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Effects of lead on the plasma electrolytes of a freshwater fish, *Heteropneustes fossilis*

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Abstract

The freshwater catfish, *Heteropneustes fossilis*, was subjected to 657.6 mg/L (0.8 of 96 h LC50) and 164.4 mg/L (0.2 of 96 h LC50) of lead nitrate for short-term and long-term experiment, respectively. Blood from fish was collected on 24, 48, 72 and 96 h in short term and after 7, 14, 21, and 28 days in long-term experiment. Plasma calcium and phosphate levels were determined at these intervals. After short-term lead exposure, the plasma calcium levels of the fish remained unaffected at 24 h. The levels exhibited a decrease after 48 h which persisted until the end of the experiment (96 h). Following 48 h of lead exposure to the fish, the plasma phosphate levels remained unchanged. The values exhibited a progressive decrease from 72 h onwards. The plasma calcium levels of the fish exposed to lead for 7 days exhibited a decrease. This decrease persisted progressively until the end of the experiment (28 days). The plasma phosphate levels of lead-exposed fish remained unaffected until day 14. The levels decreased progressively from 21 days onwards.

Keywords: Lead, Plasma calcium, Plasma phosphate, Teleosts, Hypocalcemia, Hypophosphatemia

Background

Natural and anthropogenic sources continuously release heavy metals into aquatic ecosystem. The heavy metals after reaching to freshwaters cause serious problem due to their long persistence, bioaccumulation, biomagnification in the food chain, and toxicity to the organisms. Fish, being dominant inhibitors of aquatic environment, are considered as indicators for heavy metal pollution.

Lead is a naturally occurring heavy metal which has been used in various ways including mining, smelting, refining, gasoline, battery manufacturing, electrical wiring, soldering, painting, ceramic glazing, and making of stained glass. Due to its non-degradable nature, it gets into the environment and eventually enters the human and animal's blood stream. It is accumulated in soft tissues such as liver, kidneys, nervous system, and the brain. In fishes, accumulation of lead in various tissues (Linde et al. 2004; Ashraf 2005; Spokas et al. 2006; Schmitt et al. 2007; Has-Schon et al. 2008) and alterations in biochemical and hematological parameters (Ates et al. 2008) have been reported. Moreover, lead-induced changes in the histological structure of gills and kidneys have also been reported (Martinez et al. 2004; Rabitto et al. 2005; Adeyemo, 2008; Koca et al. 2008; Palaniappan et al. 2008; Pandey et al. 2008).

In fish inhabiting a freshwater, blood ionic concentrations are maintained at much higher levels than those of the ambient water. Hence, they constantly face osmotic inflow of water and diffusion losses of ions across the body surface and gill epithelium. A disturbed hydromineral balance of the body fluids of fish is one of the most conspicuous phenomena observed during stress as there exists an intimate relationship between the surrounding water and the body fluids. Few studies exist regarding the interaction of lead with calcium homeostasis in mammals and birds. However, to the best of our knowledge, there exists a single report from fish regarding the effects of lead on plasma calcium (Rogers et al. 2003), whereas the effects of lead on plasma phosphate has not been reported. Hence, in this study, an attempt has been made to see the impact of lead nitrate (lead nitrate was used in this study because it is commonly used as a heat stabilizer in nylon and polyesters, tanning material for leather-making, photograph-promoting sensitizer/agent, as medical astringent, dyeing/textile mordant, in rodenticides, in manufacture of paint pigment, and for making fireworks, match, and explosives) on the plasma calcium and phosphate levels of a catfish, *Heteropneustes fossilis*.

Methods

Adult *H. fossilis* (both sexes, body weight 37 to 46 g) were procured locally. These fish were acclimatized to the laboratory conditions (under natural photoperiod 11.58 to 12.28 and temperature 27.2 ± 1.4°C) for 15 days in plastic pools (each pool containing 500 L of dechlorinated tap water). The physico-chemical characteristics of the tap water were as follows: pH 7.21 ± 0.06, dissolved oxygen 7.78 ± 0.30 mg/L, hardness as $CaCO_3$ 167.31 ± 5.81 mg/L, and electrical conductivity 306.18 ± 68.52 mΩ/cm. For the determination of LC_{50} values for lead, 4-day static renewal acute toxicity test (APHA et al. 1985) was used. Six replicates each containing ten fish (kept in glass aquarium (size 20 × 10 × 12 in) containing 30 L of the test solution) were subjected to each concentration of lead nitrate (700, 750, 800, 850, 900, 950, and 1,000 mg/L) for the test. Lead nitrate was dissolved in distilled water, and then the desired volume of the solution was mixed in tap water to obtain the above-mentioned heavy metal concentration. A control group with six replicates (each containing ten fish) kept in 30 L tap water was also run. The solutions of all the aquaria (control and experimental) were renewed daily. Precautions were taken to remove the dead fish immediately. Death in fish was confirmed when the movement of the operculum was stopped and the fish failed to respond when gently prodded at the caudal peduncle. Assays were terminated and results discarded if control mortality exceeds 10% at any time. At different exposure periods, the LC_{50} values were determined by the probit-log analysis (APHA et al. 1985; Swaroop 1966). The study was approved by the Animal Research Ethical Committee of DDU Gorakhpur University.

For the experiment, the freshwater catfish *H fossilis* (after 2-week acclimatization) were subjected to 657.6 mg/L (0.8 of 96 h LC_{50}) and 164.4 mg/L (0.2 of 96 h LC_{50}) of lead nitrate for short-term and long-term, respectively. Concurrently, a control group was also run. The media (both control and experimental) were changed every 24 h. The fish were killed at 24, 48, 72, and 96 h in short-term experiment and at 7, 14, 21 and 28 days in long-term experiment. Blood was collected by sectioning of the caudal

peduncle. The plasma calcium and inorganic phosphate levels were determined using a Sigma kit (Sigma-Aldrich, MO, USA). Student's *t* test (using InStat, GraphPad Software Inc., La Jolla, USA) was used to determine the statistical significance between the control and experimental groups.

Results

The LC_{50} values for lead nitrate at 24, 48, 72 and 96 h were 885, 875, 862 and 822 mg/L, respectively. After short-term lead exposure, the plasma calcium levels of the fish remained unaffected at 24 h. The levels exhibited a decrease after 48 h which persisted until the end of the experiment (96 h) (Figure 1).

Following 48 h of lead exposure of the fish, the plasma phosphate levels remained unchanged. The values exhibited a progressive decrease from 72 h onwards (Figure 2).

The plasma calcium levels of the fish exposed to lead for 7 days exhibited a decrease (Figure 3). This decrease persisted progressively until the end of the experiment (28 days) (Figure 3).

The plasma phosphate levels of lead exposed fish remained unaffected till day 14 (Figure 4). The levels decreased progressively from 21 days onwards (Figure 4).

Discussion

Lead exposure to *H. fossilis* provoked hypocalcemia. This derives support from the studies of Rogers et al. (2003) who have also reported hypocalcemia in lead-exposed rainbow trout. Other investigators have also noticed decreased blood/plasma calcium content of fish treated either with aldrin (Bano 1982; Singh et al. 1996), malachite green (Srivastava et al. 1995), cadmium (Larsson et al. 1981; Muramoto 1981; Giles 1984; Kuroshima 1987; Pratap et al. 1989, Rai and Srivastav Ajai 2003), propoxur (Singh et al. 1997), formothion (Singh et al. 1997), chlorpyrifos (Srivastav Ajai et al. 1997a), deltamethrin (Srivastav Ajai et al. 1997b), methyl-parathion (Mishra et al. 2004), cypermethrin (Mishra et al. 2005) or botanical pesticide (Prasad et al. 2011). Contrary to it, an elevation of plasma calcium concentrations has also been reported by other workers from the fish exposed to various toxicants (Sastry and Sharma 1978; Bansal et al. 1979;

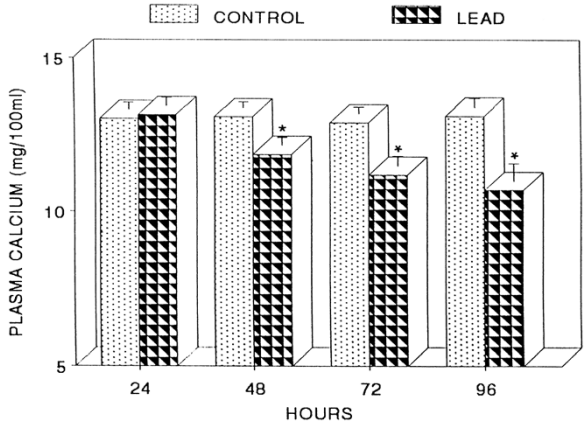

Figure 1 Plasma calcium levels of short-term lead nitrate-treated *H. fossilis*. Values are mean ± SE of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control.

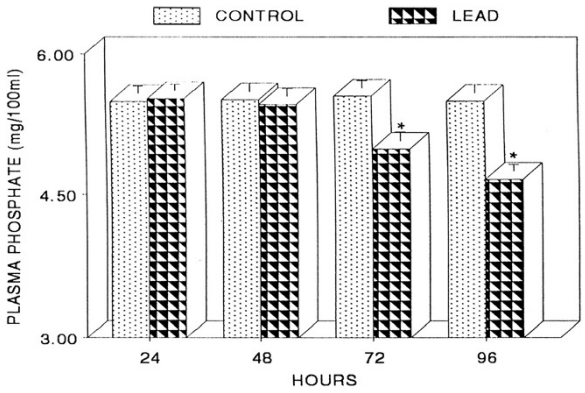

Figure 2 Plasma phosphate levels of short-term lead nitrate-treated *H. fossilis*. Values are mean ± SE of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control.

Sharma et al. 1982). However, no effect on plasma calcium level has been noticed in methoxychlor-exposed Northern puffer *Sphaeroides maculatus* (Eisler 1967) and DDT-treated flounders *Platichthys flesus* (Haux 1979).

Hypophosphatemia has been noticed in the lead-exposed *H. fossilis*. This is in agreement with other investigations in which similar effect has been observed after exposure of the fish to other toxicants: organophosphate (chlorpyrifos) (Srivastav Ajai et al. 1997a), pyrethroids (deltamethrin (Srivastav Ajai et al. 1997b and cypermethrin (Mishra et al. 2001)), cadmium (Rai and Srivastav Ajai 2003) and botanical pesticide (Prasad et al. 2011). Pratap et al. (1989) observed no effect on the plasma phosphate level of fish treated with cadmium. In contrast, few investigators have noticed hyperphosphatemia in the fish treated with various toxicants; endrin (Colvin and Phillips 1968), endosulfan (Gill et al. 1991), aldrin (Singh et al. 1996), formothion (Singh et al. 1997), and propoxur (Singh et al. 1997).

The hypocalcemia observed in lead-exposed *H. fossilis* may be attributed to the impairment of either net electrolyte influx at the gill or renal function. Rogers et al. (2003) have reported reduced calcium uptake in lead-exposed rainbow trout. Several

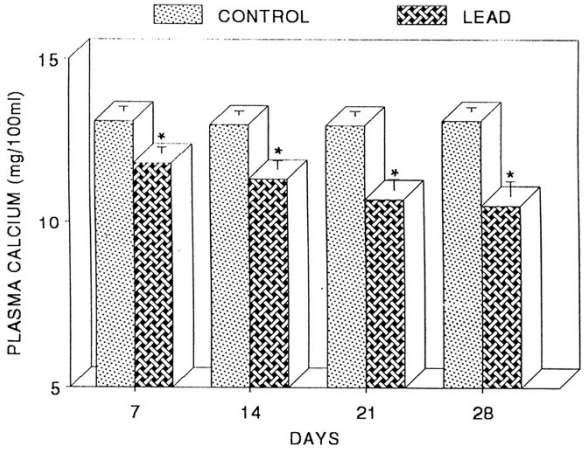

Figure 3 Plasma calcium levels of long-term lead nitrate-treated *H. fossilis*. Values are mean ± SE of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control.

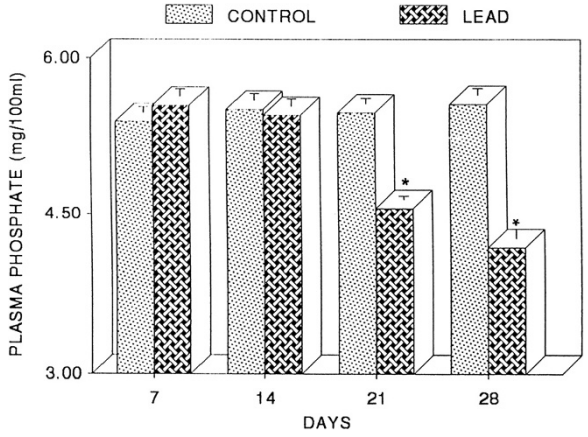

Figure 4 Plasma phosphate levels of short-term lead nitrate-treated *H. fossilis*. Values are mean ± SE of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control.

investigators have reported degenerative changes in the gills of fishes after exposure to various pesticides (Srivastava et al. 1989; Nath et al. 1997; Martinez et al. 2004; Adeyemo 2008; Koca et al. 2008; Palaniappan et al. 2008; Pandey et al. 2008). Degeneration of gills may affect the ionic permeability and cause decreased ionic levels in the blood. Tubular necrosis may be the other possible reason for the hypocalcemia and hypophosphatemia observed in lead-exposed *H. fossilis*. Kidney degeneration has been reported by several workers after the exposure of the fish to toxicants (Srivastava et al. 1990; Akram et al. 1999; Rabitto et al. 2005). The increased urine excretion rate of Ca^{2+} has been observed in lead-treated rainbow trout (Patel et al. 2006). The degeneration of kidney may lead to decreased reabsorption, thus causing increased urinary loss of these ions. This increased loss of ions through the kidney may be the possible reason for the decreased concentration of calcium and phosphate in lead-treated *H. fossilis*. In the past, Koyama and Itazawa (1977), Roch and Maly (1979), Larsson et al. (1981), and Haux and Larsson (1984) have also attributed degenerative changes in the renal tubules as one of the main causes of hypocalcemic responses in cadmium-treated fishes. Patel et al. (2006) have suggested that lead-induced ionoregulatory toxicity in rainbow trout, particularly the disturbance of Ca^{2+} homeostasis, is not exclusively a branchial phenomenon, but is in part a result of disruption of ionoregulatory mechanisms at the kidney.

Conclusion

It is concluded that lead affects the blood levels of calcium and phosphate in fish *H. fossilis.* An alteration in these electrolytes would greatly influence the physiology of the fish as calcium is vital for several biological actions including reproduction. Thus, precaution should be taken to avoid disposal of lead wastes near fish culture grounds.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
AKS designed the experiment, participated in conducting the experiment, discussed the results, and prepared the manuscript. RR, DM, and SKS conducted the experiment and helped in the processing of blood analysis. NS participated in designing the experiment, discussed the experiment, and helped in the preparation of the manuscript. All authors read and approved the final manuscript.

Authors' information
AKS (Ph.D.) is presently working as a professor in the Department of Zoology at DDU Gorakhpur University, now investigating the effects of toxicants on vertebrate calcium regulation. RR (Ph.D.) is performing researches on fish calcium regulation. NS (Ph.D.) is working at Noto Marine Laboratory, Kanazawa University, Japan, and performed experiments on fish calcium regulation. DM (Ph.D.) is a lecturer in Zoology at Govt. Girls Degree College, Ghazipur and has worked on the toxicological aspects in fishes. SKS (Ph.D.) is now working as lecturer in zoology, DDU Goralhpur University, doing researches with fish toxicology and reproduction.

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