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 Mattray, 1977). Lead also enters 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 routes 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 along with 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 2.5 µg/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
that in studies carried out with understood that waterborne – may have from Spokas. Another observation was noted accumulation Pb in that specific tissue of Pb 1987; Our investigation revealed (being at the end of food chain) various which very high 1999) their a contents higher observed water and . ppm fillet age, high various sources like 1. muscles (consumable all fresh. 0 times high - th e which is a major prod skin s and lakes alongwith and intestine was observed at first week Pb. Sci and accumulation sturgeon uptake Anim tissues water) which are closely related to heavy metal exposure and is extended 6 S are closely related to heavy metal exposure and is is due to effluents sewage waste. 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

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Temperature (C°)</th>
<th>pH</th>
<th>Hardness (mg/l)</th>
<th>Dissolved oxygen (mg/l)</th>
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<td>7.4</td>
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<td>1</td>
<td>21.8</td>
<td>7.0</td>
<td>143</td>
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<tr>
<td>6</td>
<td>21.8</td>
<td>7.3</td>
<td>139</td>
<td>6.8</td>
</tr>
<tr>
<td>Mean</td>
<td>22.01 ±0.22</td>
<td>7.17±0.14</td>
<td>140.1±2.1</td>
<td>7.15 ±0.24</td>
</tr>
</tbody>
</table>

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 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 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 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 (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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