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Bioaccumulation of lead (Pb) and remediation by calcium chelation therapy in the gills and mantle of freshwater bivalve, *Lamellidens marginalis.*

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Abstract: *The paper aims at bioaccumulation of lead (Pb) and removal of accumulated lead by calcium chelation therapy. This has been studied in the gill and mantle of the freshwater bivalve, Lamellidens marginalis. The bivalves were exposed to 1/10th of LC_{50} dose of lead acetate, different doses i.e. as 28, 56 and 84 ppm calcium acetate of and combinations of lead acetate and calcium acetate for 8 days and 16 days period. It was observed that, there was more accumulation of Pb in gills as compared to mantle. In 8 days Pb treated group accumulation of Pb in gill was 3.300 mg/ml and after 16 days exposure it was 4.129 mg/ml. It was observed that this accumulation was decreased at 56 and 84 ppm calcium treated groups for 8 days and 16 days. Similarly, in mantle Pb accumulation was 2.381 and 2.938 mg/ml on 8 days and 16 days respectively. This accumulation was decreased at 56 and 84 ppm calcium treated groups which are 1.870 and 1.581mg/ml for 8 days and 1.20 and 1.225 for 16 days mg/ml. The study also suggests that the accumulation of Pb was directly proportional to the exposure time. With increase in calcium concentration, the concentration of Pb was decreased.*

Key Words: *Lamellidens marginalis*, Lead acetate, calcium acetate, Chelation therapy.

Introduction

Aquatic ecosystems are under constant pressure of anthropogenic pollutants originating from various point and non-point sources as a result of rapid population growth, increased urbanization, increased industrial activities, extensions of irrigation and agricultural practices, exploration and exploitation of natural resources as well as the lack of environmental regulations. It is very rare for aquatic ecosystems to receive single pollutants such as heavy metals. Heavy metals represent one of the most widespread and serious agents of environmental pollution. Low metal concentrations are present in natural aquatic ecosystems but can be introduced by various anthropogenic activities and readily transported from one system to another (Biney *et al.*, 1994; Hamilton and Hoffman, 2003).

Lead is one such heavy metal with specific toxicity and cumulative effects (Ponangi *et al.*, 2000). The chief sources of lead in water are the effluents of lead and lead processing industries. In some plastic water pipes, lead is used as a stabilizer which may lead to contamination of water. Lead is also used in storage batteries, insecticides, food, beverage, ointments and medicinal concoctions for flavouring and sweeting (Sharma, 2001).

For the evaluation of toxicity, the freshwater mussels have been widely used. They play important role as bio-indicators of toxicity and as water purifier. As a consequence the use of bivalves as biomonitors of heavy metal pollution has been well established (Chaudhari and Hazra, 2001). Bioaccumulation and toxicity of Pb was also studied in some molluscs. This bioaccumulation is affected by various factors like age, dietary calcium, vitamin D, vitamin C, ascorbic acid, thiamine, iron, phosphorus etc. (Taylor *et al.*, 1962; Mahaffey *et al.*, 1972, Sorrell *et al.*, 1977). Among these all, the uptake and metabolism of lead may be modified by calcium status (Sorrell, 1977) and the study on

accumulation of Pb by different molluscs facilitates the assessment of water quality as well as the selection of suitable bioindicator species. Therefore, present investigation deals with removal of Pb toxicity by calcium chelation therapy in the freshwater mussel, (*Lamellidens marginalis)* As most of the freshwater bivalves are used as food by human beings, therefore, there is a need to eliminate Pb entering food chain from the these important carrier organisms. The freshwater bivalves are one of the most important carriers of Pb towards higher vertebrates. In the present investigation an attempt has been made to remove lead from the bivalve mussels by calcium chelation therapy.

Materials and Methods

The animals were brought to the laboratory as a single stock for complete test. They were acclimatized to the laboratory conditions for 15 days prior to the test and maintained in untreated pond water. Ten animals were kept in plastic containers. Water was changed twice daily preferably early in the morning and late evening to avoid temperature fluctuation and aeration was provided to maintain proper oxygen level. After acclimatization to laboratory conditions bivalves with weight 75-100 gms were selected. To avoid unnecessary stress animals were not subjected to rapid temperature or water quality change. All bivalves were kept at same experimental conditions in which temperature: 26.1 to 28.5°C, pH: 7.2 to 8.4, dissolved oxygen 4.2 to 5.4 mg/ml and hardness 60 to 83 mg/ml were maintained.

The LC_{50} (96 hrs.) for the bivalves were determined by using standard method (APHA, 2004). Adult bivalves (66 to 70 mm in length) were divided into eight groups, each group containing ten animals. The toxic and detoxifying medium were renewed after every 24 hrs. The bivalve were divided into groups: Group N : Control, Group A : Animals exposed to 28 ppm of lead acetate, Group B : Animals exposed to 28 ppm of calcium acetate, Group C : Animals exposed to 56 ppm of calcium acetate, Group D : Animals exposed to 84 ppm of calcium acetate, Group E : Animals exposed to 28 ppm of lead acetate + 28 ppm of calcium acetate, Group F : Animals exposed to 28 ppm of lead acetate + 56 ppm of calcium acetate, Group G : Animals exposed to 28 ppm of lead acetate + 84 ppm of calcium acetate.

The bivalves were exposed for lead acetate and calcium acetate and combination of both for 8 days and 16 days time period. From each group, 5 animals were sacrificed after 8 days and five were sacrificed after 16 days. Tissues of gills were separated from each bivalve for AAS. Samples of gills and mantles were quickly excised and cleaned off extraneous material and kept in oven at 60°C for 72 hrs. The dried samples were taken in separate test tubes (100 mg) and digested with 10 ml nitric acid and perchloric acid mixture (1:1) till a clean solution was obtained in each test tube. The digested samples were cooled at room temperature and filtered through Whattman filter paper. The filtrate was then diluted with concentrated hydrochloric acid (5 ml) and glass distilled water (35 ml). The test solution then analyzed for different trace metals using atomic absorption spectroscopy (Perkin Elmer model No.3030, USA).

Results and Discussion

The Table 1 and Fig. 1, 2 shows the accumulation of lead and effect of calcium on 8 and 16 days exposure period in gill tissue. Accumulation of lead was more in Pb exposed group after 8 days while this accumulation was increased after 16 days. The calcium level was more in all calcium exposed groups after both exposure periods. The remedial groups showed decrease in Pb accumulation with increasing Ca. After 16 days this groups showed less Pb accumulation. The Table 2 and Fig. 3, 4 shows the accumulation of lead and effect of calcium on 8 and 16 days exposure period in mantle tissue. Mantle also showed similar observations like gills. Accumulation of lead was more in Pb group after 8 days while accumulation was increased after 16 days. The calcium level was more in all calcium treated groups after both exposure

periods as compared to control one. The remedial groups showed decrease in Pb accumulation with increasing Ca as compared to 28 ppm Pb treated group. After 16 days these groups showed less Pb accumulation. Bivalve molluscs are one of the most suitable bioindicators because they are sedentary, wide spread and have a long life span (Odzak *and cossa,* 1994). Despite many studies on the uptake of stable metals in bivalves, variables affecting the results of bioindication surveys remain partly unknown. Some data is available on the effects of season, salinity, water temperature, and coexistence of several metals, organisms age, weight, size, and sex on the uptake of metals (Phillips, 1976). Accumulation of Cd, Cu, Pb and Zn in the tissue of *Mytilus edulis* and *M. planulatus* was examined under cyclic conditions of exposure in order to establish whether the rate of accumulation of heavy metal is proportional to time exposed to the elevated concentration, when exposed to a single metal, the accumulation of Pb, Zn was directly proportion to the time. Both Pb and Cd were accumulated in direct proportion to the exposure time (Eliott *et al.,* 1987). The highest concentration of lead (Pb) is in gills and the lowest values were observed in the foot Gill and mantle exhibited higher concentration of metals than adductor muscles (Mitra and Choudhary, 1994). High amount of heavy metals in mantle may be due to large surface area of the organ and in gills also there was high amount of metals as it circulates more water for respiration (Reddy *and* Kumar, 2006). It is a well known fact that the gills of aquatic animals have the ability to excrete invading heavy metals (Narayanan and Ajmal Khan, 1988). Absorption of heavy metals by crustaceans could be a process of diffusion through gills is due to absorption of metals onto the cuticle of the gills (Bryan and vysal,1964; Engel and Fowler, 1983). The metal thus entering the gills get bound to the soluble ligands like protein and then transported through blood to organs like hepatopancreas *etc.* (Engel and Fowler,1983).

Accumulation of heavy metals *i.e.* Cu and Pb were studied in the molluscs, *Bellamya bengalensis* and *Lamellidens marginalis*. Lead concentration varied from 134.80 to 1.33 µg / g in the bivalve species, it accumulated a high amount of Pb in the post-monsoon period Pb is more toxic and has high accumulating power than Cd and Zn in freshwater gastropod, *Bellamea bengalensis* (Kamble, 2008).

Bioaccumulation of lead is influenced by divalent ions like calcium, zinc, and iron *etc.* among st which calcium is naturally present and it is involved in cell signaling. The sharp increases

Table 1. Bioaccumulation of calcium and lead in the gills of freshwater bivalve, *Lamellidens marginalis.*

		8 Days		16 Days	
S. No.	**Doses**	**Calcium**	**Lead**	**Calcium**	**Lead**
1.	Control	1.038	0.204	1.038	1.038
2.	Pb^{+} 28 ppm	0.770	3.300	0.678	0.678
3.	Ca^{++} 28 ppm	1.321	0.216	1.249	1.249
4.	Ca^{++} 56 ppm	0.938	0.121	0.774	0.774
5.	Ca^{++} 84 ppm	0.861	0.083	2.049	2.049
6.	Pb^{+} + Ca^{++} 28 ppm	0.941	2.938	1.048	1.048
7.	Pb^{+} + Ca^{++} 84 ppm	1.031	1.324	1.321	1.321
8.	Pb^{+} + Ca^{++} 84 ppm	1.031	0.98	1.038	1.038

All values are expressed in mg/ml

Table 2. Bioaccumulation of calcium and lead in mantle of freshwater bivalve, *Lamellidens marginalis*.

		8 Days		16 Days	
S. No.	**Doses**	**Calcium**	**Lead**	**Calcium**	**Lead**
1	Control	0.788	0.158	0.788	0.158
2	Pb^{+} 28 ppm	1.857	2.381	0.498	2.938
3	Ca^{++} 28 ppm	1.311	0.106	2.133	0.090
4	Ca^{++} 56 ppm	1.475	0.079	1.868	0.066
5	Ca^{++} 84 ppm	1.965	0.033	2.252	0.013
6	Pb^{+} + Ca^{++} 28 ppm	1.002	2.302	1.250	1.342
7	Pb^{+} + Ca^{++} 56 ppm	1.170	1.870	2.299	1.225
8	Pb^{+} + Ca^{++} 84 ppm	1.369	1.581	1.314	1.20

All values are expressed in mg/ml

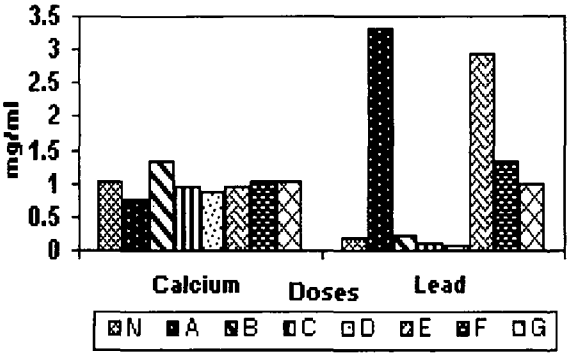

Fig. 1. Bioaccumulation of calcium and lead in gills of *Lamellidens marginalis* after 8 days exposure.

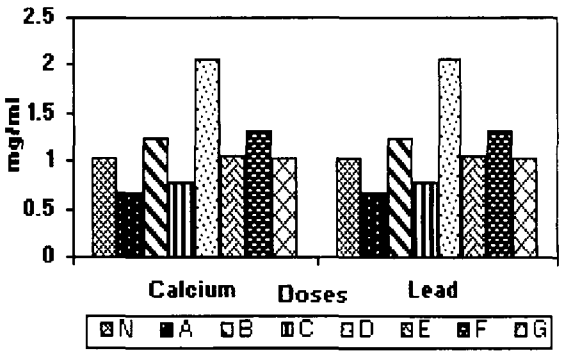

Fig. 2. Bioaccumulation of calcium and lead in gills of *Lamellidens marginalis* after 16 days exposure.

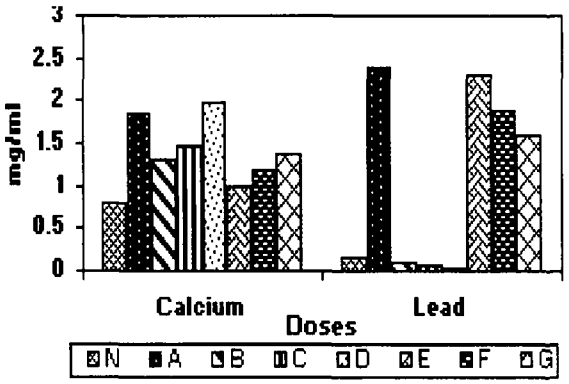

Fig. 3. Bioaccumulation of calcium and lead in mantle of *Lamellidens marginalis* after 8 days exposure.

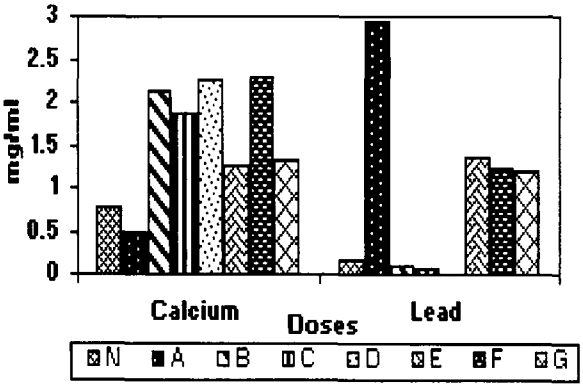

Fig.4. Bioaccumulation of calcium and lead in mantle of *Lamellidens marginalis* after 16 days exposure.

in Ca levels observed in bivalves exposed to heavy metals, indicating an alteration of Ca homeostasis as a result of heavy metal accumulation. A significant increase of total Ca content was also found in the gills of mussels exposed to Cu (Viarengo *et al.*, 1988b) and the digestive gland after exposure to Cu in presence of hydrocarbons (Viarengo *et al.*, 1988a). The pattern of Ca variations in the digestive gland was rather similar to those of the heavy metals. The increase in Ca concentrations in whole organisms exposed to Cu or to Cd, is much larger than those observed in some organs, probably reflect the high content of Ca in the kidney where numerous granules containing Ca phosphate were found to accumulate in renal cells during exposure to metals. The gills of *Donacilla cornea* are used for monitoring heavy metals in seawater; its digestive gland is particularly studied to ree the effect of heavy metals on Ca homeostasis.

Calcium, vitamin C, thiamine and iron *etc.* are used as Pb chelating agents. Ascorbic acid or thiamine greatly enhances the efficacy of calcium versenate in Pb intoxification including mobilization of Pb from central nervous system (Flora *and Tandon* 1991). The administration of ascorbic acid plus Zn or ascorbic acid plus Fe (Suzuki *et al.*, 1979) helped in amelioration of Pb intoxification.

In the present study, it was observed that, freshwater bivalve, *Lamellidens marginalis*, when exposed to chronic concentration of Pb, there was more accumulation of Pb in gills as compared to mantle. This study also suggested that the accumulation of Pb was directly proportional to the exposure time. Because, in 8 days Pb treated group accumulation of Pb in gill was 3.300 mg/ml and after 16 days exposure it was 4.129 mg/ml. It was observed that this accumulation was decreased at 56 and 84 ppm calcium treated groups *i.e.* 1.324 and 0.98 mg/ml for 8 days and 1.401 and 0.986 mg/ml for 16 days. Similarly, in mantle 8 days and 16 days Pb accumulation were 2.381 and 2.938 mg/ml respectively. This accumulation was decreased at 56 and 84 ppm Ca treated groups *i.e.* 1.870 and 1.581mg/ml for 8 days and 1.20 and 1.225 mg/ml for 16 days. It was also observed that bivalves ingesting a low Ca diet had more lead concentrations than bivalves on a normal Ca diet and with increasing calcium doses Pb level was decreased.

The foregoing discussion suggests that the exposure of the freshwater bivalve, *Lamellidens marginalis* to Pb at 1/10th of LC_{50} concentrations as 28 ppm and also its chelating agent Ca at three different concentrations (28 ppm, 56 ppm and 84 ppm) leads to the accumulation of Pb and its detoxification by Ca in the organs like gills and mantle.

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