METAL ACCUMULATION IN VARIOUS TISSUES OF *Clarias gariepinus* EXPOSED TO COPPER, ZINC, CADMIUM AND LEAD SINGLY AND IN MIXTURE

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ABSTRACT

Accumulation of copper, zinc, cadmium and lead in gill, liver, spleen, kidney and muscle tissues of *Clarias gariepinus* was studied after exposing the fish to 5 ppm Cu, 5 ppm Zn, 1 ppm Cd, 1 ppm Pb and to the same concentrations of their mixture over 1, 7 and 15 days. Atomic absorption techniques were applied in determining tissue metal levels.

Highest Cu and Zn accumulation was observed in liver and kidney tissues at all exposure periods whereas highest Cd and Pb accumulation was in gill and liver tissues. The lowest metal accumulation was observed in muscle tissue.

Accumulation of these metals in the tissues studied increased compared to control when exposed singly. Tissue accumulation of metals, however, decreased when exposed to metals in mixture compared with single exposures to metals. It was concluded that exposure to these metals in mixture had an antagonistic effect on metal accumulation.

KEYWORDS:
Copper, zinc, cadmium, lead, mixture, *Clarias gariepinus.*

1. INTRODUCTION

Heavy metal are natural components of aquatic environments and enter to these environments by natural phenomenon such as volcanic eruptions and erosion. The levels of these metals, however, increased significantly mainly by anthropogenic activities. Aquatic animals uptake these metals from water and sediment mainly via their gills and digestive track and accumulate them in various tissues. Since these metals are transferred to higher food chains in more concentrated forms they pose a danger to aquatic life. Excess amounts heavy metals lead to various physiological and biochemical abnormalities in aquatic organisms [1, 2]. Fish are frequently used in metal accumulation studies due to their adverse effects on human health and some are used as pollution indicator species [3].

Discharge of various metal mixture containing urban and industrial waste waters to freshwater environments result in a number of physiological and biochemical disturbances to organisms living in these environments [1-2]. Copper and zinc are necessary in trace amounts for the functioning various biological mechanisms. They are structural components of various enzymes and play role in a number of physiological activities such as development, growth reproduction, immunity and metabolism [4-5]. They, however, become toxic above certain levels. Cadmium and lead have no biological function and are toxic even at very low concentrations [6-7].

Gills are the target organs in accumulation metals since they are in direct contact with the external media [8]. Liver and kidneys are metabolically active organs and play a significant role in accumulating metals [9]. Muscle is not an active tissue in accumulating metals, although it plays a significant role in transferring metals through the food chain [7].

Clarias gariepinus was chosen as an experimental animal since the species is commonly found in streams and drainage channels of Mediterranean region, its consumption as a protein source in the region, its wide tolerance against pollutants and its habitat being under the effect of agricultural and industrial activities.

Accumulation of heavy metals in aquatic organisms depends on various factors including the presence of other metals in the environment. Hence the aim of the present study was to determine metal levels in gill, liver, kidney, spleen and muscle tissues of *C.garipinus* after exposing the animals to 5ppm Cu, 5ppm Zn, 1ppm Cd, 1ppm Pb and to their mixture over 1, 7 and 15 days.

2. MATERIALS AND METHODS

C. gariepinus was obtained from a private fish farm in Silifke-Turkey, placed in glass aquaria sized 40x120x40 containing 120 L of tap water and were adapted to laboratory conditions for two months.

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The same size of six aquaria were used in the experiments which contained 120 L 1 ppm Cd, 1 ppm Pb, 5 ppm Cu, 5 ppm Zn, the mixture of the same concentrations of these metals and the last one contained tap water and evaluated as control. Nine fish were placed in each aquarium totaling to 54 fish. Cadmium chloride ($CdCl_2$:H_2O), zinc sulphate ($ZnSO_4$:$5H_2O$), copper ($CuSO_4$:$5H_2O$) and the lead nitrate (Pb $(NO_3)_2$ salts were used in the experiments. Trisodium citrate ($C_6H_5Na_3O_7.5H_2O$) was added to stock solutions to prevent precipitation and adsorbtion.

Some chemical and physical parameters of the experimental water were as follows;

Temperature: 21.5 ±1^{0}C

Total alkalinity: 305 ± 0.5 mg $CaCO_3$/L

Dissolved Oxygen: 7.01 ± 0.6 mg/L

pH: 7.5 ± 0.5

Three fish were removed from each aquarium at the end of 1, 7, and 15 days of exposure periods, they were washed dried and dissected for their gill, liver, spleen, kidney and muscle tissues. Tissues were transferred to petri dishes after being wet weighted and were placed in a drying oven set at 150^{0}C for 48 hours. Dried tissues were then transferred to experimental tubes and digested in nitric acid (Merck, 65%) / perchloric acid (Merck, 60%) mixture (2/1; v/v) at 120^{0}C for three hours. Digested tissues were transferred to polyethylene tubes and their volumes were made up to 10 ml with distilled water. Metal levels in tissues were determined using Varian AA240FS atomic absorption spectrophotometer.

Data were statistically evaluated by a series of Variance Analysis, Student-Newman Keuls procedure (SNK) and Student's t-test using SPSS 16.0 statistical package.

3. RESULTS

No mortality was observed during the experiments except for those exposed to copper alone at which all fish were dead at the end of 8th day. Copper accumulation increased in fish exposed to Cu alone in liver kidney and gill tissues (P<0.05) whereas no difference was observed in spleen and muscle tissues compared to control on day one. Liver and kidney accumulation of Cu in metal mixture was lower than single copper exposure at the same period (P<0.05; Table 1). Copper accumulation increased significantly at all tissues compared to control at all the tissues tested on the 7th day (P<0.05; Table 2). Copper accumulation was lower in mixture compared to single exposure at this period (P<0.05). The following relationship was found among the tissues in accumulating copper at all the periods tested; Liver>Kidney> Gills>Spleen>Muscle (Tables 1 and 2).

Liver accumulation of zinc in single and in mixture exposures decreased, whereas gill and kidney accumulations increased compared to control on day one (P<0.05; Table 1). Zinc levels were the same as control in spleen and muscle tissues at the end of day 1 both in single and mixture exposures. Accumulation in gill and kidney tissues were higher when exposed to Zn only rather than to metal mixture (P<0.05; Table 1). Liver, gill, kidney and spleen accumulation of Zn increased compared with control on the 7th day both when exposed singly and in mixture (P<0.05; Table 2). Muscle accumulation was significantly higher when exposed to Zn singly compared with control and mixture exposures (P<0.05; Table 2). Liver, gill, kidney, spleen and muscle accumulation of Zn increased on day 15 compared to control both on single and mixture exposures (P<0.05; Table 2). The increase in single Zn exposure was higher than in mixture at all the tissues tested at this period. The following relationship was found between tissues in accumulating Zn; Liver>Kidney>Gill> Spleen>Muscle (Tables 1-3).

TABLE 1 - Accumulation of copper, zinc, lead and cadmium in liver, gill kidney, spleen and muscle tissues of *Clarias gariepinus* after exposing the animals to these metals singly and in their mixture for 1 day (µg metal / g dw).

METAL	Liver $\overline{X} \pm s\overline{x}$ *	Gill $\overline{X} \pm s\overline{x}$ *	Kidney $\overline{X} \pm s\overline{x}$ *	Spleen $\overline{X} \pm s\overline{x}$ *	Muscle $\overline{X} \pm s\overline{x}$ *
Control	85.44±3.41 sa	2.80±0.04 sb	11.23±1.16 sc	5.10±0.54 sb	2.60±0.09 sb
Cu	93.79±1.26 ta	5.98±0.65 tb	17.86±1.08 tc	6.51±0.42 sb	2.80±0.13 sd
Mixture	82.60±1.42 sa	5.16±0.08 tb	12.59±0.98 sc	5.61±0.43 sb	2.32±0.04 sd
Control	232.8±5.29 sa	108.9±5.43 sb	159.3±5.53 sc	145.2±4.87 sc	59.13±1.63 sd
Zn	209.7±3.37 ta	159.5±2.12 tb	183.7±3.96 tc	156.3±3.44 sd	59.35±0.94 sd
Mixture	202.2±4.32 ta	125.6±3.54 xb	161.6±3.32 sc	145.4±3.28 sd	58.68±0.61 se
Control	BDL	BDL	BDL	BDL	BDL
Pb	0.025±0.003 ta	1.70±0.26 tb	0.015±0.001 ta	0.004±0.001 ta	BDL
Mixture	0.01±0.001 xa	0.99±0.07 xb	0.009±0.001xa	0.003±0.001 ta	0.001±0.000ta
Control	BDL	BDL	BDL	BDL	BDL
Cd	0.07±0.003 ta	1.02±0.05 tb	0.04±0.003 ta	0.015±0.002 ta	0.002±0.001 ta
Mixture	0.004±0.005xa	0.6±0.08 xb	0.02±0.002 xa	0.016±0.001 ta	0.001±0.000xa

*SNK; Letters a, b, c and s, t, x show differences among the tissues and among control, single metal and mixture respectively. Data shown with different letters are significant at the P<0.05 level; $\overline{X} \pm s\overline{x}$: Mean ± Standard error ; BDL: Below detection limit

TABLE 2 - Accumulation of copper, zinc, lead and cadmium in liver, gill kidney, spleen and muscle tissues of *Clarias gariepinus* after exposing the animals to these metals singly and in their mixture for 7 days (µg metal / g dw).

METAL	Liver $\overline{X} \pm s_{\overline{X}}$ *	Gill $\overline{X} \pm s_{\overline{X}}$ *	Kidney $\overline{X} \pm s_{\overline{X}}$ *	Spleen $\overline{X} \pm s_{\overline{X}}$ *	Muscle $\overline{X} \pm s_{\overline{X}}$ *
Control	84.71±2.76 sa	2.82±0.13 sb	11.94±0.66 sc	4.91±0.58 sb	2.33±0.06 sb
Cu	133.8±2.13 ta	20.00±0.65 tb	27.86±1.98 tc	13.00±0.82 td	3.37±0.12 te
Mixture	74.43±3.51 xa	11.31±0.69 xb	16.85±0.77 xc	9.41±0.42 xb	2.70±0.07 xd
Control	217.3±9.00 sa	111.6±11.29 sb	147.9±5.37 sc	146.4±3.49 sc	52.84±2.57 sd
Zn	288.7±4.52 ta	197.8±3.79 tb	256.5±4.16 tc	178.3±4.88 td	74.16±1.68 te
Mixture	253.7±4.83 xa	165.5±4.53 xb	184.2±3.85 xc	165.1±2.54 xb	57.93±2.89 sd
Control	BDL	BDL	BDL	BDL	BDL
Pb	0.05±0.004 ta	2.72±0.08 tb	0.027±0.001ta	0.017±0.001 ta	0.002±0.0003 ta
Mixture	0.027±0.004xa	1.76±0.09 xb	0.007±0.001xa	0.004±0.001 xa	0.002±0.0003 ta
Control	BDL	BDL	BDL	BDL	BDL
Cd	0.10±0.004 ta	2.08±0.07 tb	0.07±0.005 ta	0.03±0.002 ta	0.003±0.0003 ta
Mixture	0.07±0.006 xa	1.22±0.07 xb	0.03±0.003 xa	0.02±0.001 xa	0.002±0.0003 xa

*SNK; Letters a, b, c and s, t, x show differences among the tissues and among control, single metal and mixture respectively. Data shown with different letters are significant at the P<0.05 level. $\overline{X} \pm s_{\overline{X}}$: Mean ± Standard error . BDL: Below detection limit

TABLE 3 - Accumulation of copper, zinc, lead and cadmium in liver, gill kidney, spleen and muscle tissues of *Clarias gariepinus* after exposing the animals to these metals singly and in their mixture for 15 days (µg metal / g dw).

METAL	Liver $\overline{X} \pm s_{\overline{X}}$ *	Gill $\overline{X} \pm s_{\overline{X}}$ *	Kidney $\overline{X} \pm s_{\overline{X}}$ *	Spleen $\overline{X} \pm s_{\overline{X}}$ *	Muscle $\overline{X} \pm s_{\overline{X}}$ *
Control	83.47±2.89 sa	2.79±0.10 sb	10.80±1.12 sc	4.75±0.18 sb	2.41±0.12 sb
Cu	-	-	-	-	-
Mixture	113.6±5.33 xa	18.64±0.48 xb	26.44±1.54 xb	16.70±2.49 xb	3.60±0.08 xc
Control	188.3±5.79 sa	118.6±2.02 sb	144.4±3.34 sc	136.1±3.17 sc	52.09±1.40 sd
Zn	334.0±6.43 ta	244.8±5.74 tb	299.9±5.77 tc	203.9±2.79 td	88.21±1.73 xe
Mixture	211.2±6.25 xa	195.6±4.22 xb	175.9±4.60 xc	158.0±2.65 xd	69.26±1.25 xe
Control	BDL	BDL	BDL	BDL	BDL
Pb	1.26±0.03 ta	3.87±0.08 tb	0.09±0.006 tc	0.03±0.001 tc	0.004±0.0005tc
Mixture	0.09±0.01 xa	2.81±0.02 xb	0.05±0.001 xa	0.009±0.0003xc	0.002±0.0003xc
Control	BDL	BDL	BDL	BDL	BDL
Cd	2.15±0.07 ta	4.10±0.13 tb	0.44±0.06 tc	0.20±0.02 td	0.005±0.0006 td
Mixture	1.20±0.09 xa	2.10±0.08 xb	0.12±0.01 xc	0.06±0.003 xc	0.002±0.0005 xc

*SNK; Letters a, b, c and s, t, x show differences among the tissues and among control, single metal and mixture respectively. Data shown with different letters are significant at the P<0.05 level. $\overline{X} \pm s_{\overline{X}}$: Mean ± Standard error. BDL: Below detection limit

No lead or cadmium was detected in tissues of control animals. Tissue levels of these metals increased to a higher level when exposed to these metals singly than in mixture. The following relationships were found between tissues in accumulating Pb and Cd; Gill>Liver>Kidney>Spleen> Muscle (Tables 1-3).

4. DISCUSSION

Accumulation and toxic effects of heavy metals in fish tissues depends on various factors such as physical and chemical properties of water [10], developmental stage [11], sex [12], species [13], metal [8], its environmental concentration [14] and presence of other metals [15].

Heavy metals accumulate in various tissues at low concentrations and may interfere with metabolic and physiologic events, however, they cause mortality at higher concentrations [16-17]. Perkins et al. [12] indicated that 354 and 465 µg Cu L^{-1} caused mortality in *Ictalarus punctatus* within the first week of exposure. All *C. gariepinus* were dead on the 8th day of experiments when exposed to 5 ppm Cu in the present study. Previously observed behavioral differences were also true for *C. gariepinus* exposed to metals at the beginning of experiments [18-19].

Gills are target tissues in accumulating metals due to their large surface areas and being in direct contact with the environment. Liver is a metabolically active tissue since it plays role in conversion of food, binding of harmful substances and in digestion of macromolecules especially lipids [8]. When the metal binding capacity of liver is exceeded, the excess amount of metals are sent to kidneys for excretion [10]. Although muscle is not an effective tissue in binding metals it is important to know metal levels in

this tissue as far as food chain and human health is concerned.

Highest metal accumulation was observed in liver, gill and kidney tissues of *O. kisutch* after long term of exposure to Pb and Cd [20]. Highest levels of Pb, Cr, Cd, Cu and Zn in gill liver and muscle tissues of five fish species caught from Mersin Bay was in liver tissue followed by gill and muscle tissues [21]. *Tilapia zillii* exposed to 1 ppm concentrations of Cu, Zn, Cd and Pb singly over 10 days accumulated higher amounts of these metals in their liver, gill and brain tissues compared to muscle tissue [22]. Juvenile and adult *O. mykiss* exposed to sublethal concentrations of Cd over long periods accumulated higher levels of this metal in their liver, gill and kidney tissues compared with brain and muscle tissues [23]. *C. gariepinus* exposed to Cu, Zn, Cd, Pb and their mixture, accumulation of Cu and Zn was higher in liver and kidney followed by gill, spleen and muscle tissues whereas the levels of Cd and Pb was higher in gill and liver followed by kidney, spleen and muscle tissues. These differences among the tissues in accumulating metals can be explained by variances in metabolic activities of these tissues, Cu and Zn being trace, while Pb and Cd being toxic elements and that metals are carried to liver for detoxification and to kidneys for excretion.

Metal mixture had antagonistic effect on Cu accumulation in *O. mykiss* exposed to Cd, Cu and Zn mixture for 28 days [15]. Metal accumulation was higher in mixture then in single exposures to Cu and Zn in *F. heteroclitus* exposed to these metals over 48 hours [24]. Cd accumulation increased whereas Pb accumulation decreased in metal mixture compared with single exposures in *O. niloticus* exposed to 1 ppm concentrations of Pb, Cd and their mixture over 7 and 15 days [25]. Metal accumulation was lower in mixture then in single exposure in *C. carpio* exposed to 0.5 Cu, 5.0 ppm Zn and the same concentrations of their mixture [7]. Liver, gill and muscle accumulations were also lower in mixture then in single exposure in *Tilapia nilotica* exposed to sublethal concentrations of Cd and Zn over 10 days [26]. No difference, however, was observed between the single and mixture exposures to sublethal concentrations of Cd, Hg and Pb in *O. aureus* [27].

Gill, liver spleen and muscle accumulations increased in *C. gariepinus* compared to control in fish exposed to Cu, Zn, Cd, Pb and singly and in mixture. Accumulation in metal mixture was lower compared with single exposures at all metals and exposure periods. In conclusion it seemed metal mixture had an antagonistic effect on metal accumulation in *C. gariepinus*, which might be due to competition between trace and toxic elements.

The authors have declared no conflict of interest.

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