Organic-specific antioxidant defenses and FT-IR spectroscopy of muscles in Crucian carp (Carassius auratus gibelio) exposed to environmental Pb\(^{2+}\)

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Abstract: Being a nonessential element, lead (Pb\(^{2+}\)) can be toxic in trace amounts for freshwater fish. The present study was designed to evaluate the induction of oxidative stress, tissue-specific antioxidant response, acetyl cholinesterase (AchE) activities, and biochemical content of muscle in Crucian carp (Carassius auratus gibelio) after 96 h of acute exposure and 21 days of chronic exposure to various environmental concentrations (5, 10, and 30 µg L\(^{-1}\)) of waterborne Pb\(^{2+}\). The results indicated that exposure to 10 and 30 µg L\(^{-1}\) Pb\(^{2+}\) significantly altered the activities of catalase, superoxide dismutase, and glutathione peroxidase in different tissues, while malondialdehyde content increased in a time- and concentration-dependent manner. Inhibition of AchE after chronic exposure also confirmed a generalized neurotoxic stress. Fourier transform infrared spectroscopy revealed mild changes in the structure of proteins and lipids, whereas the integrated biomarker response indices signified the liver, kidneys, and gills as the responsive organs to environmental Pb\(^{2+}\) exposure.

Key words: Crucian carp, heavy metal, Pb\(^{2+}\), fish, oxidative stress, Fourier transform infrared spectroscopy, integrated biomarker response

1. Introduction

Lead (Pb\(^{2+}\)) is classified to be the most toxic contaminant due to its predominant use (71%) in various industrial applications (Skerfving and Bergdahl, 2007). Recently, many of the anthropogenic sources of Pb\(^{2+}\), including paint production and leaded gasoline, have been strictly eliminated. Despite such regulations, Pb\(^{2+}\) is still continuously entering the aquatic environment, mainly through anthropogenic means, which makes it a primary pollutant in ecosystems (US EPA, 2006). The level of Pb\(^{2+}\) in freshwater systems has been estimated at 5 µg L\(^{-1}\). However, its level is much higher in aquatic environments that are near iron and steel industries and Pb\(^{2+}\) production and processing units, which are the primary cause of Pb\(^{2+}\) poisoning in fish (Rogers and Wood, 2004; ATSDR, 2007).

As a prominent xenobiotic, Pb\(^{2+}\) has no known biological role in life processes and exerts toxic effects upon prolonged exposure (Mager, 2012). Once absorbed from the surrounding environment, Pb\(^{2+}\) binds to erythrocytes and may be transferred via blood to the soft tissues, including the kidneys, liver, brain, muscles, heart, and spleen. Finally, most of it is deposited in the bones and teeth (Meyer et al., 2008) and it may cause several pathological conditions, such as anemia, kidney failure, and even death (Yao et al., 2013). Although the exact mechanism of Pb\(^{2+}\) toxicity is not clear, it is evidenced that Pb\(^{2+}\) induces oxidative stress by producing reactive oxygen species (ROS) (Dewanjee et al., 2013). These ROS can cause oxidative damage to vital compounds such as proteins, nucleic acids, and lipids. The main mechanism involves an imbalance between the generation and removal of ROS in the tissues and cellular components of exposed subjects. To neutralize the adverse effect of ROS, organisms have an effective defense mechanism of enzymatic and nonenzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), and vitamin E (Kelly et al., 1998; Trenzado et al., 2006; Jankowiak et al., 2015).

Among the antioxidant enzymes, SOD, CAT, and GPx are considered to be the main targets of Pb\(^{2+}\) toxicity because of their dependence on certain essential trace elements for proper molecular structure and activity (Basha et al.,...
2. Materials and methods

2.1. Chemicals and reagents
Lead nitrate of purity of >99%, nitric acid, acetic acid (conc. glacial), potassium iodide crystal, sodium thiosulfate, EDTA (disodium salt of EDTA), magnesium sulfate, 1,1,3,3-tetraethoxypropane (TEP), potassium chloride, sodium dodecyl sulfate (SDS), thiobarbituric acid, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), and acetyltetriacholine iodide were obtained from Sinopharm (Beijing, China). All other chemicals used were of analytical grade. ICP-multielement certified reference materials were obtained from PerkinElmer (No. N9300281, Shelton, CT, USA).

2.2. Animals and experimental conditions
Crucian carp of average size (14 ± 3.2 cm) and weight (112 ± 8.4 g) were obtained from a commercial fish farm and acclimated for a period of 1 week in a 50-L glass aquarium containing dechlorinated tap water. Water quality parameters were assessed prior to the experimentation and thereafter on daily basis according to the standard methods of the APHA (1992). The aquarium was aerated 1 day before starting and later throughout the experiment with a stone aerator connected to a compressed air pump. Water quality parameters (total hardness: 153.44 ± 6.21 mg L–1 as CaCO3, temperature: 23 ± 2 °C, pH: 7.2 ± 0.51, dissolved oxygen: 8.43 ± 0.59 mg L–1) were maintained until the end of the experiment. During acclimatization, fish were fed with artificial feed twice a day. Feeding was stopped 1 day before starting the experiment. All the experimental protocols for fish maintenance and experimentation were approved by the ethical committee for animal handling, Huazhong Agricultural University, Wuhan, P.R. China.

2.3. Pb exposure and sampling
During the experiment, no distinction was made between sexes and all fish were divided into 4 groups (10 fish per group). Group 1 was assigned as a control under similar experimental conditions, but with no Pb2+ exposure. The remaining 3 groups were exposed to Pb2+ in the form of Pb(NO3)2, at respective rates of 2.5, 10, and 30 µg L–1 for a period of 96 h as acute exposure and 21 days as a chronic assay under semistatic conditions. These concentrations were selected on the basis of Pb2+ contamination reported in rivers and lakes earlier (Zhou et al., 2008; Yang et al., 2009; An et al., 2010; Wang et al., 2012; Bing et al., 2013; Li et al., 2013) and considered to be environmentally relevant. The aquarium water was replaced every day to remove the wastes produced by the fish. Pb2+ concentrations were carefully monitored in the stock solutions and the aquarium water with the help of inductively coupled plasma-optical emission spectrometry (PerkinElmer-Optima 8000 Series) to maintain the required concentrations. At the completion of the 96-h and the 21-day exposure periods, all fish were decapitated. Livers, brains, kidneys, gills, and...
muscle tissues were isolated, immediately frozen in liquid nitrogen, and stored at –80 °C until complete analysis.

2.4. Oxidative stress assay

Tissues were homogenized in cold physiological saline (0.9% NaCl; 1:10 w/v) using a glass homogenizer. The homogenates were then centrifuged at 4000 × g for 15 min at 4 °C and supernatants were recovered for further analysis. SOD, CAT, and GPx were assayed using commercial diagnostic reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions.

SOD (EC 1.15.1.1) activity was measured at 550 nm by the method of ferricytochrome c via xanthine/xanthine oxidase as a source of superoxide radicals (Orbea et al. 2002). One unit of SOD activity (U mg–1 protein) is the amount of enzyme needed to cause 50% inhibition of the cytochrome c reduction. CAT (EC 1.11.1.6) activity was examined at 405 nm using the spectrophotometric method of H2O2, based on the formation of a stable complex with ammonium molybdate (Goth, 1991). One unit of CAT activity was defined as the degradation of 1 µmol H2O2 per second per milligram protein and was expressed as U mg protein–1. GPx (EC 1.11.1.6) activity was estimated at 412 nm based on the rate of NADPH oxidation by means of glutathione reductase (Feng et al., 2013). AchE activity in the tissues was measured according to the method of Ellman et al. (1961). The activity was recorded at 412 nm and was expressed as nmol acetylcholine iodide hydrolyzed min–1 protein–1. MDA content as a biomarker of LPO was estimated according to the method of Ohkawa et al. (1979) with little modification. Briefly, 200 µL of 100 mg L–1 tissue homogenate in 1.15% KCl buffer was added to a reaction mixture consisting of 1.5 mL of 0.8% thiobarbituric acid, 200 µL of 8.1% (v/v) SDS, 1.5 mL of 20% (v/v) acetic acid (pH 3.7), and 600 µL of distilled water. The content was heated at 90 °C for 45 min and read at 532 nm. The results were expressed as nmol mg–1 protein. The concentration of protein in the tissue homogenate was determined according to the method of Bradford (1976), using bovine serum albumin as the standard. All measurements were done with an ultraviolet-visible spectrophotometer.

2.5. FT-IR analysis

The tissue was lyophilized for 12 h to make it moisture-free, and was then ground in an agate mortar and pestle to bring it to powdered form. The fine powdered tissue was mixed with potassium bromide in a ratio of 1:150 and again dried to remove any traces of moisture. The finely powdered mixture was then compressed in an evacuated die to produce sample pellets of 1 mm in thickness and 13 mm in diameter. FT-IR spectra were recorded on a NEXUS 470 spectrophotometer installed at the Central Laboratory of the Food Science and Technology College, Huazhong Agricultural University.

The pellets were scanned at room temperature in the spectral range of 4000–500 cm–1 at a resolution of 4 cm–1, with air as the background. Special care was taken during pellet preparation by taking equal amounts of sample and applying the same pressure to maintain the same thickness of pellets. Thus, the spectra were most probably related to the intensities of the absorption bands and to the concentration of the corresponding functional groups (Cakmak et al., 2006; Dogan et al., 2007). All the spectra obtained were analyzed by ORIGIN 9.0 software.

2.6. Integrated biomarker response

A method for combining all the measured biomarker responses into one general "stress index", termed the "integrated biomarker response" (IBR; Beliaeff and Burgeot, 2002), was employed to estimate the potential toxicity of Pb2+ in the major tissues by the studied biomarkers. The IBR values were calculated as follows: 1) mean and standard deviation (SD) were calculated for each treatment; 2) data were standardized for each treatment such that \( F_i' = (F_i - \text{mean } F)/S \), where \( F_i' \) is the standardized value of the biomarker, \( F_i \) is the mean value of a biomarker from each treatment, \( \text{mean } F \) is the mean of the biomarker calculated for all treatments, and \( S \) is the treatment-specific SD calculated for each biomarker; 3) by using the standardized data, \( Z \) was calculated as \(+F_i' \) or \(-F_i' \) in the case of activation or inhibition, respectively. The minimum value for all the treatments of each biomarker was then obtained and added to \( Z \). Finally, the score \( B \) was calculated as \( B = [\text{min } F_i'] + Z \), where \( B \geq 0 \) and \( \text{min } F_i' \) is the absolute of the minimum \( F_i' \). The corresponding IBR values were calculated as \( (\sum (B_i \times B_j) + \sum (B_i \times B_j)/2)/2 + \sum (B_i \times B_j)/2) \).

2.7. Statistical analysis

Data were statistically analyzed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The experiments were repeated three times. All data were expressed in terms of mean ± SD. The data from all the groups were checked for the assumption of normality by the Kolmogorov–Smirnov test and for homogeneity of variance by Levene test. One-way analysis of variance followed by Duncan post hoc test were then used to find significance (\( P < 0.05 \)) between the control and Pb2+-treated groups. All the graphs were constructed by ORIGIN 9.0 software (Origin Lab Co., Northampton, MA, USA).

3. Results

The physicochemical parameters (temperature, pH, dissolved oxygen, and total hardness) of the aquarium and the exposure to different Pb2+ concentration had no visible effects on the overall activity, swimming ability, or color of the exposed fish. No mortality was observed during the acute and chronic exposure regimes.
3.1. CAT activity
The activity of CAT in various organs of Crucian carp was slightly decreased following acute and chronic exposure (Figures 1a and 1b). Significant (P < 0.05) reduction in CAT activities was observed in the liver, kidneys, and gills following acute exposure to Pb\(^{2+}\) as compared with the respective control. The decrease in activity was 32% and 61% in the livers and 19.45% and 33.6% in the kidneys of fish exposed to 10 and 30 µg L\(^{-1}\) Pb\(^{2+}\), respectively. Under chronic exposure, the fish exposed to 10 and 30 µg L\(^{-1}\) Pb\(^{2+}\) revealed 36% and 66.35% inhibition of CAT in the liver, 36.64% and 78.53% in the gills, and 55% in the muscles.

Figure 1. Response of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities in the major tissues of Crucian carp after 96 h of acute exposure (a, c, e) and 21 days of chronic exposure (b, d, f) to Pb\(^{2+}\). Data are presented as mean ± SD (n = 5). Bars with different letters represent significant differences (P < 0.05) that resulted from the Duncan test in comparison with the respective control group.
The kidneys showed an upregulation of CAT with a 49.31% increase at 30 µg L\(^{-1}\) Pb\(^{2+}\) exposure (P < 0.05). The highest decrease in CAT activity (5.52 U mg protein\(^{-1}\)) was observed in the gills of fish exposed to 30 µg L\(^{-1}\) Pb\(^{2+}\) under the chronic exposure when compared with the control (25.71 U mg protein\(^{-1}\)).

3.2. GPx activity
Referring to the individual response of each organ, it was noted that the liver and gill GPx activities of fish acutely exposed to 10 and 30 µg L\(^{-1}\) Pb\(^{2+}\) were increased significantly (P < 0.05) when compared with the control, while the brain did not show any significant change in GPx activity. On the other hand, the muscles and kidneys GPx activities were significantly reduced following acute exposure, with maximum inhibition of 33% in the muscle tissue (Figure 1c). Contrary to acute Pb\(^{2+}\) exposure, the chronic exposure regime resulted in a decline of GPx activity (20%) in the liver of fish exposed to 30 µg L\(^{-1}\) Pb\(^{2+}\), while a significant increase in GPx activity was observed in kidney and gill tissues. A comparison of GPx activity among the studied organs revealed that the kidneys (170.93 U mg protein\(^{-1}\)) and livers (130.22 U mg protein\(^{-1}\)) had the highest GPx activity, whereas the brain responded maximally (35.5% increase in GPx activity) to chronic Pb\(^{2+}\) exposure (Figure 1d).

3.3. SOD activity
Variable responses for SOD activity were observed in the major tissues of Crucian carp. Exposure to 10 and 30 µg L\(^{-1}\) Pb\(^{2+}\) for 96 h caused a significant upregulation of SOD in the brain, kidneys, gills, and muscles of Crucian carp. The increase in SOD activity was 28.5% and 42% in the brain, 22% and 40% in the muscles, and 28% in the gills, respectively. The liver had a 10% downregulation of SOD (Figure 1e). Under the chronic exposure regime, a significant decline in SOD activity was observed in the liver (34.6%), kidneys (23.4%), and muscles (28.2%) of Crucian carp exposed to 30 µg L\(^{-1}\) Pb\(^{2+}\), whereas the brain and gills showed an increase of 38% and 26% in SOD activity, respectively (Figure 1f). The liver and kidneys remained the sites with highest SOD activity. The brain, gills, and muscles were revealed to be responsive tissues to SOD under Pb\(^{2+}\) intoxication.

3.4. Lipid hydroperoxide (LPO)
The tissue-specific LPO gradually increased after acute and chronic Pb\(^{2+}\) exposure and the extent of increase in MDA content was concentration-dependent (Figures 2a and 2b). Exposure to 30 µg L\(^{-1}\) Pb\(^{2+}\) caused a significant (P < 0.05) increase in MDA content. The increase was 29.5% in the liver, 64.9% in the brain, 36.76% in the kidneys, and 47.64 % in the gills, while no significant change was noted in the muscle tissue for acute Pb\(^{2+}\) exposure as compared with the respective controls (Figure 2a). The fish exposed chronically to 10 and 30 µg L\(^{-1}\) Pb\(^{2+}\) had significantly boosted LPO levels in each studied tissue. The MDA content was elevated by 32.6% and 47.7% in the liver, 41.35% and 55.1% in the brain, 33.9% and 61.65% in the kidneys, 40.9% and 52.5% in the gills, and 31.9% and 48.46% in the muscles (Figure 2b). Intertissue comparison revealed that the brain and the kidneys were the most vulnerable sites for higher LPO due to Pb\(^{2+}\) in the acute and chronic regimes, respectively.

3.5. AchE activity
AchE activities in the major tissues of Crucian carp are presented in Figure 3. There were no prominent changes in

Figure 2. Malondialdehyde (MDA) content in the major tissues of Crucian carp after 96 h of acute exposure (a) and 21 days of chronic exposure (b) to Pb\(^{2+}\). All values are expressed as mean ± SD (n = 5). Bars with different letters respresent signicant differences (P < 0.05) that resulted from the Duncan test in comparison with the respective control group.
the enzyme activity under acute exposure of Pb\textsuperscript{2+} (Figure 3a). However, chronic exposure to 30 µg L\textsuperscript{-1} Pb\textsuperscript{2+} caused a significant inhibition of AchE in the studied tissues (Figure 3b). Among the tissues, the muscles had the highest AchE inhibition (157.1%), followed by the kidneys (43.58%), the gills (33.3%), the brain (20.6%), and the liver (18.8%), whereas no significant effect was noticed for 2.5 µg L\textsuperscript{-1} and 10 µg L\textsuperscript{-1} Pb\textsuperscript{2+} in either exposure regime.

3.6. FT-IR spectroscopy of muscles

FT-IR spectroscopy was employed to investigate the effect of environmental Pb\textsuperscript{2+} on the biochemical constituents of the Crucian carp’s muscles. Shifts in peak positions, changes in intensities, and integrated areas of infrared bands were used to elucidate structural and functional information about the system of interest. The representative FT-IR spectra of acute and chronic Pb\textsuperscript{2+}-exposed fish in the region of 4000–500 cm\textsuperscript{-1} are presented in Figure 4. The spectra comprised several bands corresponding to various functional groups belonging to proteins, lipids, nucleic acids, and amino acids. The band assignments were done according to the available literature (Toyran et al., 2005; Akkas et al., 2007) and the top bands are labeled in Figure 4. The FT-IR spectra revealed significant variations in the absorbance intensities between the control and the Pb\textsuperscript{2+}-intoxicated muscles, reflecting

![Figure 3. Acetylcholine esterase activity (AchE) in the major tissues of Crucian carp after 96 h of acute exposure (a) and 21 days of chronic exposure (b) to Pb\textsuperscript{2+}. All values are expressed as mean ± SD (n = 5). Bars with different letters represent significant differences (P < 0.05) that resulted from the Duncan test in comparison with the respective control group.](image)

![Figure 4. The average FT-IR spectra of Crucian carp’s muscles, representing the control and Pb\textsuperscript{2+}-intoxicated groups after 96 h of acute exposure (a) and 21 days of chronic exposure (b) in the 4000–400 cm\textsuperscript{-1} region.](image)
major biochemical changes in the tissue of exposed fish. The band areas corresponding to amide A (N–H stretching (3372 cm⁻¹), CO–O–C asymmetric stretching (1158 cm⁻¹), PO₃ symmetric stretching (1088 cm⁻¹), and C–O stretching (1036 cm⁻¹) decreased significantly (3301, 1155, 1081, and 1033 cm⁻¹) with increasing Pb²⁺ concentration and time of exposure, respectively (Figures 4a and 4b), while the asymmetric C–H stretching and COO⁻ symmetric stretching shifted from 2927 to 2930 cm⁻¹ and 1393 to 1395 cm⁻¹ after chronic exposure to 30 µg L⁻¹ Pb²⁺, respectively (Figure 4b). However, the band area corresponding to amide I (1653 cm⁻¹), amide II (1542 cm⁻¹), and CH₂ bending (1455 cm⁻¹) remained intact following both acute and chronic Pb²⁺ exposure.

3.7. Integrated biomarker response
To investigate and compare the total physiological response of Crucian carp’s major tissues following acute and chronic exposure to various environmental concentrations of Pb²⁺, the IBR method was applied to the 5 measured biomarkers (Figure 5). Star plots using IBR values instead of biomarker data make it possible to visualize sensitivity differences among the major tissues for the studied biomarkers. Generally, the IBR values showed great variability for different organs at different concentrations and exposure regimes. The star plot areas, differently scaled for each organ, revealed that the liver had the maximum response (15.9) of all the measured biomarkers, followed by the kidneys and gills, while the muscles and the brain responded least under acute exposure to 30 µg L⁻¹ Pb²⁺ (Figure 5a). The organ-specific IBR values and star plot areas for chronic exposure show that the gills (56.6), liver (34), muscles, and brain are sensitive organs with decreasing IBR indices in the fish exposed to 30 µg L⁻¹ Pb²⁺, whereas the kidneys and liver proved to be the sensitive sites at the lowest level (2.5 µg L⁻¹) of Pb²⁺ exposure (Figure 5b).

4. Discussion
The aim of the present study was to investigate the potential effects of environmentally relevant concentrations of Pb²⁺ on tissue-specific oxidative stress and antioxidant defense after 96 h and 21 days of waterborne exposure of Crucian carp. The acute and chronic exposures induced oxidative damage and differential antioxidant responses for SOD, CAT, GPx, AChE, and LPO in the major tissues. The tissues were selected on the basis of their vital functions in xenobiotic metabolism: uptake by the gills, clearance by the kidneys, detoxification by the liver, and the neurotoxic effects on the brain, which ultimately affect the biochemistry of the tissue of interest for human consumption, the muscles. The antioxidant enzymes (SOD, CAT, and GPx) are the first line of defense against ROS-mediated injury caused by xenobiotics. Thus, changes in the levels of these antioxidant enzymes along with other enzymatic and nonenzymatic biomarkers can help us in revealing a prooxidant condition.

CAT activity is thought to be crucial for detoxification of ROS when high levels of H₂O₂ are produced. In our study, CAT activity was inhibited in the liver and gills and finally activated in the kidneys, but it did not change in the brain and muscles. The highest CAT inhibition in the liver illustrated that this organ has enormous antioxidant capacity. Organ-specific inhibition and stimulation of CAT in fish due to metal (Cd, Ag, Cu, Zn, and Cr) exposure was also reported earlier (Atli et al., 2006; Prieto et al., 2006). Similarly, inhibition of CAT activity was also observed in the heart and spleen of rainbow trout exposed to Cd and Cr (Orun et al., 2008). Hepatic CAT inhibition was also noted in Cr-exposed carps after 4 days, whereas the activity increased on day 8 of exposure. It was also
stated that inhibition in CAT activity was mainly due to the generation of CAT inhibitors by the metal (Velma and Tchounwou, 2010). CAT and SOD activities are often found correlated because both enzymes function simultaneously to constitute a first line of defense against oxidative stress (Asagba et al., 2008). In the present study, an increase in SOD activity was observed in the brain, gills, and muscles after 96 h of exposure to Pb²⁺, while it was unaffected in the liver. However, the 21-day exposure period revealed inhibition of SOD in the liver, kidneys, and muscles of Crucian carp. Previously, 35% inhibition in SOD activity in the liver tissue of *S. aurata* was attributed to the extension of lesions in the liver and drastic lipid infiltration in adipocytes due to Cd exposure (Souid et al., 2013). SOD activation may be due to excessive generation of ROS or inhibition as a result of direct damage to the SOD protein by increased \( \text{H}_2\text{O}_2 \) content in the cell, whereas tissue-specific changes in SOD activity might be species-dependent (Ahmad et al., 2004; Puerto et al., 2010; Ekoue and Diamond, 2014). The activity of GPx can also be considered complementary to the activity of CAT, which is supported by the present data. Saglam et al. (2014) reported that it was GPx (rather than CAT) with the secondary ability to decompose peroxides in the liver of *P. mesopotamicus* following exposure to hypoxia and Cu. In our study, the GPx activity did not change significantly, except for a slight increase in the gills and kidneys following chronic Pb²⁺ exposure. It was also reported earlier that exposure to Co²⁺ did not change GPx activity in goldfish organs (Kubrak et al., 2011). Contrary to the present findings, Vieira et al. (2012) suggested that the lack of CAT stimulation by Mn in the organs of goldfish was compensated by higher stimulation of GPx activity. Atli et al. (2006) also found an inverse relationship between CAT and GPx activities in the different organs of fish exposed to metals. Thus, the activation and inhibition in the antioxidant enzyme system under xenobiotic stress mainly depends on the intensity and duration of the applied stress as well as on the susceptibility of the exposed species (Ballesteros et al., 2009). However, all organisms have a complex defense mechanism of enzymatic and nonenzymatic antioxidants that act collectively to mimic the deleterious effects of ROS. The interactions among them in different organs of a model animal may be the subject of future studies.

Lipid hydroperoxides are produced as a result of oxidative injury to saturated and unsaturated lipids and have widely been used as markers of oxidative damage in fish suffering from metal induced environmental stress (Kubrak et al., 2011; Vieira et al., 2012). Our data revealed a higher content of LPO for all the studied organs under the two exposure regimes except for the muscles, which did not show any change following acute exposure. This strongly suggests that Pb²⁺ provoked a generalized oxidative stress in the fish, which could be a manifestation of Pb²⁺ toxicity for higher production of ROS. Among the studied organs, the brain and kidneys had the highest LPO content, showing their susceptibility to oxidative damage, which might be due to higher contents of unsaturated lipids in the two tissues or to a proactive defense mechanism of the other tissues.

Inhibition of AchE activity is a commonly used biomarker of neurotoxicity. Studies have shown that metal ions such as mercury, copper, cadmium, and zinc inhibit the activity of AchE (Frasco et al., 2005; Badiou and Belzunces, 2008; Tu et al., 2012; Varo et al., 2012). In the present study, short-term exposure did not show any change in the AchE activity of various organs. However, significant inhibition of AchE activity was observed in all the organs following long-term exposure to Pb²⁺ higher concentration. Our results are comparable to the findings of Souid et al. (2013). One of the reasons for the highest inhibition of AchE activity in kidneys and gills might be the greater contact or permeability of metal ions with these organs.

In order to better understand the toxicological effects of Pb²⁺, FT-IR spectroscopy was employed to determine the biochemical alterations in muscles of Crucian carp. FT-IR spectroscopy is a widely accepted nonperturbing rapid technique to get useful information about biomolecules such as proteins, carbohydrates, lipids, DNA, and RNA by monitoring their functional groups (Palaniappan and Renju, 2009; Palaniappan and Pramod, 2011). In our study, a downshift of amide A : N–H stretching and CO–O–C asymmetric stretching might imply alterations in proteins, amide hydrogen bonding, glycogen, and nucleic acids. A downshift of PO₄⁻ symmetric stretching and C–O stretching may suggest changes in the phosphodiestri backbone of cellular nucleic acid and alterations in intramolecular hydrogen bonding of the interfacial region of the phospholipid structure with some other molecules. The asymmetric C–H stretching shift might be due to an alteration in the acyl chains of lipids, while a shift of COO⁻ symmetric stretching shows changes mainly in the fatty acids and amino acids. In conclusion, the FT-IR analysis also verifies oxidative stress, thereby increasing lipid oxidation and alteration in the biochemical integrity of muscles.

IBR has been used previously in fish models with different biomarkers to assess the cumulative response of environmental pollution at different sites (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006; Kim et al., 2010). However, in our study we have applied it to the studied biomarkers to understand the sensitivity of various tissues to Pb²⁺ toxicity. Beliaeff and Burgeot (2002) stated that there was a rational agreement between the concentrations
of PAH and PCB and star plot patterns of the Seine Estuary. Broeg and Lehtonen (2006) demonstrated good agreement between the IBR and tissue levels of organochlorine. In another study, Kim et al. (2010) proved that the IBR values tended to increase with increasing concentrations of perfluorooctanoic acid and perfluorooctane sulfonate. The maximum IBR values for the liver and gills in the current study might suggest that these two organs are the most sensitive to inciting Pb2+ toxicity response. Moreover, it can also be presumed that under acute Pb2+ exposure, the response of the studied biomarker would be high in the liver and kidneys, while under the chronic condition the gills may have the maximum response, followed by the liver. Such information might be useful during sampling to assess Pb2+ toxicity in the aquatic environment.

The current study indicated that acute and chronic exposure to environmentally relevant concentrations of Pb2+ provoked significant oxidative stress and differential tissue-specific antioxidant response in Crucian carp. Among the studied organs, the liver showed a marked defense mechanism against oxidative stress, followed by the kidneys and gills in terms of SOD, CAT, and GPx activities, whereas the brain and muscles revealed weak antioxidant potential. Lead exposure caused increased LPO production in all the studied tissues in a time- and concentration-dependent manner, indicating that prolonged exposure to Pb2+ may cause elevated production of ROS. Significant inhibition of the AchE activity was also observed in all the studied tissues following chronic exposure, suggesting a general neurotoxic stress level in the fish under the tested concentrations. In addition, FT-IR spectroscopy demonstrated a mild alteration in the biochemical integrity of the muscles. The IBR indices also confirmed that the liver, kidneys, and gills are sensitive to the oxidative stress posed by Pb2+. Based on these findings, Pb2+ exposure may potentially affect multiple biological processes in the organism. Further studies are needed to unveil more data on the mechanism of Pb2+ toxicity.

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References


