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UPTAKE AND BIOACCUMULATION OF WATER BORNE LEAD (Pb) IN THE FINGERLINGS OF A FRESHWATER CYPRINID, *CATLA CATLA* L.

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ABSTRACT

A study was conducted to evaluate the uptake and accumulation of waterborne lead (Pb) in various tissues of *Catla catla* when exposed to lower doses. Single breed fingerlings (6-8 cm) of *C. catla* were obtained from a commercial fish seed hatchery. Six groups (in duplicate) of fish (40 each) were maintained at 22^o C, pH 7.0, hardness 140 mg/l and DO 7.0 mg/l in 90 liters of water in glass tanks. Each group was exposed to a sub-lethal dose of waterborne Pb at 0.0 µg/l, 1.0 µg/l, 2.5 µg/l, 5.0µg/l, 7.5 µg/l and 10.0 µg/l for 6 weeks. Fish sampling was done on day zero and weekly thereafter for six weeks. Ten fish from each treatment were sacrificed; tissue samples were collected and prepared for analysis of Pb using graphite furnace atomic absorption spectrophotometer. Fish tissues that were directly exposed to waterborne Pb, the absorption and accumulation was significantly high ($p<0.05$) in the skin (including mucus) (4.92 ±0.25 µg/g dry wt.), gills (4.71 ±0.33 µg/g dry wt.), and eyes (4.51 ±0.19 µg/g dry wt.), as compared to the tissues from control group. While, Pb had accumulated significantly high ($p<0.05$) in internal tissues like liver (4.79 ±0.11 µg/g dry wt.), muscles (4.41 ±0.23 µg/g dry wt.) and intestine (4.21 ±0.22 µg/g dry wt.) as compared to the tissues from control group. This study indicates that the fishes living in water bodies receiving industrial effluents and city waste water containing various heavy metals, had absorbed and accumulated in various tissues like skin, gills and intestine directly from water as well as alongwith the food during feeding. With the passage of time as the fishes grow, these metals accumulate in various tissues like liver, muscles and intestine to a significantly high concentration not suitable for human consumption.

Key words: Cyprinids, fish tissues, industrial effluents, Pb bioaccumulation, sub-lethal dose, topical absorption, water pollution,

INTRODUCTION

Lead, a non-essential and toxic metal, is released into the aquatic environment by industrial sources such as chemical and fertilizer industries, refining of ores (Handy, 1994), the plating process, and gasoline containing Pb that leaks from fishery boats (Pascoe and Mattery, 1977). Lead also enters in the aquatic environment through erosion and leaching from the soil, domestic and industrial waste discharges, Pb-dust fallout from the atmosphere and combustion of petroleum products. It is mainly soluble in soft and slightly acidic water (Moore and Rainbow, 1987). Generally, Pb present in the environment is in its inorganic forms and exists in several oxidation states (0, I, II and IV). Pb II is the most stable ionic species present in the aquatic environment and get accumulated in the aquatic organisms. Moreover, Pb is also present in our environment in an organic form such as alkyl Pb which is obtained from auto emissions (Nussey *et al*., 2000).

When fishes are exposed to high level of metal ions in aquatic environment, their tissues tend to take up these metal ions through various routes from their surroundings. There are two main routs of metal acquisition; directly from the water and from the diet (Bury *et al*., 2003). Pb ions enter in the body of fish through gills after binding to the mucus layer. It is also ingested alongwith the food and water and is finally absorbed in the intestine and other tissues (Kotze *et al*., 1999; Ay *et al*., 1999; Macdonald *et al.,* 2002, Hensen *et al*., 2007). But the metal accumulation in tissues of aquatic animals is dependent upon exposure concentration and period as well as some other factors such as salinity, temperature, interacting agents and metabolic activity of the tissue in concern. Similarly, it is also known that the metal accumulation in the tissues of fish is dependent upon the rate of uptake, storage and elimination (Roesijadi and Robinson, 1994; Longston, 1990). Various metal ions get biologically magnified when taken up from the surrounding water in their various tissues as they grow. This uptake and bioaccumulation is well documented in skin, gills, stomach, muscles, intestine, liver, brain, kidney and gonads but their main target organs are liver, kidney and muscles depending on the exposure concentration and time (WHO, 1980; Chen and Chen, 2001; Allinson *et al*., 2002; Alam *et al*. 2002; Spokas, *et al*. 2006; Fabris *et al*. 2006). Pb is metabolized via the Ca^{2+} metabolic pathway and therefore accumulates in the skeletal tissues (Seymore, 1995). However, Pb is also known to biologically accumulate in other tissues of fish, including skin and scales, gills, eyes, liver, kidneys and muscles (Rashed, 2001; Nussey *et al*., 2000; Alves *et al*,, 2006, Spokas *et al*. 2006). The primary mode of uptake of aqueous Pb^{2+} in freshwater fishes is through their gills into the blood stream (Seymore, 1995) and during the sub-lethal exposure, the amount of Pb taken up by the fishes, have induced behavioral deficits due to disruptions in the integrative functioning of the medulla, cerebellum and optic tectum (Rademacher *et al*. 2003). Lead is cancer-causing agent and adversely effects reproduction, liver and thyroid function and disease resistance (Eisler 1988). Fishes exposed to high levels of lead exhibit a wide-range of effects including muscular and neurological degeneration and destruction, growth inhibition, mortality, reproductive problems, and paralysis (U.S. EPA 1976; Eisler 1988). In the present investigation, a study was conducted to estimate the uptake and accumulation of waterborne Pb in various tissues like skin, gills, eyes, liver, intestine and muscles of fingerlings of a freshwater fish, *Catla catla*.

MATERIALS AND METHODS

Experimental design: Single breed fingerlings of *Catla catla* measuring 6-8 cm (±1.2cm) were purchased from a commercial fish seed hatchery. All fish were acclimatized to 12 hrs light/dark regimen in 90 liters of water in glass tanks (aquaria) for two week prior to Pb exposure in a flow through system. Six glass tanks (in duplicate), five treatments and a control, each containing 40 fingerlings were maintained at 22.01 ±0.22°C, pH 7.17 ±0.14, hardness 140.01 ±2.1 mg/l and DO 7.15 ±0.24 mg/l (Table 1). Lead acetate (E. Merck, Darmstadt, Germany) was dissolved in deionized water and a clear solution was obtained by adding few drops of acetic acid. The concentrations of lead (Pb) in the water of experimental tanks were adjusted to nominal values as 0.0 (control), 1.0 µg/l, 2.5 µg/l, 5.0 µg/l, 7.5 µg/l and 10.0 µg/l in the tanks. All fish were fed with commercial fish feed (Miracle: Pb level 0.05 – 0.08 µg/g dry weight) to an equivalent of 2 % body weight twice daily. Uneaten food and the feces were removed at 30 minutes after feeding from all tanks daily.

Tissue sampling and Pb analysis: Fish tissue sampling was done on day zero and weekly thereafter from all treatments for 6 weeks. Five fish from each aquarium (10 fish / treatment) were sacrificed and various tissues like skin (alongwith mucus), gills, eyes (eye balls), liver, muscles and intestine were removed, wrapped in Teflon grade polythene bags and kept at –40° C until analyzed. Fish tissue sample were pulverized in liquid nitrogen with glass mortar and a pestle (precleaned with 10% HNO_3) and allowed to air-dry overnight at room temperature to a constant weight (5 gram). The dried samples were then acid-digested by adding 5 ml of concentrated $H\ NO_3$ (metal grade) according to Csuros and Csuros (2002) and analyzed by using graphite furnace atomic absorption spectrophotometer (1275 GF-AAS). Standard curves were established by measuring different dilutions of Pb standards, the lowest dilution being 0.05 µg Pb/l. The accuracy and integrity of the sample analysis was monitored by regularly running check standards and deionized water blanks.

Statistical analysis: One-way nested ANOVA followed by the Tukey-Kramer multiple comparison test were used. An overall α value of 0.05 was used to assess significant differences. Data are presented as mean ± SEM. Most statistical analyses were conducted with STATISTICA data mining software, version 8 (StatSoft, Inc., Tulsa, OK). Bartlett's test was used to assess the homogeneity of variables.

RESULTS

The amount of Pb uptake and absorption in the skin (including mucus) at treatments 1.0 µg/l and 2.5µg/l was high but not significantly different from zero treatment (control). At treatments 5.0 µg/l it was significantly different (p<0.05) and at 7.5 µg/l and 10.0 µg/l, highly significantly different (p<0.001) from control (Figure 1). In the gills, Pb uptake and absorption was similar like in skin at all treatments (Figure 2). In the eyes, Pb uptake and absorption was significantly high (p<0.01) from control only at treatments 7.5 µg/l and 10.0 µg/l (Figure 3). The uptake and accumulation of Pb in liver at treatments 1.0 µg/l, 2.5 µg/l and 5.0 µg/l was not significantly different from zero treatment (control) but at treatments 7.5 µg/l it was significantly different (p<0.05) and at treatment 10.0 µg/l highly significant (p<0.001) (Figure 4). At treatments 7.5 µg/l and 10.0 µg/l, Pb accumulation in muscle was highly significant (p<0.001) only on day 42 of exposure (Figure 5). Similar patterns of Pb accumulation were observed in the intestine at all treatments (Figure 6). The data and statistical analysis are presented in Tables 2-7.

The maximum values of Pb uptake and accumulation in each tissue (Pb µg/g dry wt.) were calculated for the highest treatment. The data indicated the following rank order of Pb uptake and accumulation (from highest to lowest mean Pb in each tissue) in skin (including mucus) (4.92 ±0.25 µg/g dry wt.) > in liver (4.79 ±0.11 µg/g dry wt.) > gills (4.71 ±0.33 µg/g dry wt.) > eyes (4.51 ±0.19 µg/g dry wt.) > muscles (4.41 ±0.23 µg/g dry wt.) > intestine (4.21 ±0.22 µg/g dry wt.), (Figure 7).

DISCUSSION

The accumulation of heavy metals by aquatic organisms involves tissues that serve as the site for uptake and absorption like gills, skin and intestine. These tissues have the ability to concentrate metals and therefore exhibit relatively high potentials for accumulation. Significant Pb accumulation occurred in the skin (including mucus) and was followed by liver, gills, eyes, muscles, and intestine. Skin, gills and eyes are tissues which were directly exposed to waterborne Pb have showed high uptake and accumulation which increased significantly high with the passage of time. The Pb uptake and accumulation in these tissues was highest at higher concentration with longer exposure whereas minimum Pb uptake and accumulation was observed at lower dose during the first week of exposure. This is because high dose and longer exposure resulted into high uptake and accumulation. Another observation was noted that the uptake and accumulation of Pb in tissues like skin and gills was high which were directly exposed to the waterborne Pb and lower in the tissues which were not directly exposed like liver. Our investigation revealed that fingerlings of *C. catla* (6-8 ±1.2cm) were exposed to a nominal concentration of waterborne Pb for 6 weeks only. During this exposure time fish has already accumulated significant level of Pb in their various tissues. If the exposure time is extended for 1-2 year, a normal time for a fish to grow to a consumable size, will accumulate significantly high level of Pb in its various tissues including muscles (consumable tissue). These results are in accordance with finding of Pouring *et al*. (2005) who documented Pb accumulations in edible tissues of five species of sturgeon and it was high in those tissues which were in immediate contact with water carrying heavy metal ions. Rashed (2001) also observed very high amount of Pb and Cd accumulation in the skin (scales) of *Tilapia nilotica* captured from a polluted lake in Egypt receiving industrial effluents and city sewage. Tao *et al*. (2000) described that Pb ions from water do bind to the mucus layer present on general body surface as well as on the gills of the fish leading to high uptake and absorption of metal ions in the skin and gills. It is also evident that Pb uptake and accumulation in the gills also become high because of its high affinity with ion transport activity of various mineral ions (Roesijadi and Robinson, 1994; Allen, 1995; Spokas, *et al*. 2006). While Pb uptake and accumulation in the intestine can be correlated with ingestion of food (feed pallets absorb Pb when put in treatment tanks to feed fish) and small amount of water containing Pb alongwith the food during feeding. The continuous process of food ingestion, digestion and absorption may have leaded the accumulation of Pb in intestine with the passage of time. Similar findings of Pb accumulation in various fish tissues were also observed in studies carried out with various fish species from number of polluted water bodies (Reichert *et al*. 1979; Amiard *et al*. 1987; Allen, 1995; Tulasi, *et al*. 1987 & 1992; Spokas, *et al*. 2006).

It is well understood that metal ions taken up by a fish through any route are not totally accumulated because fish can regulate metal concentrations to a certain extent, after which accumulation occurs. Therefore, the ability of each tissue to either regulate or accumulate metal ions can be directly related to the total amount of metal uptake in that specific tissue. This metal regulation is due to the induction of low molecular weight metal-binding proteins, such as metallothionein which are closely related to heavy metal exposure and metals taken up from the environment can be detoxified by binding on these proteins (Roesijadi and Robinson, 1994; Canli *et al.* 1997; Kotze, 1997). Therefore, tissue like liver, which is a major producer of metal-binding proteins, show high concentrations of most heavy metals detoxification (Thomas *et al*. 1983; Amiard *et al*. 1987; Roesijadi and Robinson, 1994; Allen, 1995; Heath, 1995) which eventually result in clearance of heavy metal ions from the body. Furthermore, the physiological differences and the position of each tissue in the fish can also influence the accumulation of a particular metal (Heath, 1995; Kotze, 1997). In other words, the amount of a metal accumulated is influenced by various environmental, biological and genetic factors, leading to the differences in metal accumulation between different individuals, species, age, tissues, seasons and sites (Kotze *et al*. 1999).

It is important to know that fishes from water reservoirs like rivers and lakes receiving industrial effluents containing variable concentration of toxicants including heavy metals from various sources like industries, agriculture runoff or domestic wastewater, may have accumulated heavy metals in their tissues as they grow and these toxicants and metals will be transferred to humans (being at the end of food chain) when consumed and may impair body metabolism (WHO, 1980; Ceirwyn, 1995; Pourang *et al*. 2005). *Catla catla*, is a preferred freshwater fish for human consumption in Indian sub-continental region. It is found naturally in all freshwater bodies including rivers and lakes which are receiving untreated industrial effluents and city wastewater containing various toxicants and heavy metals that may lead to the accumulation of toxicants and heavy metal including Pb in their tissues especially muscles (fish fillet). River Ravi is located in the vicinity of Lahore, Pakistan which is continuously receiving untreated industrial effluents and city wastewater containing high contents of various heavy metals (Ahmad, 2001). *C. catla* is the most common fish among other commercial fishes. While being in river Ravi until they reach the consumable size (1-2 years) would have already accumulated very high contents of Pb in its various tissues including muscles (consumable part). Our earlier studies (Ahmad, 2001) have revealed that Pb level in the water of river Ravi is 2.4-3.55 ppm (more than 50 times higher than WHO standards for drinking water) and the fish species including *C. catla* (± 1 year old) had accumulated very high level of Pb (31.2 – 38.3 ppm) in the muscles which is more than 600 times

Table 1. Physico-chemical parameters maintained in the water for fingerlings of *Catla catla* during Pb exposure. Data is presented as ±SEM, n=12

Weeks	Temperature (C°)	pH	Hardness (mg/l)	Dissolved oxygen (mg/l)
0	22.2	7.1	142	7.4
1	21.8	7.0	143	6.9
2	22.2	7.3	140	7.5
3	21.7	7.4	138	7.1
4	22.1	7.1	142	7.3
5	22.3	7.0	137	7.3
6	21.8	7.3	139	6.8
Mean	22.01 ±0.22	7.17 ±0.14	140.1 ±2.1	7.15 ±0.24

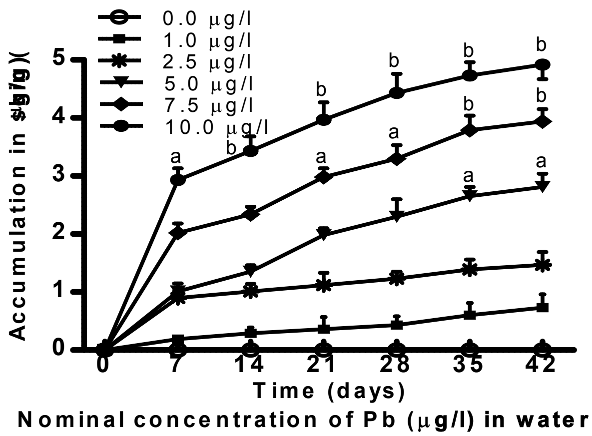

Figure 1. Uptake and bioaccumulation of total lead (Pb µg/g dry weight) at different time intervals in the skin (including mucus) of fingerlings of *Catla catla* when exposed to various concentrations of Pb in water. Data is presented as ±SEM, the letter 'a' on the line represent (P<0.05) and 'b' (P<0.01), n=10.

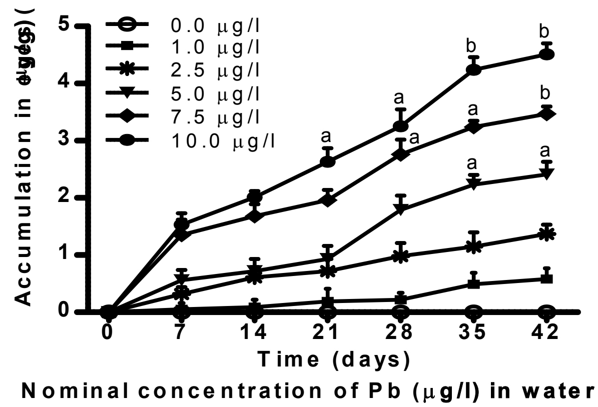

Figure 3. Uptake and bioaccumulation of total lead (Pb µg/g dry weight) at different time intervals in the eyes of fingerlings of *Catla catla* when exposed to various concentrations of Pb in water. Data is presented as ±SEM, the letter 'a' on the line represent (P<0.05) and 'b' (P<0.01), n=10.

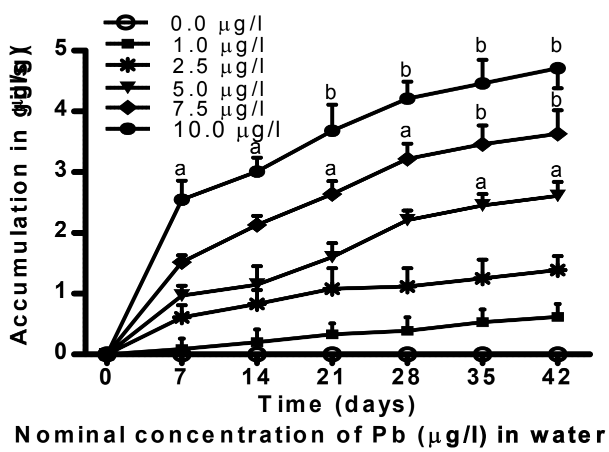

Figure 2. Uptake and bioaccumulation of total lead (Pb µg/g dry weight) at different time intervals in the gills of fingerlings of *Catla catla* when exposed to various concentrations of Pb in water. Data is presented as ±SEM, the letter 'a' on the line represent (P<0.05) and 'b' (P<0.01), n=10

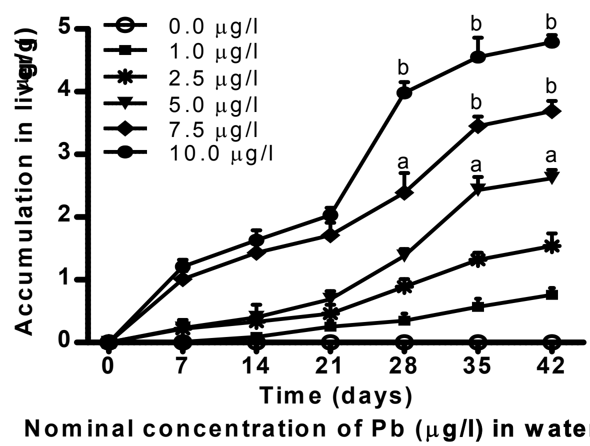

Figure 4. Uptake and bioaccumulation of total lead (Pb µg/g dry weight) at different time intervals in the liver of fingerlings of *Catla catla* when exposed to various concentrations of Pb in water. Data is presented as ±SEM, the letter 'a' on the line represent (P<0.05) and 'b' (P<0.01), n=10.

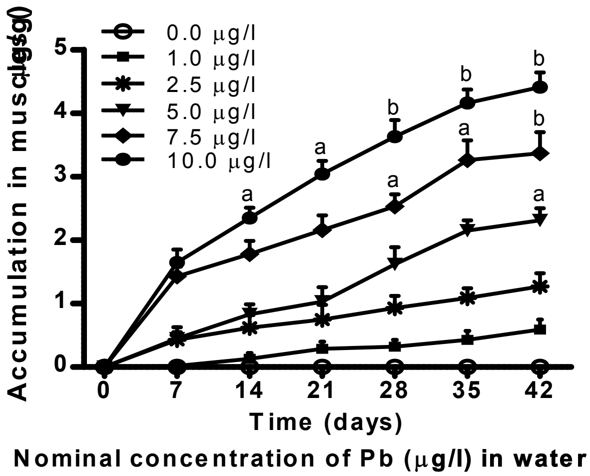

Figure 5. Uptake and bioaccumulation of total lead (Pb µg/g dry weight) at different time intervals in the muscles of fingerlings of *Catla catla* when exposed to various concentrations of Pb in water. Data is presented as ±SEM, the letter 'a' on the line represent (P<0.05) and 'b' (P<0.01), n=10.

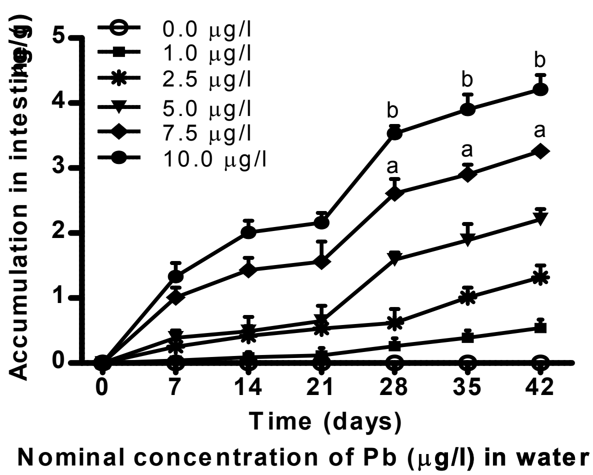

Figure 6. Uptake and bioaccumulation of total lead (Pb µg/g dry weight) at different time intervals in the intestine of fingerlings of *Catla catla* when exposed to various concentrations of Pb in water. Data is presented as ±SEM, the letter 'a' on the line represent (P<0.05) and 'b' (P<0.01), n=10.

higher than the permissible level of Pb in food recommended for human consumption (WHO, 1980). It is therefore, recommended that the consumption of *C. catla* and similar other freshwater fishes obtained from contaminated water bodies like river Ravi should be prohibited.

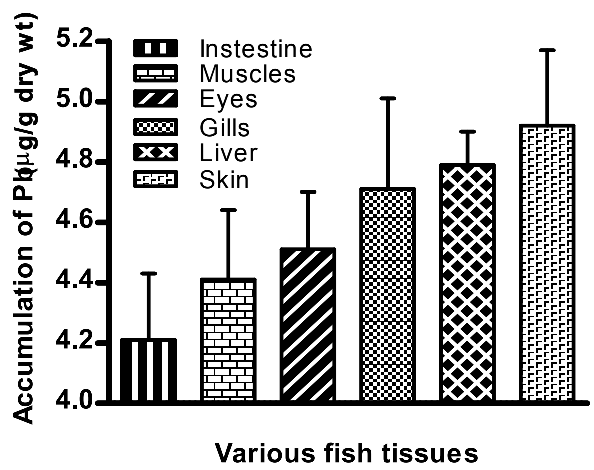

Figure 7. The rank order of uptake and bioaccumulation (from highest to lowest) of total lead (Pb µg/g dry weight) in various tissues (P>0.05) of fingerlings of *Catla catla* at the highest concentration and maximum exposure time. Data is presented as ±SEM, n=10.

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