. Fresh stock solutions were prepared daily. These solutions were serially diluted to get the experimental concentration for the toxicity test. The experimental method includes static renewal (24-hour renewal) test. Moreover, for all tests (exposure, exposure-depuration), the mussels were divided into rectangular polystyrene trays 42 × 28 × 19 cm (15 mussels per tray) in a total volume of 20 L of seawater/tray. Besides, in exposure test, the specimens mussels were exposed to cadmium (Cd), lead (Pb), and copper (Cu) at different concentrations (Cd, Pb: from 0.05 to 8 $mg{\cdot}L^{-1}$; Cu: from 0.005 to 0.025 $mg{\cdot}L^{-1}$) for up to 72 h (exposure phase). A tray with pure seawater had served as control. However, in exposure-depuration test, mussels were exposed to cadmium (C_1 = 2 $mg{\cdot}L^{-1}$), lead (C_2 = 2 $mg{\cdot}L^{-1}$), copper (C_3 = 0.015 $mg{\cdot}L^{-1}$), and mixture ($C_1 + C_2 + C_3$) for up to 72 h and were then transferred in a clean water for 10 days (depuration phase). In parallel, a tray with pure seawater had served as control.

2.1. Some Physicochemical Properties and Nutrients Salts Dissolved of Aqueous Medium. Physicochemical properties and nutrients were measured daily in medium, before and after each water renewal (24-hour renewal). Determination of temperature (°C), salinity (Psu), dissolved oxygen ($mg{\cdot}L^{-1}$), and pH is done using a multiparametric YSI 556. Ammonia ($NH_{3,4}$), nitrogen nitrous ($N\text{-}NO_2^-$), nitrate nitrogen ($N\text{-}NO_3^-$), and orthophosphate ($P\text{-}PO_4^-$) were estimated by following standard analytical methods [18].

2.2. Biochemical Determinations. In order to determine proteins levels and catalase activity, the tissue samples of 15 survived test animals were collected from each tray at the final stages of the exposure test (72 h). Moreover, in exposure-depuration test 3 to 4 mussels were collected from each tray after 72 h of exposure phase. In the second phase of exposure-depuration test (depuration phase), mussels were collected in selected days (1st, 4th, 7th, and 10th). To ensure the stability of organelles and pH, 5 g mussel's tissue initially underwent a homogenization in chilled mortar by mixing at a rate of 1/10 w/v in Tris (hydroxymethyl) aminomethane 20 mM buffer, pH 7.8, and then was centrifuged at 10000 g for 30 min at 4°C. The obtained supernatant (S9 fraction) was used to determine the proteins levels, the CAT activity.

2.2.1. Total Protein. Proteins were assayed by the method of Lowry et al. [19] 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%.

2.2.2. Catalase Activity. The CAT activity was determined by the method of Lartillot described by Atli et al. [20]. 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.

2.3. Metal Analysis. For metal analysis, the soft tissues from each individual were dried in an oven at 70°C for 48 h [21] and then digested with aqua regia or pseudototal digestion ($HCl\text{-}HNO_3\text{-}H_2O$) at 95°C for two hours until the solution was clear [5]. The solution is diluted to 100 mL with deionized water (double distilled) and used for the chemical analysis. Cadmium, lead, and copper were then analysed by atomic absorption spectrophotometry type Perkin Elmer Analyst 700; air-acetylene oxidizing flame source was used. The determination limits levels of the AAS machine for Cd (II), Pb (II), and Cu (II) ions were 0.03 $mg{\cdot}L^{-1}$, 0.2 $mg{\cdot}L^{-1}$, and 0.2 $mg{\cdot}L^{-1}$, respectively.

The quality of the attacks was monitored by analysis of international standards which are certified concentrations, provided by the Japan International Cooperation Agency (JICA) in Algeria. We used as standards cod fish tissue (CRM 74026-a, with certified concentration, Cu: 1.25±0.07 $mg{\cdot}Kg^{-1}$ dry weight).

2.4. Statistical Analysis. All data were processed using SPSS 17.0 for Windows software. All parameters detected were statistically compared by one-way analysis of variance and LSD multiple comparison test amongst the means. The relationships among the biochemical and chemical parameters were calculated by Spearman's rank correlation. Statistical analysis was expressed as means ± SD, with $P < 0.05$ being considered significant and $P < 0.01$ being considered extremely significant.

Table 1: Means of some physicochemical properties of the aqueous medium before and after the renewal. (+): augmentation, (−): diminishment.

Parameters	Control Cd	Test Cd	Control Pb	Test Pb	Control Pb	Test Cu
ΔT (°C)	+0.810	−0.116	+1.350	−0.106	−0.260	−0.087
ΔpH	+0.030	+0.056	+0.006	+0.150	+0.740	+0.217
ΔSalinity (Psu)	−0.010	+0.033	−0.016	−0.110	+0.860	+0.242
ΔDissolved oxygen	nd	nd	+0.070	+0.226	nd	nd
$\Delta NH_{3,4}$ ($mg{\cdot}L^{-1}$)	+0.003	−0.006	−0.003	−0.014	+0.001	−0.017
$\Delta N\text{-}NO_2^-$ ($mg{\cdot}L^{-1}$)	−0.073	−0.169	+0.030	−0.009	+0.511	+0.563
$\Delta N\text{-}NO_3^-$ ($mg{\cdot}L^{-1}$)	+0.032	−0.068	+0.048	+0.009	+0.236	+0.193
$\Delta P\text{-}PO_4^-$ ($mg{\cdot}L^{-1}$)	−0.030	−0.110	+0.070	−0.423	−0.020	−0.957

nd: not determined.

Table 2: Means (±SD) of bioaccumulation of different concentrations of cadmium, lead, and copper after 72 h exposure and Pearson correlation coefficients between metals exposure and metals accumulation.

Tests of cadmium and lead			Test of copper	
Concentration ($mg{\cdot}L^{-1}$)	Tissue Cd content ($mg{\cdot}Kg^{-1}\ dw^{-1}$)	Tissue Pb content ($mg{\cdot}Kg^{-1}\ dw^{-1}$)	Concentration ($mg{\cdot}L^{-1}$)	Tissue Cu content ($mg{\cdot}Kg^{-1}\ dw^{-1}$)
Control	<3	<20	Control	31.92 ± 0.0001
0.05	<3	<20	0.005	51.48 ± 0.0002
0.20	10.68 ± 0.0013	80.12 ± 0.0001	0.015	78.64 ± 0.0001
1	72.46 ± 0.0007	325.26 ± 0.0003	0.025	91.99 ± 0.0001
2	73.45 ± 0.0012	481.80 ± 0.0004	—	—
4	249.01 ± 0.0016	982.14 ± 0.0002	—	—
8	381.57 ± 0.0016	922.72 ± 0.0001	—	—
Correlation	0.794[a]	0.891[a]	0.985[a]	

[a]Highly significant correlation ($P < 0.01$).

3. Results and Discussion

3.1. Acute Toxicity

3.1.1. Some Physicochemical Properties and Nutrient Salts Dissolved of Aqueous Medium. Table 1 shows any significant differences of the average difference of physicochemical properties and nutrient salts dissolved of seawater before and after the tests. Besides, the effects of the variation of the abiotic parameters have been reported on CAT activity and bioaccumulation of heavy metals [12, 22–26]. Moreover, temperature and salinity affected the filtration rate of mussels and the amount of oxygen consumed. As a result, this influences nutriments uptake and therefore xenobiotics uptake [7, 13]. Volodymyr (2011) [12] reported the variation of the environmental conditions (temperature, pH, oxygen level, salinity, transition metal, ions, and iron) known to be inducers of oxidative stress in aquatic animals. In addition, Khessiba et al. [24] reported that CAT activity increased in *Mytilus galloprovincialis* with the increase of the temperature and salinity. In the present study, the abiotic factors may be less important effects when compared to the stress level of the heavy metals contamination for our specimens. In fact, the measurements obtained of biomarker (CAT) and bioaccumulations of heavy metals of mussels are the direct result of the effect of metals contamination.

3.1.2. Tissue Metal Content. Table 2 shows that the accumulation of Cd, Pb, and Cu in *P. perna* is concentration-dependent. Besides, the metal accumulation in mussels increased linearly with increasing concentration of metals in the medium. The coefficient of correlation R^2 values between metal concentration in solution and that accumulated by mussels for Cd, Pb, and Cu were 0.794, 0.891, and 0.985, respectively.

Moreover, during the accumulation processes of heavy metals, the accumulation mechanism is an overall combination of uptake, metabolism, and excretion processes [22]. In this paper, an increase in the bioaccumulation in exposed specimens suggested the ability of *P. perna* to bioaccumulate these metals in their tissues. Further, the bioaccumulation capacity of bivalves exposed to metal contamination in the laboratory has been demonstrated in other marine mussels such as *Mytilus galloprovincialis* [22], *Perna viridis* [27], and *Mytilus edulis* [28]. Moreover, the apparent biological accumulation indicated that partitioning of this compound between seawater and mussels was an important accumulation mechanism, and a steady state was reached after a short treatment time. Furthermore, Kamaruzzaan et al. [27] reported that the rate of bioaccumulation of essential heavy metals in *Perna viridis* is faster than nonessential metals. In this paper, based on accumulated levels in the tissue of *Perna perna*, the bioaccumulation of cadmium, lead, and copper followed this order $Cu^{2+} > Pb^{2+} > Cd^{2+}$. These results are

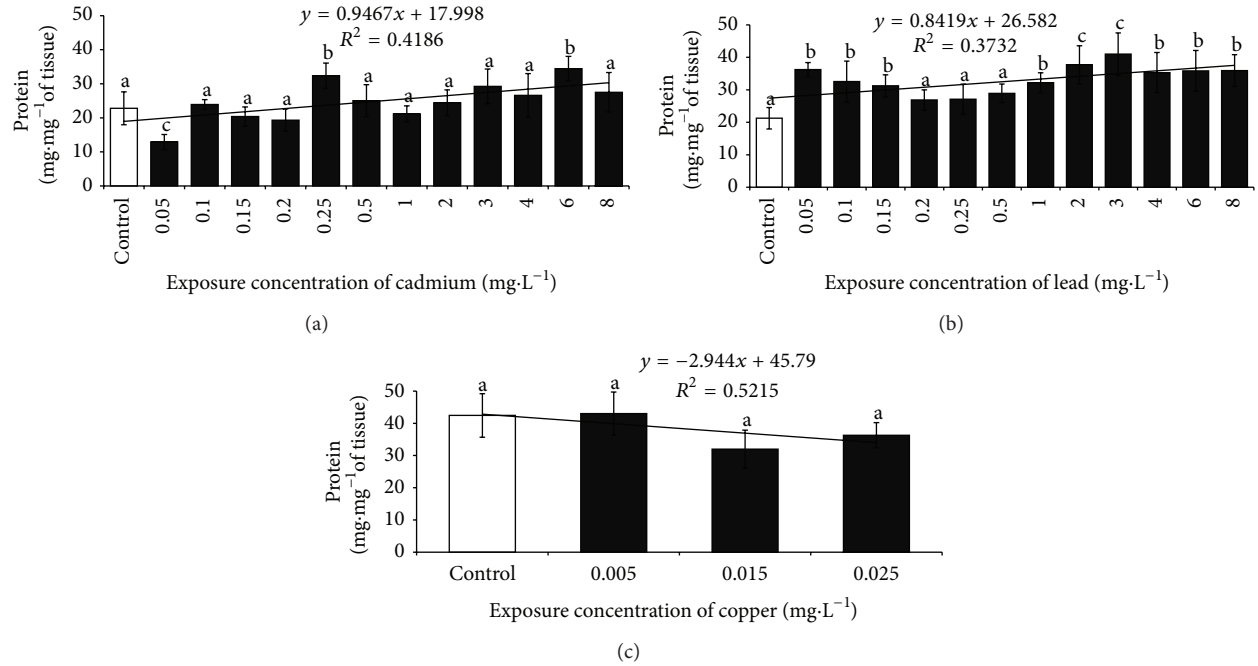

Figure 1: Mean (± SD) variation of total protein in *Perna perna* of control and Cd-exposed (a), Pb-exposed (b), and Cu-exposed mussels (c) after 72 h exposure. Values followed by the same letter are not statistically different ($P > 0.05$) but values followed by the different letter are statistically significant ($P < 0.05$).

comparable to the study where the rate of bioaccumulation followed this order Fe > Zn > Cu > Co > Pb > Cd [27].

3.1.3. Total Protein. *Perna perna* exposed to cadmium, lead, and copper showed changes in metabolites. The values of total protein were not significantly higher ($P < 0.05$) in the exposed concentrations (0.25 and 6 mg·L^{-1}) of cadmium when compared with control (Figure 1(a)). Furthermore, the total level of protein significantly decreased ($P < 0.05$) to cadmium concentration (0.005 mg·L^{-1}). Besides, concentrations of cadmium (0.1, 0.15, 0.20, 0.50, 2, 3, 4, 6, and 8 mg L^{-1}) showed no significant difference after 72 h of exposure when compared with control (Figure 1(a)). However, the values of total protein were significantly higher ($P < 0.01$) in all the exposed concentration of lead when compared with control (Figure 1(b)). Though, the mean values of proteins in the tissues of *P. perna* mussels at 0.005 mg·L^{-1} of copper exposure showed no significant difference after 72 h exposure. Moreover, the values of total protein were not significantly lower ($P > 0.05$) in exposed concentrations (0.015 and 0.025 mg·L^{-1} of copper) when compared with control (Figure 1(c)). Moreover, a weak correlation between the levels of total protein and the exposure concentrations ($R^2 = 0.418$, $R^2 = 0.373$, and $R^2 = 0.521$ for Cd, Pb, and Cu, resp.) indicates that the total level of protein had variation with no linearity in the Cd, Pb, and Cu treated *P. perna*.

Protein reserves appear to be affected by the chemical pollutant [25, 26]. However, Rajkumar (2013) [26] reported that the metabolites of mussels given by the level of total protein can be affected by heavy metal exposures. At low or height cadmium, lead and copper concentrations, and physiological concentrations of H_2O_2, protein damage is restricted to amino acid modification at specific metal binding sites [25]. Moreover, Rajkumar and John Milton [25] observed an increase of levels of total proteins in *Perna viridis* exposed to deferent concentrations of lead, cadmium, and zinc, but no significant changes in copper exposure. Besides, no significant difference in protein contents was reported by Borković et al. [29] in mussels *M. galloprovincialis* collected in a place which is known for intensive industrial pollution during both winter and spring seasons. However, Rajkumar (2013) [26] reported that the total level of protein in *Perna indica* significantly decreased with increase in concentration of a trivalent arsenic in short toxicity test. Though, according to Mosleh et al. [30], 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 [31].

3.1.4. Catalase Activities. *Perna perna* exposed to cadmium, lead, and copper showed changes in CAT antioxidant enzyme. Furthermore, all treatment groups show the catalase activity significantly increased with increase in concentration in the medium (Figures 2(a), 2(b), and 2(c)). Moreover, after 72-hour exposure, a very significant increase ($P < 0.01$) in catalase activity was observed at 8, 8, and 0.025 mg·L^{-1} cadmium, lead, and copper groups, respectively (72.05 ± 4.5,

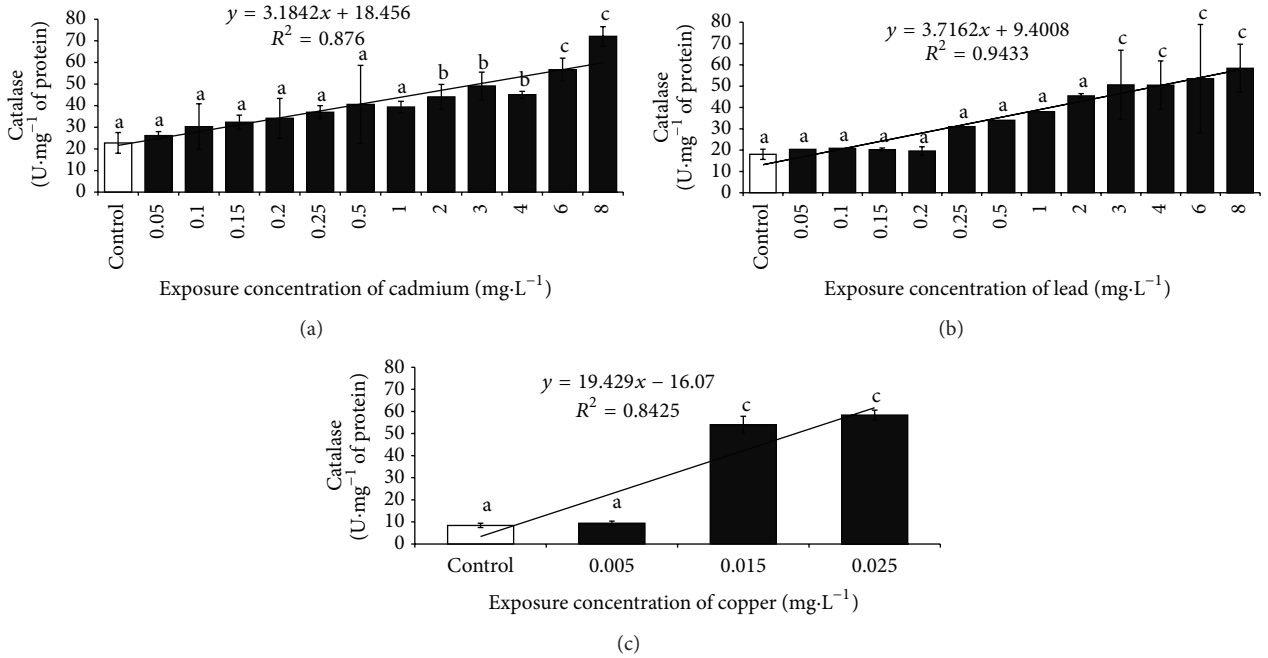

Figure 2: Mean (± SD) variation of catalase activity in *Perna perna* of control and Cd-exposed (a), Pb-exposed (b), and Cu-exposed mussels (c) after 72 h exposure. Values followed by the same letter are not statistically different ($P > 0.05$) but values followed by the different letter are statistically significant ($P < 0.05$).

58.41 ± 11.27, and 58.32 ± 2.27 U·mg^{-1} of protein, resp.), compared to the control group.

Besides, in bivalves increased CAT activity is associated with tissue burden accompanied by heavy metals [32]. CAT is the primary scavengers of H_2O_2 in the cell. Increased CAT activity presently seen exposed to trace metals in *P. perna* further indicates that pollution stress has elevated the formation rate of H_2O_2. These results are in agreement with those reported by Rajkumar and John Milton [25] who observed a significant induction of catalase in *Perna viridis* by Cd, Cu, Pb, and Zn under short toxicity test. Moreover, these results are comparable to those found in other studies, where CAT activity increases at sites contaminated with metals [33]. Also, elevated activity of CAT was reported in *Mytilus galloprovincialis* in the Adriatic Sea [29]. Seasonal alterations in the CAT activities were measured in the digestive gland of the brown mussel *P. perna* [16].

However, some heavy metals are required at structural and catalytic sites in protein and are regarded as essential metals; others do not have a known biological function and are thus considered as nonessential metals [34]. Furthermore, the CAT mechanism could also be better understood by controlling the speciation of cadmium, lead, and copper which plays a fundamental role in the interactions between metal ions and living organisms [35]. Moreover, the effects of trace metals on antioxidant defence enzymes are more evident when different species are compared. Trace metals seem to influence directly the antioxidant defence enzyme due to biological factors, which regulate fluctuations of defences [36].

Therefore, metal ions are well known inducers of oxidative stress [12, 25, 26]. They can stimulate ROS production via two different mechanisms. The first one is related with the interference of metal-related processes and the second one with the generation of free radicals by ions with changeable valence (transition metal ions) via Haber-Weiss reaction [12]. Additionally, cadmium and lead, the typical nonessential and essential metals, are often found together in the environment because of similar properties, but biologically they have diverse properties. Both of them can be toxic when present above the critical level [37]. In addition, cadmium and lead are not transition metals; on the contrary, copper (redox active metals) indirectly induces ROS production and lipid peroxidation by affecting the antioxidant systems and affects a physiology of mussels [12, 25]. Determinations of sulfhydryls group levels that metals have high affinity towards and total protein levels could be beneficial in estimating the toxicity of metals [38].

Besides, presence of contaminants is likely responsible for elevated CAT activity in tissues of *P. perna*. In this paper, an induction of CAT observed in the tissues exposed to cadmium, lead, and copper suggests greater utilization of CAT in order to combat metals present in these marine media. Moreover, lead alters filtration and assimilation rates [39], changes expression of stress proteins [40], and decreases immunity in mussels [41]. In addition, lead binds with glutathione and decreases GSH level [42]. Similar responses of decreased GSH level have been shown in the mussel *Perna perna*, as an indicator of oxidative stress [15]. Lead induces oxidative stress in mussels, suggesting that lead, along with other metals, can induce LPO and production of ROS [43]. Contrary to cadmium and lead, copper homeostasis in aquatic animals entails on regulated uptake, transport, and excretion. However, there

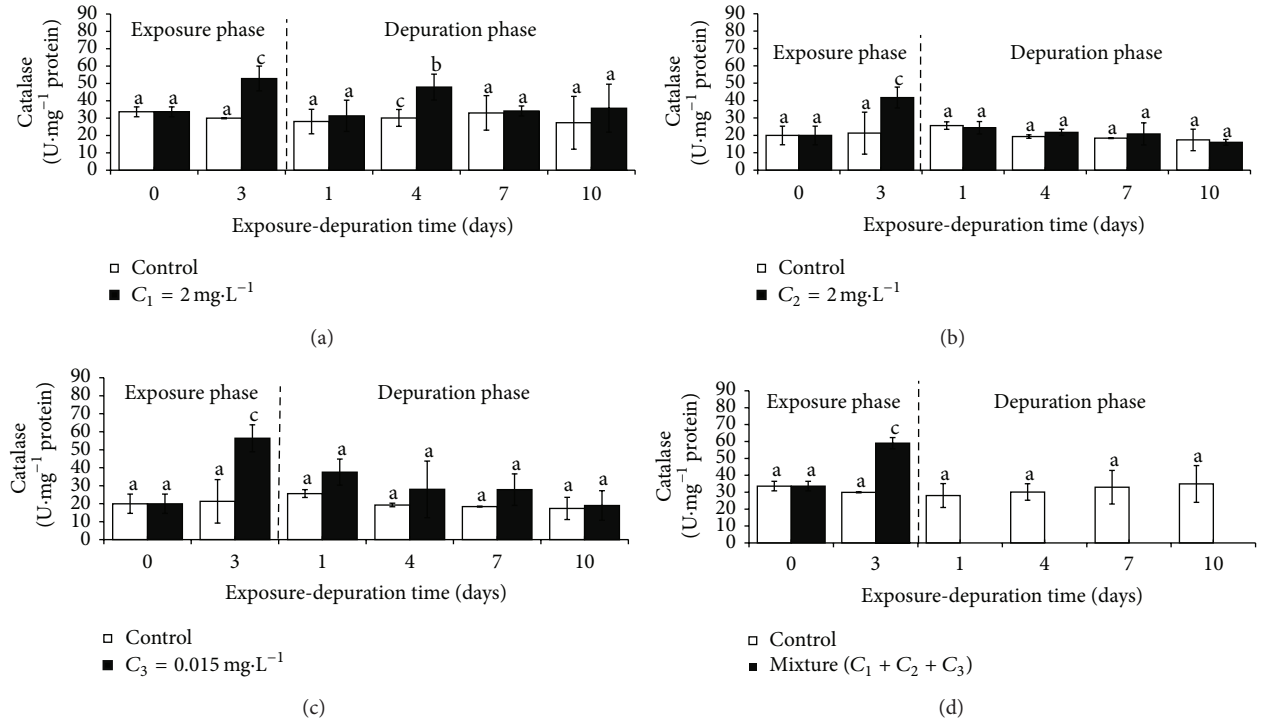

Figure 3: Mean (± SD) variation of catalase activity in *Perna perna* of control and Cd- (a), Pb- (b), Cu- (c), and mixture-exposed mussels (d) during 3 days of exposure and 10 days of depuration. Values followed by the same latter are not statistically different ($P > 0.05$) but Values followed by the different latter are statistically significant ($P < 0.05$).

is some specificity of these processes in hydrobionts related with the possibility of brachial uptake along with intestinal. Both ways are efficient but depend on many factors and are highly regulated processes [44].

In this study, lower concentrations of copper at $0.035\,mg{\cdot}L^{-1}$ were lethal since no mussels survived during 24 h of exposition. On the contrary, Cd and Pb do not cause acute physiological or biochemical damage to *Perna perna* compared with copper. It seems that the toxicity of these two metals occurs only during chronic exposures. However, cadmium and lead, different from copper, are redox inactive metals, whose toxic effects are induced mainly by acting on the sulfhydryls groups of the proteins [12, 45]. Consequently, it is possible that the concentrations of metals required for Cd^{2+} and Pb^{2+} to induct this response enzyme are well below those that cause a crisis in the organism or a visible degradation of the marine ecosystem. Besides, variations of CAT activity are consistent with other findings, showing decreased and increased activities exposed to cadmium, copper, lead, and zinc [46]. In this study, the degree of induction of CAT activity can be classified as follows: CAT (Cu^{2+}) > CAT (Cd^{2+}) > CAT (Pb^{2+}). However, statistical results showed the CAT in tissue was significantly positively correlated ($R_s = 0.921$, $R_s = 0.949$, and $R_s = 0.949$ for Cd, Pb and Cu, resp.) with the accumulated Cd, Pb, and Cu in the tissue of *Perna perna* after 72 h exposure (Table 3).

3.2. Exposure-Depuration Test. Figures 3(a), 3(b), 3(c), and 3(d) show the induction and the return of catalase activities to their initial level after exposure-depuration test (72 h/10 days). Overall, in all the exposure-depuration tests, a highly significant ($P < 0.01$) increase in CAT activity was observed during the phase of exposure for all tested xenobiotics (Cd, Pb, Cu, and mixture). The high significant induction ($P < 0.01$) was recorded after 72 h exposure ($59.04 \pm 3.33\,U{\cdot}mg^{-1}$ protein) in mixture of copper cadmium ($C_1 = 2\,mg{\cdot}L^{-1}$), lead ($C_2 = 2\,mg{\cdot}L^{-1}$), and copper ($C_3 = 0.015\,mg{\cdot}L^{-1}$), when compared to controls ($29.24 \pm .28\,U{\cdot}mg^{-1}$ of protein).

Table 3: Pearson correlation coefficients between catalase activity and metals accumulation.

Parameters	Pearson correlation
Cadmium accumulated	0.921[a]
Lead accumulated	0.949[a]
Copper accumulated	0.949[a]

[a]Highly significant correlation ($P < 0.01$).

Moreover, the lowest average difference of physicochemical properties and nutrient salts dissolved of seawater before and after the exposure-depuration tests does not represent a possible source of physiological perturbation for mussels and catalase activity (Table 4).

Furthermore, these results support the reproducibility of results in acute exposure test. Besides, it appears that the induction of CAT activity is higher in mixture than in metal only. Therefore, the nonspecific nature of its response as a biomarker is an advantage of a state of mixed metal

Table 4: Means of abiotic parameters in exposure-depuration tests (exp: exposure, dep: depuration, (+): augmentation, and (−): diminishment).

Parameters	Cd-exp-dep		Pb-exp-dep		Cu-exp-dep		Mixture-exp-dep	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
ΔT (°C)	−0.530	−0.280	+0.250	+0.500	+0.250	+0.440	−0.530	+0.070
ΔpH	+0.010	−0.090	+0.010	−0.010	−0.420	−0.640	+0.010	+0.050
ΔSalinity (Psu)	−0.550	+0.030	−0.910	−0.170	−0.910	+0.360	−0.550	+0.010

pollution [15]. Moreover, in this paper, the degree of induction of CAT activity can be classified as follows: CAT $(Cu^{2+} + Cd^{2+} + Pb^{2+})_{mixture}$ > CAT $(Cu^{2+})_{only}$ > CAT $(Cd^{2+})_{only}$ > CAT $(Pb^{2+})_{only}$.

The return of catalase activities to their initial level after decontamination test illustrated the adaptability of molluscs responses which allows us to qualify them as good bioindicators. The catalase seems to adapt to environment changes, which make us think that this biomarker may be used to monitor chemical pollution. In this paper, the enzymatic activity decreased for all the groups after the transfer of mussels in a pollutant-free water (decontamination phase).

Moreover, after 1 day, 3 days, 7 days, and 10 days of depuration, CAT activity decreased in mussels exposed to 2 mg L^{-1} of lead to values of 24.39 ± 3.59, 21.74 ± 1.8, 20.83 ± 6.37, and 1.63 ± 15.99 U·mg^{-1} protein, respectively. Reductions are 38.82%, 47.95%, 50.13%, and 61.71%, respectively. Similarly, CAT activity decreases in mussels exposed to 0.015 mg L^{-1} of copper to values of 37.55 ± 7.23, 27.96 ± 15.76, 27.81 ± 8.76, and 19.00 ± 8.27 U·mg^{-1} protein after 1 day, 3 days, 7 days, and 10 days, respectively. Reductions are 38.82%, 47.95%, 50.13%, and 44.07%, respectively. Also, after 1 day, 3 days, 7 days, and 10 days of depuration, CAT activity decreased in mussels exposed to 2 mg L^{-1} of cadmium to values of 31.31 ± 9.01, 47.88 ± 7.49, 34.11 ± 2.92, and 13.83 ± 35.71 U·mg^{-1} protein, respectively. Reductions are 33.38%, 50.39%, 50.66%, and 66.29%, respectively.

However, in case of mixture, the results showed the percentages of mortality which are 26, 20, and 20% after 24, 48, and 72 h exposure, respectively, and 100% after one day of depuration phase. It appears that a lethal effect occurs in decontamination phase in mussels exposed to mixture. Several authors have noted that the lethal effect occurs in depuration phase after the exposure; Sunila (1981) [47] reported that the mortality of *Mytilus edulis* occurs in 6 days of depuration after the exposure at 0.4 mg·L^{-1} of copper, indicating that the biological damage is irreversible.

Though, CAT has proved capability of reflecting the state of the surrounding environment in a short time (10 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. [48] after six days of depuration cycle with the mussels *Bathymodiolus azoricus* that had followed 24 days of exposure to 25 μg·L^{-1} copper; CAT activities recorded in gills were found below those of contamination phase. Also, Leonie et al. [49] observed a return of catalase activity to initial levels after 7 days of depuration in the gills of oysters exposed to different concentrations of copper.

4. Conclusion

In conclusion, the metabolites and antioxidant enzymes activity was affected by heavy metal exposures and they strongly have the potential as indicators of heavy metal contamination and cadmium, lead, and copper in specific. Besides, the induction of catalase in *P. perna* is metal and/or mixture concentration-dependent. Hence, the determination of oxidative stress biomarker (CAT) in *Perna perna* may serve as a convenient approach during pollution biomonitoring programme.

Conflict of Interests

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

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