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Comparative and Interactive Biochemical Effects of Sub-Lethal Concentrations of Cadmium and Lead on Some Tissues of the African Catfish (*Clarias gariepinus*)

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Abstract

Cadmium is a strong toxic heavy metal which presents in paints and liquid wastes and causes oxidative stress in fish. On the other hand, lead is widely used for different purposes, e.g. lead pipes, it targets vital organs such as liver and kidney causing biochemical alterations. The present study evaluates the effects of 60 days exposure to Cd and Pb either single or combined together in African catfish. Sixty-four fishes were divided into 3 groups and exposed to $CdCl_2$ (7.02 mg/L) or $PbCl_2$ (69.3 mg/L) or a combination of them along with control group. Activities of acid phosphatase (ACP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) were estimated. Moreover, gill, liver and kidney were assayed for activities of superoxide dismutase (SOD), catalase (CAT) and levels of glutathione (GSH) and malondialdehyde (MDA). Individual exposure showed that both Cd and Pb significantly decreased LDH activity and SOD activity in the kidney. Pb significantly increased G-6-PDH activity and decreased GSH level in the gill. CAT activity in liver and kidney elevated significantly on Cd exposure while lead caused a significant depletion in the liver and significant elevation in the kidney. Both Cd and Pb significantly increased MDA levels in liver and kidney while Pb increased its level in gills. The combined exposure resulted in normalization of LDH, G-6-PDH activity, and CAT activity in liver and kidney as well as GSH level in both tissues and MDA in gill and kidney. The combination increased SOD activity and MDA level in liver and decreased SOD activity in kidney and GSH level in gills. In conclusion, the antioxidant system of African catfish was adversely affected by prolonged exposure to Cd and Pb. The combined exposure caused less damage than individual exposure and returned most parameters to those of controls.

Key words: Cadmium, Lead, Catfish, Catalase, SOD, G-6-PDH

INTRODUCTION

Heavy metals are a persistent component of the environment and are difficult to remove completely by biological degradation. Freshwater habitats are mostly affected by heavy metals that come from the discharge of untreated wastes from industrial facilities, some agricultural processes and daily life activities, problems resulting from aquatic pollution with toxic heavy metals are of a great concern as it adversely affects both aquatic biota and human (1).

Cadmium is well known as strong toxic heavy metal which presents in paints, mining extracts and liquid wastes (2), it usually targets the kidney and liver (3), on the other hand, lead is widely used for different purposes, e.g. lead pipes and print shops and lead batteries, which increase its

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List of abbreviations: ACP, acid phosphatase; CAT, catalase; $CdCl_2$, cadmium chloride; G-6-PDH, glucose-6-phosphate dehydrogenase; GSH, glutathione; LDH, lactate dehydrogenase; MDA, malondialdehyde; OECD, organization for economic co-operation and development; $PbCl_2$, lead chloride; PBS, phosphate buffered saline; SOD, superoxide dismutase.

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presence in the biological system and may cause lead poisoning at exposure by targeting vital organs such as liver, kidney and brain. Previous studies on fishes have shown that exposure to cadmium or lead induces biochemical changes in tissues and oxidative stress by interfering with the enzymes activity (4-6) and increasing lipid peroxidation in many organs such as brain, liver, kidney and gills (7-10).

Physiological changes in Freshwater fishes are widely used as a biomarker of any stressor component in the aquatic environment (11,12). The changes in activities of antioxidants enzymes (SOD, CAT, G-6-PDH and LDH), glutathione level and lipid peroxidation product could be used as a biomarker of water pollution. When aquatic organisms are exposed to a potential pollutant, these enzymes are usually affected either by activation or inactivation, these effects have been reported in many studies on fishes (13,14).

African catfish (*Clarias gariepinus*) is commercially important fish because it is considered as a cheap food source in many African countries including Egypt, due to the lack of toxicity studies on catfish in Egypt, this study aimed to evaluate the biochemical effects of sub-lethal concentration of cadmium or lead alone or combined with each other on liver, kidney and gills of African catfish using antioxidants enzymes, glutathione level and lipid peroxidation product.

MATERIALS AND METHODS

Chemicals. Cadmium as $CdCl_2 \cdot H_2O$ (monohydrate, molecular weight 201.32 g/mol, purity 98%) and lead as in the form of $PbCl_2$ (molecular weight 278.1 g/mol, purity 98%) manufactured by Oxford Lab Chem (Maharashtra, India).

African catfish. Sixty-four adult African catfish (mean body length = 28 ± 2.8 cm, mean body weight = 150 ± 25 g) were collected from Manzala Lake, Damietta governorate, Egypt; apparently healthy fishes were transported immediately to the lab and handled according to animal welfare guide. They were allowed to acclimatize for 15 days in glass aquaria (40 × 35 × 70 cm) containing 60 L of dechlorinated tap water which was replaced every day. Fishes were fed daily on a commercial basal fish diet.

Experimental design. The experiment was done according to the Organization for Economic Co-operation and Development guidelines (15). After acclimation, 64 fishes were divided into four groups (16 fishes/group); Group 1: fishes were kept in dechlorinated water and served as control, while fishes of Group 2, 3 and 4 were exposed to 30% of 96 hr LC_{50} value of $CdCl_2$ (7.02 mg/L), $PbCl_2$ (69.3 mg/L), and a combination of $CdCl_2$ (7.02 mg/L) plus $PbCl_2$ (69.3 mg/L), respectively, at constant temperature 25°C and 12 hr light/dark cycle for 60 days.

Blood sampling. Fishes were not fed for 24 hr before sampling, blood samples of eight fishes were collected from the caudal vein using 3 mL heparinized syringe within less than 3 min to minimize handling stress. The collected blood samples were centrifuged for 30 min at 1,207 ×g at 4°C within one hour of sampling. The plasma samples were collected for determination of acid phosphatase and LDH, while erythrocytes were diluted with phosphate buffer saline (1:1) and mixed by vortex for few minutes on ice, erythrocyte lysates were used for determination of G-6-PDH using commercial kits (Biodiagnostics Co., Giza, Egypt).

Tissue sampling, homogenate preparation and biochemical measurements. The remaining eight fishes from each group were sacrificed. Gills, liver and kidney were weighed separately and rinsed with phosphate buffered saline (PBS), pH 7.4, to remove any erythrocytes or clots. In order to prepare a 10% homogenate from each tissue, a specific weight from each organ was taken, minced and homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were then centrifuged at 1,500 ×g at 4°C, the collected supernatants were stored at −80°C for determination of SOD, glutathione (GSH), catalase (CAT) and malondialdehyde (MDA) using commercial kits (Biodiagnostics Co.).

Statistical analysis. The data from each group were calculated and represented by Mean ± standard error (n = 8). The Tukey-HSD test was used for multiple comparisons, while the Dunnett-two sided test was used to compare the control with other treated groups. All statistical analyses were performed using XLSTAT program (Addinsoft, NY, USA).

RESULTS

Acid phosphatase (ACP). Statistical analysis of the data shows that there is no significant change in the activity of acid phosphatase of African catfish exposed to Cd or Pb or a combination of Cd plus Pb (Fig. 1).

Glucose-6-phosphate dehydrogenase (G-6-PDH). Fig. 2 shows that the activity of glucose-6-phosphate dehydrogenase increased significantly only in the group exposed to lead compared to cadmium and control groups ($p = 0.0001$), the addition of cadmium to lead decreased G-6-PDH activity to those levels in both cadmium and control groups.

Lactate dehydrogenase (LDH). Lactate dehydrogenase activity in all treated groups is shown in Fig. 3. Cad-

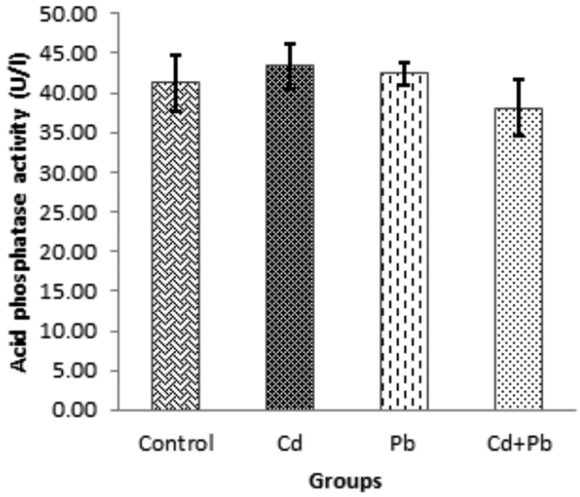

Fig. 1. Acid phosphatase (ACP) activity of African cat fish in control, cadmium, lead and cadmium-lead combination exposed groups. Bars represent means ± SE, letters on each bar indicate significantly different groups.

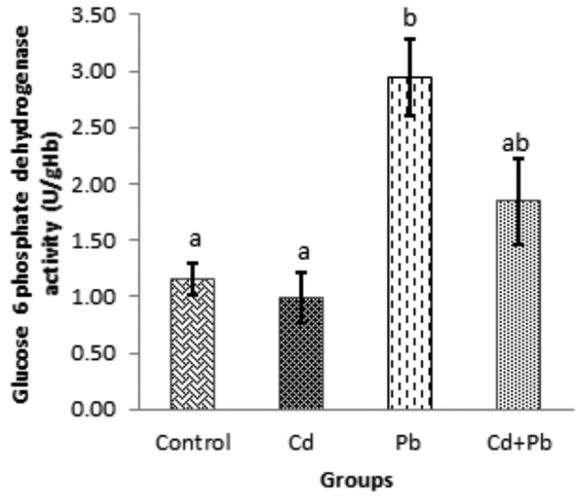

Fig. 2. Glucose-6 phosphate dehydrogenase (G-6-PDH) activity of African cat fish in control, cadmium, lead and cadmium-lead combination exposed groups. Bars represent means ± SE, letters on each bar indicate significantly different groups.

mium-exposed group and the lead-exposed group showed a significant decrease in LDH activity as compared to the control group ($p = 0.0001$ and 0.02, respectively). The lead-exposed group had a significant elevation in LDH activity as compared to the Cd-treated group ($p = 0.02$). The combination between Cd and Pb resulted in LDH activity close to those of the control group and Pb treated group but significantly higher than its activity in the Cd-treated group ($p = 0.02$).

Superoxide dismutase (SOD). Superoxide dismutase activity in gill, liver and kidney of African catfish exposed

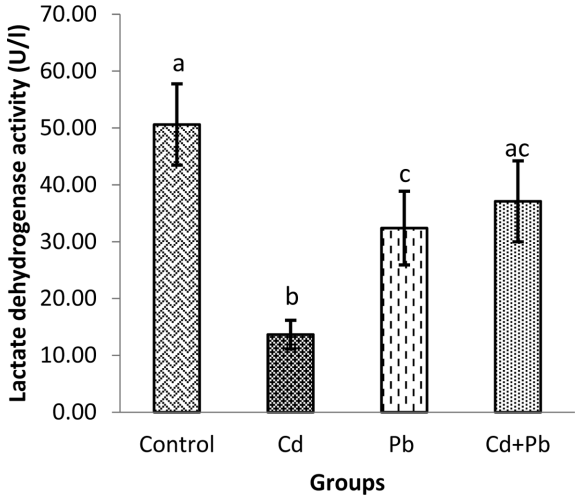

Fig. 3. Lactate dehydrogenase (LDH) activity of African catfish in control, cadmium, lead and cadmium-lead combination exposed groups. Bars represent means ± SE, letters on each bar indicate significantly different groups.

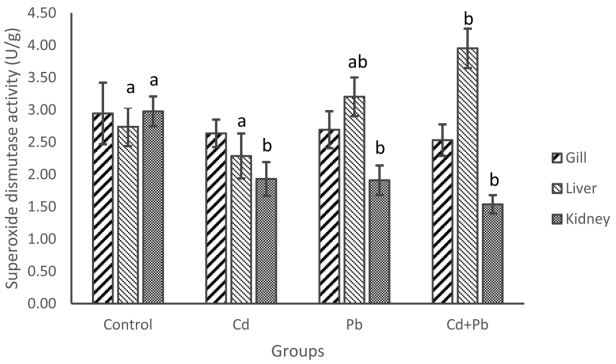

Fig. 4. Superoxide dismutase (SOD) activity in gill, liver and kidney of African catfish in control, cadmium, lead and cadmium-lead combination exposed groups. Bars represent means ± SE, letters on each bar indicate significantly different groups.

to sub-lethal concentrations of cadmium or lead or a combination of cadmium plus lead is shown in Fig. 4. In gill, no significant alterations were found in all treated groups compared to control group, while a significant elevation was found in the liver of Cd plus Pb-exposed group as compared to control group and Cd-exposed group ($p = 0.01$ and 0.001, respectively). On the other hand, there was a significant decrement in the SOD activity of the kidney in all treated groups as compared to the control group ($p = 0.01$).

Glutathione (GSH). Fig. 5 shows the GSH level in different organs. In gill, GSH level was significantly lower in Pb and Cd plus Pb treated groups than the control group and Cd-exposed group ($p = 0.002$). In liver, however, no

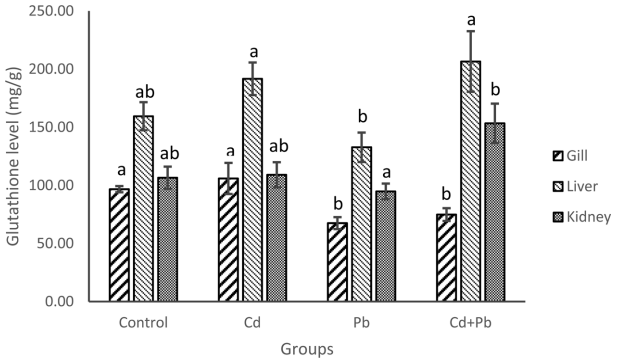

Fig. 5. Glutathione level (GSH) in gill, liver and kidney of African catfish in control, cadmium, lead and cadmium-lead combination exposed groups. Bars represent means ± SE, letters on each bar indicate significantly different groups.

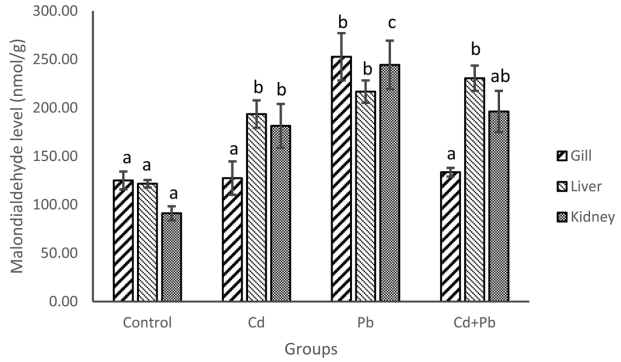

Fig. 7. Malondialdehyde (MDA) in gill, liver and kidney of African catfish in control, cadmium, lead and cadmium-lead combination exposed groups. Bars represent means ± SE, letters on each bar indicate significantly different groups.

significant alteration was observed between all treated groups and control group, the lead-exposed group showed a significantly lower GSH level than Cd-exposed group and Cd plus Pb treated group ($p = 0.01$ and 0.005, respectively). In the same manner, no significant effects were observed between all treated groups and control group in kidney homogenate, but GSH levels showed significant elevation when combining Cd and Pb compared to the group treated with Pb alone ($p = 0.001$).

Catalase (CAT). Catalase activity of all groups is shown in Fig. 6. No significant changes were found in the catalase activity of gill for all groups. In the liver, the group exposed to Cd exhibited a significant elevation in catalase activity while lead exposed group showed a significant depletion as compared to the control group ($p = 0.02$ and 0.01, respectively). Combining cadmium with lead caused a significant increase in catalase activity compared to lead-exposed group ($p = 0.0001$) and very close to its activity in controls. On the other hand, fishes exposed to cadmium or lead showed significant elevations in catalase activity in kidney compared to control group ($p = 0.002$ and 0.001, respectively) and Cd plus Pb exposed group ($p < 0.001$) which was very similar to controls.

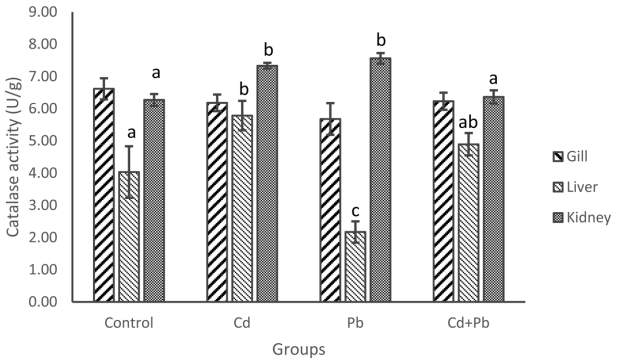

Fig. 6. Catalase activity (CAT) in gill, liver and kidney of African catfish in control, cadmium, lead and cadmium-lead combination exposed groups. Bars represent means ± SE, letters on each bar indicate significantly different groups.

Malondialdehyde (MDA). The effects of sub-lethal doses of cadmium or lead or cadmium plus lead on malondialdehyde level are shown in Fig. 7. In gill, MDA levels increased significantly in the lead-exposed group compared to all other groups ($p < 0.001$), while MDA levels in liver showed significant elevations in all treated groups compared to controls ($p < 0.001$).

In the kidney, a significant increment of MDA was observed in both Cd or Pb exposed group as compared to control ($p < 0.001$ and $= 0.02$, respectively). The lead-exposed group showed a progressive elevation in MDA levels as compared to Cd-exposed group ($p = 0.01$), while the co-exposure to cadmium and lead significantly decreased MDA level as compared to lead-exposed group ($p = 0.01$).

DISCUSSION

The performance of aquatic organisms especially fishes are associated with the quality of their environment. Heavy metals, such as cadmium and lead, are entering the aquatic ecosystem from multiple sources including industrial and agricultural and other human activities. The toxicity induced by heavy metals force fishes to acclimatize by modifying their behavioural and physiological activities to coup with the oxidative stress they face (10). Changes of biochemical parameters have been widely used for early prediction of potentially damaging effects in fish under stress (16,17). The current study investigates the toxicological effect of exposure to sub-lethal doses of Cd and Pb either solitary of combined with each other on African catfish (*C. gariepinus*).

The Acid phosphatase activity in the present study

showed no significant differences between different groups. It is a plasma membrane-derived enzymes which are associated with lysosomal activities, phagocytosis, digestion and transport of nutrients (18), it is also involved in creating phosphate buffer system to maintain buffer system in the blood (19).

Both G-6-PDH and LDH are metabolic enzymes and were used as a bioindicator in stressed fishes (20). G-6-PDH is considered a very important antioxidant enzyme as its activity is usually affected by different heavy metals (21,22). In the current study, G-6-PDH activity increased significantly in the lead-exposed group in comparison to controls. A similar effect of lead exposure on G-6-PDH activity has been reported before in different fishes (22,23), this increase is a protective mechanism against the elevated production of reactive oxygen species (ROS) in stressed cells (24).

On the other hand, LDH is significantly affected in the case of tissue damage induced by heavy metals toxicity (25). Both Cd and Pb significantly decreased LDH activity as compared to control. In consistence with our results, LDH activity was depleted in Nile tilapia, *Oreochromis niloticus*, when exposed to cadmium (26) and in African catfish treated with lead at different developmental stages (22), this inhibition may be due to formation of a complex that inhibits the enzyme (25), direct interaction between heavy metals and LDH (27) or inhibition of metabolic processes (26). Although both metals have an inhibitory effect on LDH activity. Although Cd causes the lowest LDH activity in all groups, the interaction between Cd and Pb caused no significant effect on LDH activity and its level was close to those of controls which might suggest a synergetic effect of the mixture.

Alteration in SOD, GSH, CAT and MDA have been used as indicators of cadmium (10) and lead toxicity (5) in fishes. The results of the present study showed that exposure to cadmium or lead did not affect SOD activity compared to the control group in the liver but significantly decreased its activity in the kidney. Combining both metals significantly increased SOD activity in liver and decreased it in the kidney. Similar results were reported in previous studies where SOD decreased in the kidney of zebrafish after exposure to Pb (5) and of African catfish exposed to Cd (16). The non-significant effect of Cd and Pb may reflect the ability of the liver to detoxify both elements efficiently, but the corresponding increase in MDA (Fig. 7) in liver indicates oxidative stress and failure to maintain adaptive response (28).

On the other hand, significant elevation of SOD activity in liver on exposure to a mixture of both metals may be due to summation effect of both metals which result in accumulation of free radicals where SOD acts as a protective antioxidant enzyme (29). Unlike liver, the significant depletion of SOD reflects the inability of the kidney to metabolize Cd which results in its accumulation (30) and/or the accumulation of ROS because Pb releases Zn^{++} and Cu^{++} from SOD thus preventing it from binding to any substrate and decreasing its activity (31). This decrease of SOD activity results in accumulation of ROS which is proven by the significant increase in MDA.

SOD-CAT system is the first defensive line heavy metal-induced oxidative stress (32). In the present study, no significant changes were found in gills, this is in accordance with those results of *Oreochromis niloticus* where CAT activity did not change after exposure to different heavy metals (33). On the other hand, the activity of CAT increased significantly in the Cd-exposed group in both liver and kidney and in the kidney of the Pb-treated group, while CAT activity in the latter group showed a significant decrease in the liver. The elevation of CAT activity in different tissue on exposure to heavy metal had been recorded before in Cyprinidae fishes living in Seyhan Dam lake (34) and in rainbow trout, Oncorhynchus mykiss (35). The inhibition of CAT activity may be due to the generation of superoxide radicals which strongly decreases CAT activity (32,36). The inhibition of CAT activity previously reported different fishes; *Oreochromis niloticus* (33) and in starlet, *Acipenser ruthenus*, living in the Danube river of Serbia (37).

GSH is a very important antioxidant which has a great ability to bind to free radicals and different heavy metals, e.g. Cd and Pb and protect cells against their adverse effects (38). GSH levels, in the current study, depleted significantly in gills after exposure Pb and Cd plus Pb, the decreased level of GSH usually indicate high lipid peroxidation and elevated level of MDA (Fig. 7). In line with this findings, GSH level decreased in zebrafish when exposed to dimethoate (39) and juveniles of African catfish exposed to lead (17).

In the current study, exposure to cadmium, or lead, alone or combined with each other did not change GSH levels in liver, similar results were reported in previous studies where hepatic GSH levels in *Oreochromis niloticus* were not altered by Cd and/or Pb pollution (40) and in zebrafish exposed to 2 ppm of $Pb(NO_3)_2$ (5). Generally, fishes have very low levels of hepatic GSH, studies on fathead minnow and trout showed the ability of these fishes to maintain normal levels of hepatic GSH during exposure to pollutants (41). The significantly increased GSH levels in the kidney of Cd plus Pb group compared to the Pb-exposed group may be an evidence that Cd abolish or minimize Pb effect suggesting a protective and adaptive role against oxidative stress.

The presence of malondialdehyde (MDA), a product of lipid peroxidation, in tissue is an indicator of oxidative stress. Current study results showed that Cd exposure increased MDA significantly in both liver and kidney while exposure to Pb increased its level significantly in

gill, liver and tissue when compared to controls. There were no changes in MDA levels in gills and kidney when fishes exposed to a combination of Cd and Pb when compared to their levels in controls except liver. In agreement to those results, MDA level increased in liver of fishes collected from polluted sites in Lagos, Nigeria, compared to control sites (42). Similarly, the MDA level increased significantly in all tissue of obscure puffer, *Takifugu obscurus*, when exposed to Cd (43). Conversely, MDA levels did not change in post juvenile catfish exposed to sublethal concentration of Pb for 28 days (17). This difference can be attributed to the duration of exposure.

The current results showed that cadmium alone could not induce any changes in SOD in gills and liver; GSH level in gill, liver and kidney, and CAT and MDA in kidney, these finding have been found before where Cd failed to cause any significant alterations in sea bass Cd failed to cause any significant alterations (44). Exposure to different heavy metals at the same time may potentiate or minimize their toxicity, such interactive effect has been reported in fish (45). In the current study, Cd minimizes the adverse effects of Pb on G-6-PDH, GSH and CAT in both liver and kidney, and MDA in gills and kidney suggesting a protective role of Cd. Similar effect of Cd has been proven in previous studies (46,47).

The results from present research may be used as a useful tool in the assessment of pollution stress in the aquatic environment and its inhabitants so that policies and strategies could be made to reduce the discharge of chemical compounds and heavy metals in freshwater. Moreover, the toxic effect of two combined metals could not be clearly determined because it is not equal to their single effect.

CONFLICT OF INTEREST

The author declares has no competing interests.

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