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IMPACT OF ACUTE TOXICITY OF LEAD ACETATE ON THE LEVEL OF ESSENTIAL TRACE METALS AND HISTOPATHOLOGICAL CHANGES IN CRUCIAN CARP. *(CARASSIUS AURATUS GIBELIO)*

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ABSTRACT

This study evaluated the acute toxicity effects of Pb^{2+} on essential trace metals behavior and histopathology of Crucian carp *(Carassius auratus gibelio).* The median lethal concentration (96 h LC_{50}) of Pb^{2+} was determined to be 29.07 mgL^{-1} in a semi-static bioassay. The fish were exposed to sublethal concentration of $5mgL^{-1}$ as environmentally relevant Pb^{2+} for a period of 96 h. Trace metals (Pb, Cu, Zn, Fe and Ca) levels were determined in the gill, liver, kidney and muscles tissue by ICP-OES. Regression analysis revealed that Pb^{2+} exposure had negative impact on the level of Cu, Zn, Fe and Ca in certain tissues ($P<0.05$;$P<0.01$). Histopathological changes in the gills of exposed fish were characterized by lamellar shrinkage, disruption of cartilaginous core, epithelial lifting, lamellar shortening with desquamation and curling of the secondary lamella. The trunk kidney had severe shrinkage of glomeruli, hypoplasia of hemopoietic tissue as well as mild glomerular and tubular necrosis. The liver showed cellular edema, necrosis of hepatocytes with nuclear degeneration and pyknotic nuclei. The brain exhibited severe proliferation of glial cells, cellular necrosis, severe perivascular edema and satellitosis. These results clearly depict the deleterious effects of Pb^{2+} on trace metal metabolism and tissue architecture of Crucain carp.

Key words: Acute toxicity, 96 h LC_{50}, environmentally relevant Pb^{2+}, trace metals, histopathology.

INTRODUCTION

Lead (Pb) is a well known heavy metal found naturally in the Earth's crust, and exists in soils, plants and water in trace amounts. Nowadays it became a widely distributed toxic metal in the world due to manmade actions (Cheng and Hu, 2010). The main anthropogenic sources of Pb pollution include mining, smelting, industrial uses, waste incineration, coal burning, and use of leaded gasoline. Lead can get into the human body through various routes by exposure to contaminated air, water and food. The main target of Pb poisoning in adults is peripheral and central nervous systems (Needleman, 2004) while in children it results neuropsychological abnormalities associated with reduced IQ and impaired learning abilities**.** In addition to this, Pb accumulates in bones, brain, kidney and muscles and may cause several pathological conditions including anemia, kidney failure and even death (Ozdes *et al.,* 2009).

Toxicity of Pb has similar adverse implication despite of their route of entry to the body. Once absorbed from the surrounding environment, Pb binds to erythrocytes and via blood it may transfer to the soft tissues including kidney, liver, brain, muscles, heart and spleen. Finally most of it is deposited in the bones and teeth (Meyer, *et al.,* 2008). Lead affects almost every organ of the living body, the mechanism of Pb toxicity involved alteration of several biochemical processes. It can inhibit or change the action of calcium and interact with proteins (Bellinger and Bellinger, 2006). The most studied mechanism is the adverse effects on hematopoietic system and heme biosynthesis. In connective tissue like blood Pb binds with erythrocytes where plasma is the active portion which makes it available to the adjacent cells (Cavalleri *et al.,* 1978). Lead may also have adverse effect on the absorption and metabolism of essential trace metals concentration in various organs (Jin *et al.,* 2008).

Fish play a vital role in the food-web, however they are prone to be contaminated by heavy metals dissolved in their surrounding water (Guven *et al.,* 1999). The bioaccumulation and magnification is capable of leading to toxic level of these metals in fish, even when the exposure level is low. The presence of toxic metals in fresh water is known to disturb the delicate mineral balance of the aquatic systems which may adversely affect the freshwater fish (Adeniyi and Yusuf, 2007). To study the direct toxic effect of certain environmental pollutants in different organs of fish, histopathology can serve as a sensitive tool (Leonardi *et al.,* 2009; Yasser and Naser, 2011). The gills, liver and kidney are considered to be the responsive organs that respond to toxic pollutants and can be used as biomarker for environmental pollution assessment (Hughes, 1984; Mallat, 1985; Moon *et al.,* 2006; Figueiredo-Fernandes *et*

al., 2006). Crucian carp is one of the famous food fish in China and in other parts of the world. Due to its good taste, fast growth and suitability for mono and polyculture in fishponds, they are considered to be one of the most intensively cultured freshwater fish in the world (Gui and Zhou, 2010). Moreover it has also been used as a bio-indicator of persistent organic pollutants (Moon *et al.*, 2006).

Several studies have reported the effect of Pb on the behavior of essential trace elements in blood using human and animal models (Ahamed *et al.*, 2007; Jin *et al.*, 2008). Lead has also been reported for its acute toxicity and histopathological alteration in different fish species (Hermana *et al.*, 2009; Ergonul *et al.*, 2012; Binkowski *et al.*, 2013). However, the knowledge pertaining to acute toxicity effects and histopathological alteration due to Pb exposure in Crucian carp *(Carassius auratus gibelio)* is limiting. While the relationship between Pb and essential trace elements is also not clear in some vital organs like kidney, liver, gill and muscles.

In the present study we determined the 96 h median lethal concentration (LC_{50}) of Pb^{2+} and examined the histopathological changes in liver, kidney, gill and brain of Crucian carp *(Carassius auratus gibelio)*. Moreover, Pb accumulation and its impact on essential trace metal variation in different organs of Crucian carp were investigated to better understand Pb toxicity and organ specific trace metal behavior.

MATERIALS AND METHODS

Chemical and reagents: All the chemical and reagents used were of analytical grade and were purchased from Sinopharm (Beijing, China). Lead nitrate of purity > 99 %, nitric acid, Acetic acid (conc. glyacial), Potassium iodide crystal, Sodium thiaosulphate, Starch indicator, Ammonium chloride, Ammonium hydroxide, EDTA (disodium salt of EDTA), Erichrome black T and Megnisum sulphate.

Aquarium water quality and Pb^{2+} exposure: Crucian carp (*Carassius auratus gibelio*) were used as model animal for this study. Healthy adult fish with average size (15±4.4 cm) and weight (109±5.7g) were obtained from a nearby commercial fish farm and immediately transported to laboratory in plastic buckets having pond water. On arrival all the fish were released to the glass aquarium containing dechlorinated tape water. Water quality parameters were determined prior to experimentation and thereafter on daily basis according to the standard method of APHA (1992). The water were aerated for one day bfore starting and later throughout the experiment with stone aerator connceted with compressed air pump. Water quality parameters (total hardness 156.32±4.43 mgL^{-1} as $CaCO_3$, temp. 22.41±2.11 ^oC, pH 7.6±0.31 , dissoved oxygen 8.26±0.68 mgL^{-1}) were maintained till the end of the experiment. The experimental protocols for fish maintanance and experimatation were approved by The Ethical Committee of animal handling Huazhong Agricultural University, Wuhan P.R. China.

No distinction was made between sexes and fish were acclimatized to laboratory condition in a glass aquarium containing 50 L water for a period of 1 week. All the fish were fed with artificial feed twice a day during the period of acclimatization while feeding was stopped 24 h earlier of actual experiment and no feeding were administered during the experiment. After acclimatization only healthy fish were selected for LC_{50} determination. Median lethal concentration based on 96 h acute toxicity was determined according to the guidelines of OECD (1992) in a semi-static condition. A series of preliminary experiments were conducted to determine the concentration range for Pb^{2+}. Finally the acclimatized fish were divided into 7 groups with each group having 10 fish and exposed to different concentration of Pb^{2+} in mg L^{-1} (0.00, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2) as $Pb(NO_3)_2$. Stock solutions were prepared by dissolving the test chemical in distilled water. Fish were examined after each 12 h interval and mortalities were noted at logarithmic intervals (24, 48, 72 and 96 h) of exposure. No mortality was observed in the control group. Mortality data were statistically analyzed by Finney's Probit Analysis (Finney, 1978). For environmentally relevent Pb exposure the fishes were divided into 2 groups. Group 1 was kept as control under same experimental condition but without any addition of Pb^{2+} while the second group was exposed to Pb^{2+} at a rate of 5 mg L^{-1} as $Pb(NO_3)_2 \approx 17$ % of LC_{50}. This concentration was selected on the basis of Pb contamination levels in the river and lakes of China reported earlier (Zhou *et al.*, 2008; An *et al.*, 2010; Yang *et al.*, 2009; Wang, *et al.*, 2012; Li, *et al.*, 2013; Bing, *et al.*, 2013) and considered to be environmentally relevant. The exposure medium was replaced every day and Pb concentrations were monitored carefully in the stock solutions and aquarium water to maintain the required concentration and remove the waste produced by fish. At completion of 96 h exposure period all the fish from treated and control group were sacrificed by decapitation and the abdominal part was opened by making a cut from anus to the gill. The different organ like kidney, liver, gill and brain were exercised on an ice-cold plate and washed with physiological saline.

Metal analysis: Determination of metals (Pb, Cu, Zn, Fe and Ca) were carried out in various tissues (kidney, liver, gill and muscles) using ICP-OES model PerkinElmer-Optima 8000 Series (Software – WinLab 32). Dry ashing method was used for digestion of all the samples as described by Mendil *et al.*, (2010). Briefly 1 g dried sample was transferred to a procelin crucible followed by

ashing in a furnace at gradual increasing temperature till 550 ºC for 6 h. The white gray ash was then dissolved in 1:1 HNO_3 solution and heated if needed to be dissolved completely. The contents were then filtered off and marked to 25 ml with Milli-Q Millipore water. The precision of the analytical procedure was checked by running certified reference materials (CRM) of commercially available standards (PerkinElmer No. N9300281, Shelton, Connecticut 06484 USA, ICP-Multi-element quality control standards, 21 elements in nitric acid). The instrumental operating condition and analytical parameters for each element are listed in Tables 1 and 2. Axial view was used for metals determination, while 2-point background correction and 3 replicates were used to measure the analytical signal. The emission intensities were obtained for the most sensitive lines free of spectral interference.

Histopathology: For histopathological examination, the tissue samples (kidney, liver, gill and brain) after isolation were directly immersed in Bouin's solution for 24 h followed by paraffin (m. p. 62ºC) embedding (Roberts, 2001). Paraffin block were then cut to 6 µm thickness and stretched on clean glass slide. All the sections were stained with Haematoxylin–Eosine after deparaffinization and were examined under light microscope for pathological alterations. The histological changes were marked as none (-), mild (+), moderate (++) and severe (+++) according to Ahmed, *et al.,* (2013).

Statistical analysis: Statistical analysis was performed by SPSS 16.0 Chicago USA. All the experiments were replicated three times. Data were recorded for each parameter in triplicates and expressed in terms of mean±SD (standard deviation). Linear regression was applied to study the relationships between Pb and individual trace metal in each studied organ. Significant differences were calculated by one way ANOVA following t-test. Significance was considered at $P<0.05$ and $P< 0.01$.

RESULTS AND DISCUSSION

No objectionable changes were noticed for physico-chemical parameters (temperature, pH, dissolved oxygen and total hardness) of the aquarium water during the experimental work. There was no mortality observed during the period of acclimatization and control group.

The 96 h lethal concentrations of Pb^{2+} for Crucian carps are summarized in Table 3 with value 29.07 mgL^{-1} as LC_{50}. During the acute toxicity experiment abnormal fish behavior was noticed in response to different Pb^{2+} exposure like erratic swimming, jumping to the surface, rapid operculum movement, convulsion and thick mucus secretion. Fish of the control group maintained normal behavior till the end of the experiment.

Table 4 shows the concentration of Pb, Cu, Zn, Fe and Ca in various organs (kidney, liver, gill and muscles) of control and exposed fish as determined by ICP-OES. Lead accumulation was much prominent ($P<0.01$) in all the organs of exposed group. The organs showed an increasing Pb level in the order of gill, kidney, liver and muscles with accumulation of 3.31 ± 0.30, 2.53 ± 0.23, 1.44 ± 0.14 and 0.54 ± 0.07 mg kg^{-1} respectively. Among other trace metals the highest level of Cu and Zn was found in the liver while the concentration of Fe and Ca was high in the gill with mean concentration of 25.96 ± 0.99, 133.63 ± 0.60, 30.25 ± 2.56 and 1646.09 ± 24.53 mg kg^{-1} respectively. The impact of Pb exposure on essential trace metals were further explained by regression analysis as shown in Fig 1. It was observed that Pb^{2+} exposure had no significant effect ($P>0.05$) on the level of Cu in muscles, kidney and gills while it had significantly negative effect on Cu in the liver of Crucian carp ($P<0.05$). Zinc concentration in the muscles, kidney and gill was negatively affected by Pb^{2+} exposure ($P<0.01$), however, the relationship between Zn and Pb^{2+} was significantly positive in the liver of Crucian carp ($P<0.05$). Similarly Pb^{2+} had significantly negative effect on Fe levels in the muscles, gill and liver ($P<0.01$, $P<0.05$) except kidney of Crucian carp where no significant relationship was observed ($P>0.05$). In the same way Pb^{2+} had no significant effect on the Ca level of muscles and kidney ($P>0.05$) while Ca concentration in the gill and liver of Crucian carp was negatively affected by Pb^{2+} exposure ($P<0.05$).

Table 5 represents the histopathological changes in gill, kidney, liver and brain of control and exposed groups of Crucian carp. The overall histopathological examination revealed a mild to moderate and severe changes in the various tissues. There was no histopathological alteration in the gills, kidney, liver, and brain of fish in the control group. Gills of the acute Pb^{2+} exposed fish showed shrinkage of primary and secondary lamella and severe disruption of the central cartilaginous core at several points. A moderate epithelial lifting and lamellar shortening with desquamation and curling of the secondary lemella were also observed (Fig 2). The trunk kidney of exposed fish had severe shrinkage of glomeruli, having increased space between glomerulus and Bowman's capsule. The renal tubules showed moderate separation, hypoplasia of hemopoietic tissue as well as mild glomerular and tubular necrosis (Fig 3). The liver cells of exposed fish showed moderate cellular edema and necrosis of hepatocytes with nuclear degeneration and pyknotic nuclei (Fig 4). Moreover brain of the exposed fish showed severe proliferation of glial cells, cellular necrosis, severe perivascular edema and satellitosis (microglial cells surrounding neurons with swollen and prenecrotic neurons) (Fig 5).

Table 1. Operating parameters for the Inductively Coupled Plasma Optical Emission Spectrometer.

Parameters	Condition
Plasma captivity (W)	1300
Plasma flow rate (L min^{-1})	10
Nebulizer gas flow rate (L min^{-1})	0.70
Sample flow rate (mL min^{-1})	1.50

Table 2. Detection limits and wavelength of each element used for ICP-OES analysis.

Elements	Wavelength (nm)	Detection limit μgL^{-1}
Pb	220	1.0
Cu	327	0.04
Zn	206	0.02
Fe	238	0.01
Ca	317	0.05

Table 3. The 96 h LC values of Pb^{2+} for Crucian carp (*Carassius auratus gibelio*)) as determine by Fenny's Probit analysis (95% confidence limits).

Points	Exposure concentration mgL^{-1}	95% Confidence limits	
		Lower	Upper
LC_1	23.89	19.12	25.62
LC_5	25.30	21.61	26.65
LC_{10}	26.09	23.05	27.23
LC_{20}	27.08	24.88	28.00
LC_{30}	27.81	26.21	28.65
LC_{50}	**29.07**	**28.15**	**30.20**
LC_{70}	30.39	29.45	32.68
LC_{80}	31.21	30.09	34.49
LC_{90}	32.39	30.91	37.26
LC_{95}	33.40	31.57	39.76
LC_{99}	35.38	32.83	44.94

Table 4. Lead and essential trace metals concentrations in various organs of control and Pb^{2+} exposed Crucian carp (*Carassius auratus gibelio*) in $mgkg^{-1}$.

Metal	Control				Exposed			
	Muscles	Kidney	Gill	Liver	Muscles	Kidney	Gill	Liver
Pb	0.07±0.02	0.67±0.07	0.81±0.12	0.29±0.02	0.54±0.07**	2.53±0.23**	3.31±0.30**	1.44±0.14**
Cu	1.37±0.42	8.72±0.56	3.33±0.46	25.96±0.99	1.15±0.03	8.61±0.41	3.11±0.12	24.91±0.56
Zn	42.53±0.79	113.65±0.59	97.49±0.63	131.52±0.81	36.24±0.66**	92.13±0.72**	70.19±0.93**	133.63±0.60**
Fe	2.72±0.38	16.29±1.01	30.25±2.56	22.62±2.54	1.70±0.65*	16.52±0.71	20.05±0.93	16.49±0.59*
Ca	235.20±14.00	208.03±6.48	1646.09±24.53	223.63±11.52	236.12±6.54	209.01±9.24	1431.52±22.51**	125.32±12.22*

Significant differences are shown with *(*P<0.05*) and **(*P<0.01*). Significant differences are shown with *(*P<0.05*) and **(*P<0.01*).

Table 5. Histopathological changes in different organs of Crucian carp (*Carassius auratus gibelio*) exposed Pb^{2+} for 96 h.

Tissue	Histopathological effects	Control	Exposed
Gill	Disruption of cartilaginous core	-	+++
	Epithelial lifting	-	++
	Desquamation	-	+
	Lamellar shorting	-	++
	Curling of secondary lamellae	-	+
Kidney	Tubular necrosis	-	+
	Shrinkage of glomerulus	-	+++
	Renal tubular separation	-	++
	Hypoplasia of hemopoietic tissue	-	+
	Glomerular necrosis	-	+
Liver	Necrotic hepatocytes	-	+
	Cellular edema	-	++
	Nuclear degeneration	-	+
	Pyknotic nucleus	-	+++
Brain	Glial cells proliferation	-	+++
	Cellular necrosis	-	+
	Perivascular edema	-	+++
	Satellitosis	-	+++

None (−), mild (+), moderate (++), and severe (+++).

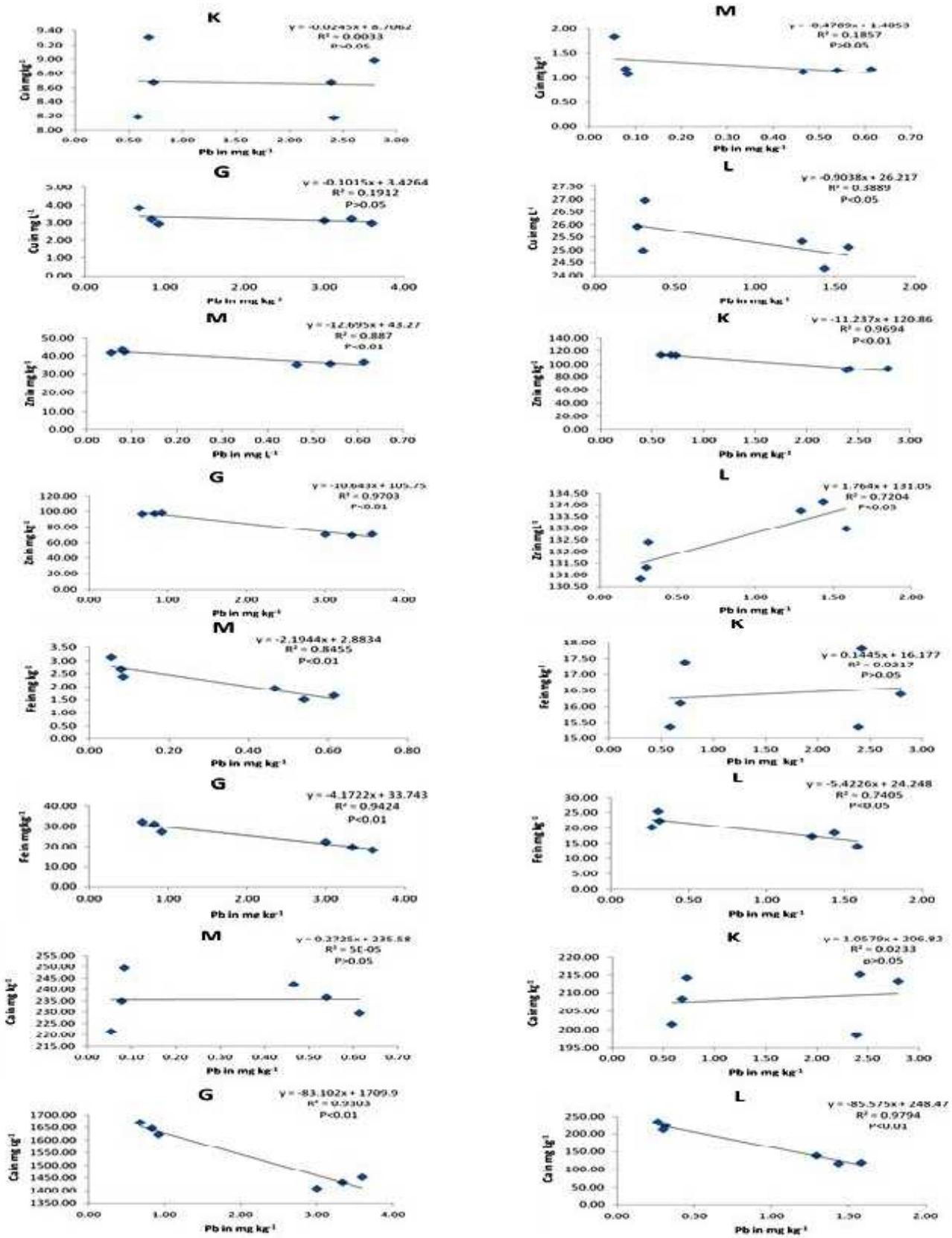

Figure 1. Relationship between Pb and Cu, Zn, Fe or Ca in the muscles (M), Kidney (K), gill (G) and liver (L) of Crucian carp (*Carassius auratus gibelio*) after 5mgL^{-1} Pb^{2+} exposure for 96 h.

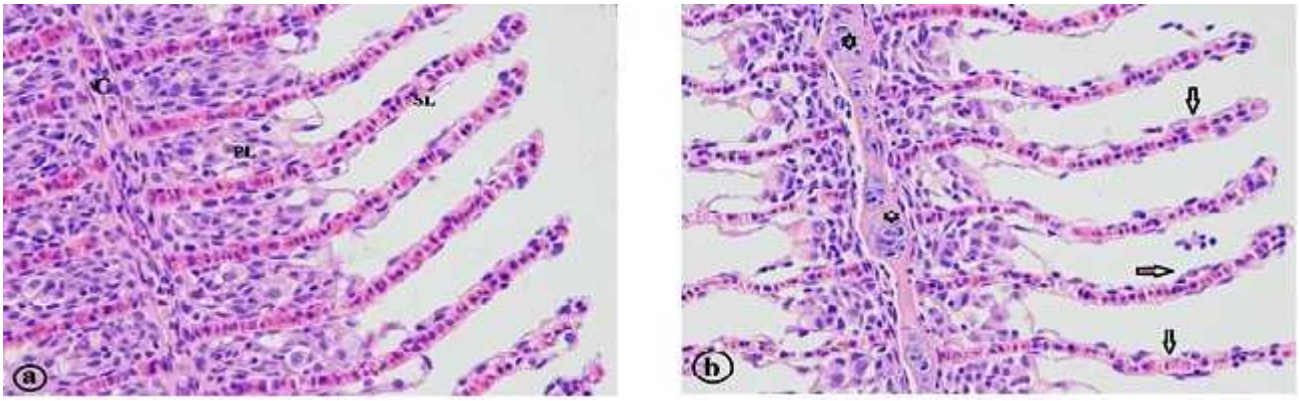

Figure 2. Longitudinal sections of gill showing histopathology of Crucain carp a) Gill section of control group (400×) showing normal gill architecture with primary lamellae (PL), secondary lamellae (SL) and cartilaginous core (C). b) Disruption of cartilaginous core(*), epithelial lifting, desquamation, lamellar shorting as well as curling of secondary lamellae in fish exposed to 5 mgL^{-1} group (400×).

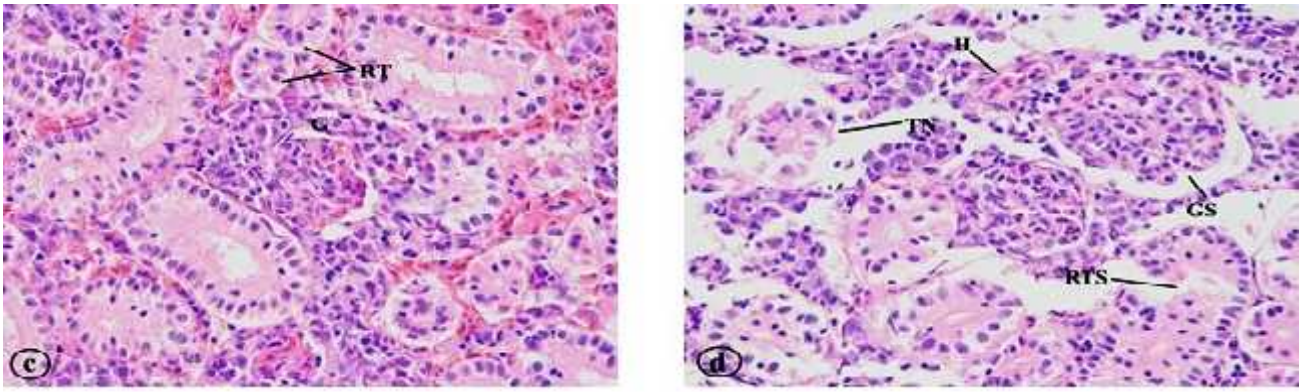

Figure 3. Transverse sections representing histopathology of kidney after 5mgL^{-1} Pb^{2+} exposure of Crucian carp. C) Kidney section of control group (400×) showing normal renal tubule (RT) and Glomerulus (G). d) Exposed group (400×) revealed hypoplasia of interstitial hemopoietic tissue (H), tubular necrosis (TN), shrinkage of glomerulus (GS) and separation of renal tubule (RTS).

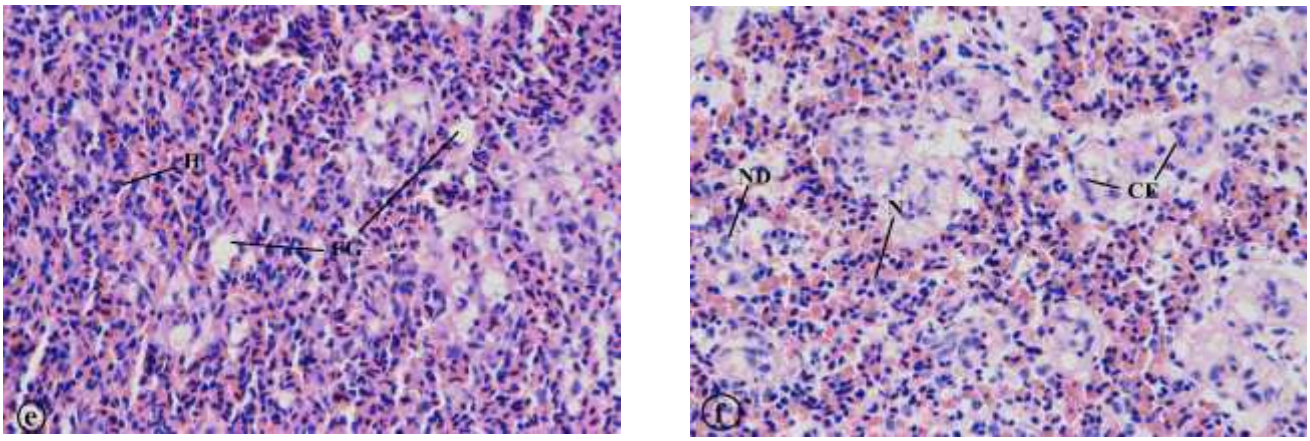

Figure 4. Transverse sections of Crucian carp's liver. e) Liver section of control group (400×) representing normal hepatocytes (H) and fatty granules (FG). f) Exposed group (400×) showing nuclear degeneration (ND), necrosis of hepatocytes (N) and cellular edema (CE).

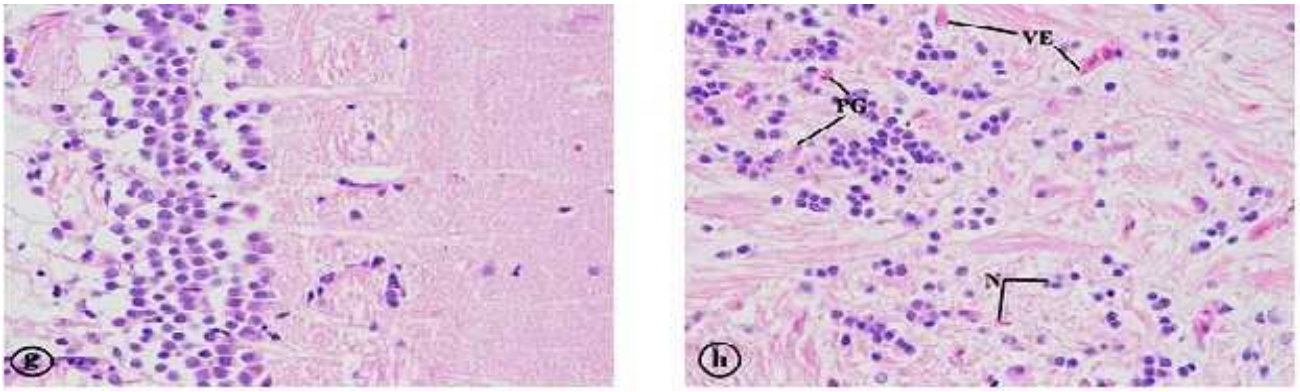

Figure 5. Histopathological changes in the tissue of brain exposed to 5mgL^{-1} of Pb^{2+}. g) Control group show the normal structure of brain (400×). h) The exposed group shows proliferation of the glial cells (PG), cellular necrosis (N) and severe perivascular edema (VE) with satellitosis (400×).

Lead has yet no known role in the living organism and may cause several pathological conditions in exposed population. In the present study we have evaluated the acute effect of environmentally relevant Pb^{2+} exposure on organ specific essential trace metals variations and histopathology. The 96 h LC_{50} value for Pb^{2+} as $Pb(NO_3)_2$ was calculated to be 29.07 mgL^{-1} by the use of Fenny's Probit analysis. Ergonul *et al.,* (2012) reported 96 h LC_{50} value of Pb^{2+} in the range of 17.43 - 24.29 mg L^{-1} for *Cyprinus carpio*. In *Clarias gariepinus* the 96 h LC_{50} value of Pb^{2+} was estimated as 22.65 mg L^{-1} while the 96 h LC_{50} value of Pb^{2+} for *Oreochromis niloticus* was reported to be 12.45 mg L^{-1} by Al-Akel and Shamsi, (2000). In some studies higher concentrations of Pb^{2+} such as 95.00 and 300 mgL^{-1} were estimated as LC_{50} of *Prochilodus lineatus* and *Tinca tinca* respectively (Martinez *et al.,* 2004; Shah, 2006).

A great variability has been observed regarding the acute toxicity and 96 h LC_{50} values of Pb^{2+} for fish. These variations may be attributed to species specific response to the toxic metals. However, there are several other factors that influence LC_{50} values such as aquarium water quality and static or semi-static bioassays (Datta and Das, 2003; Rogers *et al.,* 2003; Martinez *et al.,* 2004; Mager *et al.,* 2011). In addition to this, during Pb^{2+} exposure fish exhibeted abnormal behaviour including erritec movemnet, jumping, excessive secretion of mucus and loss of balance which may be due to Pb neurotoxicity. These results are consistent with ealier studies of fish exposed to toxic metals (Ahmed *et al.,* 2013; Akter *et al.,* 2008; Mishra and Mohanty, 2008).

Adequate supply of essential trace elements is crucial for the normal maintenance of animal health. They have distinct structural, catalytic and regulatory functions as well as a marked role in the immune system. However, some heavy metals including Pb may drastically affect their mechanisms in various tissues (Jankovská, *et al.,* 2012; Sharma, *et al.,* 2010). The present study elucidated the effect of Pb^{2+} exposure on the levels of Zn, Cu, Fe and Ca in gill, liver, kidney and muscles. The data revealed that Pb^{2+} exposure had significantly negetive effect on the level of Cu in liver, Zn in muscles, kidney and gills, Fe in muscles, gills and liver and Ca in gills and liver. Previously it was observed by Jankovská, *et al.,* (2012) that Pb administration to *Ovis aries* for 7 days showed significant decrease of Fe and Zn in the liver, kidney and muscles respectively while muscles showed increase level of Cu as compared to control. Jin *et al.,* (2008) reported that Pb exposure significantly decreased the concentration of Zn and Fe in the blood. In another study Srivastav *et al.,* (2013) found that Pb exposure for 96 h and 28 days progressively decreased the plasma Ca and phosphate level in a freshwater fish *Heteropneustes fossilis.* In children low level of Zn, Fe, Cu and Ca was indicated as a result of elevated blood Pb which may be due to the interaction of Pb with these elements at the absorptive as well as enzymatic sites or they may share similar binding sites on the transferring protein like metellotheonin (Ahamed, *et al.,* 2007; Kerper and Hinkle, 1997). Some investigators have reported degenerative changes in the gills as a result of xenobiotics exposure, which may affect the ionic permeability and became a reason for the depleted level of essential trace elements in various tissues (Palaniappan *et al.,* 2008; Koca *et al.,* 2008; Pandey *et al.,* 2008; Rabitto *et al.,* 2005). The increase urinary excretion observed in Pb exposed rainbow trouts and kidney degeneration with impaired re-absorption (Patel *et al.,* 2006; Srivastav *et al.,* 2013) may be the possible cause of lower essential trace elements in various tissues.

The acute Pb^{2+} exposure produces marked histopathological alteration in the gills, kidney, liver and brain of Crucian carp. The gills of Pb^{2+} exposed fish exhibited cartilaginous core disruption, shrinkage of primary and secondary lamella, desquamation and curling of secondary lamella. Similar toxicological effects were observed in earlier studies of fish exposed to xenobiotics

and heavy metals (Olojo *et al.,* 2005; Figueiredo-Fernandes *et al.,* 2007; Mishra and Mohanty, 2008; Ahmed, *et al.,* 2013). Gills are the critical organs that carryout osmoregulatory functions and exposure to water born Pb^{2+} may cause reduced oxygen consumption and disturbed osmoregulatory functions in Crucian carp. Trunk kidney is one of the important organs in fish to evaluate the toxicological effects of environmental pollutants because it receives bronchial blood and could be a good indicator of heavy metals and pesticides induced histopathological changes (Ortiz *et al.,* 2003; Velmurugan *et al.,* 2007; Xing, *et al.,* 2012). Histopathological changes like glomerular shrinkage, renal tubule separation, hypoplasia of hemopoietic tissue and tubular necrosis were observed in the trunk kidney. The aberrant histopathological changes in kidney tissue are usually associated with the presence of toxic pollutants in filtrate of glomeruli (Silva and Martinez, 2007). Boran *et al.,* (2010) had observed similar findings in rainbow trout exposed to sublethal concentrations of maneb and carbaryl. The abnormal renal cells may adversely affect the metabolism of the organism, thereby inducing pathological condition that may even lead to death. Liver mainly carryout the detoxification of toxins and pollutant to which organisms are exposed through various routes. In the present study exposure to 5 mgL^{-1} Pb^{2+} induced pathological changes including nuclear degeneration, hepatocytes necrosis, cellular edema and pyknotic nuclei in the liver tissue of Crucain carp. Similar observations were made by Ahmed, *et al.,* (2013) in freshwater fish, tilapia exposed to sublethal arsenic for 96 h. Nuclear degeneration, necrosis and nuclear condensation were also noticed in the liver tissue of teleost fish, after exposure to Cr or Cu (Mishra and Mohanty, 2008; Figueiredo-Fernandes *et al.,* 2007). Brain is a sensitive organ which provokes abrupt response to toxicant in the surrounding environment. In our study Pb^{2+} exposure caused obvious changes to brain architecture such as glial cells proliferation, cellular necrosis, satellitosis and perivascular edema. Almost the same histopathological alterations were recorded for acute nitrate toxicity in *Rachycentron canadum* by Rodrigues, *et al.,* (2011). The neurotoxic effect of Pb is well recognised in the preveous studies (Neala and Guilarte, 2013), however the actual machnism of Pb^{2+} toxicity in brain is still not clear.

Conclusion: Lead is an environmentally reactive metal that exhibits a high degree of toxicity to living organisms. The results of the present study indicated that water born Pb^{2+} had lucid deleterious effects on Crucian carp. It not only cause histopathological alterations in the vital organs of the fish but also interact with essential trace elements and may have drastic effects on mineral homeostasis and vital organs architecture. Studies regarding Pb^{2+} toxicity may be crucial to understand their toxicological mechanism and its impact on trace metals metabolism in freshwater fish.

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