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Accumulation of Heavy Metals in Freshwater Fish-An Assessment of Toxic Interactions with Calcium

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Abstract: The present investigation deals with the interaction between accumulation of waterborne lead, cadmium and chromium with different calcium concentration (1.0, 2.0, 3.0 and 4.0 mM L⁻¹) in *Cirrhina mrigala* fingerlings in the laboratory. There was no mortality occurred in metal alone and metal treated with calcium. At the beginning there was no significant differences observed between Pb, Cd and Cr alone and combination with calcium in muscle as well as whole body at lower calcium concentration, but with increasing exposure period marked differences were observed. In muscle tissue accumulations was very much lower compared to whole body and significantly lower below human consumption level. In binary mixtures of Pb, Cd and Cr with calcium, the calcium compound was found to consistently reduce the toxic effect as well as accumulation of Pb, Cd and Cr compounds. Increased Ca levels showed lower transfer of Pb, Cd and Cr from water to the gills which resulted slower transfer of metal from the gills to the blood indicated lower accumulation rates in muscle tissue compared to metal without Ca. An increase in calcium concentration of approximately 3 and 4 mM L⁻¹ resulted in a 46 and 54% decrease of Pb uptake, 55 and 58% of Cd and 41 and 53% of Cr uptake in whole fish at 28 days exposure period. There is an inverse relationship between calcium concentration in the water and metal uptake in whole body, muscle as well as gills of the *Cirrhina mrigala*. Calcium has strong antagonistic effect on Pb, Cd and Cr accumulation and toxicity. Predicted and experimental values of Pb, Cd and Cr concentration in fish body treated with calcium verified in terms of root mean square percent deviation and correlation coefficient which exhibit fair agreement.

Key words: Lead, cadmium, chromium, calcium, *Cirrhina mrigala*

Introduction

Diverse industrial wastes have aggravated the problem of water pollution. This problem becomes complex because of the non-degradability of inorganic pollutants like heavy metals. Metals have received attention other non-degradable toxic chemicals because of their adverse effects on aquatic life forms. The presence of heavy metals in ecosystems becomes dangerous for organisms when the concentration rises above the natural background in water, sediment and the food supply. To control water pollution, the problems have to be solved by adopting alternative technologies to chemical-specific tools which suit low capital availability and minimum manpower. It has become increasingly evident that the environmental impact of a particular metal species is dependent on the chemistry of water. Among various chemical parameters namely pH, salinity, organic compounds and complexing agents, calcium concentration play an important role in the interaction between heavy metals and to reduce bioavailability of heavy metals in aquatic organisms. The environmental water has been shown to be the major source of calcium for fish (Mugiaya, 1980). Payan *et al.* (1981) have suggested that the chloride cells of the gills are the primary site of calcium uptake. In sea water, the

uptake of calcium also occurs across the gastro-intestinal tract but at a much slower rate than branchial uptake (Mayer-Gostan *et al.*, 1983). Several studies have shown that increasing water hardness reduces heavy metal toxicity to fish (Waiwood and Beamish, 1978; Bradley and Sprague, 1985; Pascoe *et al.*, 1986). The reduction in heavy metal toxicity with increasing calcium concentration may partly explain that calcium has a positive effect on fish survival and productivity in acidified freshwaters (Brown, 1982). There is a competition between calcium and divalent metal ions for influencing metal uptake and toxicity to fish (Hunn, 1985). Pantani *et al.* (1989) reported accumulation of chromium was greater at low hardness of water where as, Wicklund (1990) investigated that Cd^{2+} enters the gills through calcium channels on the apical side and is further translocated to the circulation interactions with Ca-ATPases. However, reports about the influence of calcium concentration on Pb, Cd and Cr toxicity and accumulation to freshwater fish *Cirrhina mrigala* is not existent. For fish, Gill is the potential sites of absorption of water borne chemicals because fish have to extract oxygen from the medium by passing enormous volumes of water over their gills. So, it is necessary to study the transfer rate of Pb, Cd and Cr from water with different calcium concentration to the gills and also accumulation in muscle tissue because muscle is consumed by people.

Therefore, the aim of the present study was to investigate the toxicity and accumulation of Pb, Cd and Cr in *Cirrhina mrigala* fingerlings at various concentrations of calcium and their interactive effects.

Materials and Methods

Experimental Set-up

Freshwater fish e.g., *Cirrhina mrigala* were acclimatized in the laboratory at Central Institute of Freshwater Aquaculture, Bhubaneswar, India for one month at $27\pm 1^{\circ}C$, which was the temperature of experimental conditions. After the acclimatized period mean length and weight of fish were measured 7.7 ± 0.42 cm and 6.0 ± 0.32 g, respectively. A total of 15 sets of glass jars 100 L capacity of each were maintained and in each jar 15 numbers of fish were released in a dechlorinated tap water.

Case-I: Five glass jars contained 0.15 mg L^{-1} Pb, 0.15 mg L^{-1} Pb + 1 mM L^{-1} Ca, 0.15 mg L^{-1} Pb + 2 mM L^{-1} Ca, 0.15 mg L^{-1} Pb + 3 mM L^{-1} Ca and 0.15 mg L^{-1} Pb + 4 mM L^{-1} Ca.

Case-II: Five glass jars contained 0.05 mg L^{-1} Cd, 0.05 mg L^{-1} Cd + 1 mM L^{-1} Ca, 0.05 mg L^{-1} Cd + 2 mM L^{-1} Ca, 0.05 mg L^{-1} Cd + 3 mM L^{-1} Ca and 0.05 mg L^{-1} Cd + 4 mM L^{-1} Ca.

Case-III: Five glass jars contained 0.2 mg L^{-1} Cr, 0.2 mg L^{-1} Cr + 1 mM L^{-1} Ca, 0.2 mg L^{-1} Cr + 2 mM L^{-1} Ca, 0.2 mg L^{-1} Cr + 3 mM L^{-1} Ca and 0.2 mg L^{-1} Cr + 4 mM L^{-1} Ca.

All chemicals used for sample preparation were in analytical grade. Stock solution of 1000 mg L^{-1} of K_2CrO_4 was prepared in deionized water. The chromate solution was acidified with H_2SO_4 (pH=3.5) to maintain a relatively stable Cr (VI) species. High purity chloride and nitrate salts of Cd and Pb were made from pure metals by dissolving them, respectively in 1:1 (v: v) HCl (12 M) and 1:1 (v: v) HNO_3 (16 M), followed by dilution to 1000 mg L^{-1} in deionized water. Calcium stock solution of 500 mM L^{-1} was prepared from analytical grade $CaSO_4$.

Experimental Procedure

The experiment was continued for 28 days. During exposure period chemical parameters of water such as pH, total alkalinity, dissolved oxygen were analysed (APHA, 1992). The water used for the

experiments such as pH, DO, total alkalinity and calcium were in the range of 7.7 ± 0.40 , $5.4 \pm 0.32 \text{ mg L}^{-1}$, $114 \pm 5.4 \text{ CaCO}_3 \text{ mg L}^{-1}$ and $0.45 \pm 0.06 \text{ mg L}^{-1}$, respectively. Calcium concentration in the water was analysed in Flame photometer. Fish samples were collected from each respective jar at 7, 14, 21 and 28 days period for accumulation studies. Fish samples were dissected to remove muscle and gills.

Analysis of Total Metal Contents in Fish

Dried fish samples were transferred to porcelain basin and put to a Heraeus 'Thermicon P' muffle furnace at a temperature of about 550°C for 4 to 5 h. When all the carbon was destroyed, it was taken to 125 mL Erlenmeyer flask. Samples were digested with tri-acid mixture ($\text{HNO}_3:\text{HClO}_4:\text{H}_2\text{SO}_4 = 10:4:1$) at a rate of 5 mL per 0.5 g of sample and was placed on hot plate at 100°C temperature. Digestion was continued until the liquor was clear (AOAC, 1990). All the digested liquors were filtered through Whatmann 42 filter paper and diluted to 25 mL with distilled water.

Measurement of Heavy Metals by Atomic Absorption Spectrophotometer

All heavy metals were measured with a Perkin-Elmer Atomic Absorption Spectrophotometer (Model No. 3110) by specific cathode lamp using wavelength and potential detection limit for respective heavy metals as follows:

Heavy metals	Wavelength (nm)	Detection limit ($\mu\text{g g}^{-1}$)
Pb	283.3	1.0
Cd	228.8	0.08
Cr	357.9	1.5

Heavy metal concentration was calculated as follows:

$$\mu\text{g/g in metal in tissue material} = \frac{\mu\text{g g}^{-1} \text{ of metal in solution} \times \text{volume of acid}}{\text{g of sample}} \quad (1)$$

Concentration factors (C_f) were used as a measure of the metal availability in the water to fish. Concentration factor ($\mu\text{L g}^{-1}$ wet wt.) was calculated as

$$C_f = C_o / C_w \quad (2)$$

Where C_o = metal concentration ($\mu\text{g g}^{-1}$ dry wt.) in the organ and C_w = water metal concentration ($\mu\text{g } \mu\text{L}^{-1}$). Metal concentrations in gills are expressed on a dry weight basis and can be transformed to wet weight concentration by multiplying by 0.19.

Statistical Analysis

ANOVA and Duncan's Multiple Range Test

Data were tested by regression analysis for gills tissue concentration factor. Data was analyzed in terms of metal accumulation in fish between metal alone and metal + calcium treatment using one way analysis of variance (ANOVA) and multiple comparisons were made with Duncan's Multiple Range test. Differences were considered at ($p < 0.01$) levels.

Coefficient of Correlation (r)

When predicted values are validated with the experimental data then correlation between predicted and experimental values is presented with a coefficient known as coefficient of correlation. The coefficient of correlation can be evaluated with the following expression (Chapra and Canale, 1989).

$$r = \frac{N \sum x_i y_i - \sum(x_i) \sum(y_i)}{\sqrt{N \sum x_i^2 - (\sum x_i)^2} \sqrt{N \sum y_i^2 - (\sum y_i)^2}} \quad (3)$$

Root Mean Square of per Cent Deviation (e).

The prediction is done with the help of experimental values. The predicted values are validated with experimental data. The closeness of predicted values and experimental data can be presented in terms of root mean square of per cent deviation. The expression used for this purpose is as follows (Chapra and Canale, 1989).

$$e = \sqrt{\frac{\sum(e_i)^2}{n}} \quad \text{where} \quad e_i = \frac{X_{\text{pred}} - Y_{\text{exp}}}{X_{\text{pred}}} \quad (4)$$

Results

The water quality parameters namely, pH, total alkalinity, dissolved oxygen and calcium concentration are given in Table 1. Lead+calcium and cadmium+calcium did not show any marked differences with metal alone whereas Cr with calcium showed slight differences with Cr alone treatment in pH and total alkalinity. The accumulation of Pb, Cd and Cr concentration in whole body and muscle tissue exposed to metal alone and metal + calcium mixture are presented in Table 2-4, respectively. The lead, cadmium and chromium uptake in the whole body and muscle decreased with increasing Ca concentration. There was no mortality occurred in Pb, Cd and Cr single exposed and with calcium. Lead, cadmium and chromium accumulates in whole fish exposed to Pb+Ca, Cd+Ca and Cr+Ca for 14 days as well as 28 days were significantly lower ($p < 0.01$) when compared with those exposed to Pb, Cd and Cr alone. At lower (1 mM L^{-1}) Ca concentration, there was no significant differences of Pb, Cd and Cr accumulation between Pb, Cd and Cr alone and combination with Ca at 14 days but with increasing exposure period accumulation consistently decreases (Table 2- 4). With increasing calcium concentration Pb, Cd and Cr accumulation reduces with exposure days. An increase in Ca concentration of approximately 3 and 4 mM L^{-1} resulted in a 46 and 54% decrease of Pb, 55 and 58% of Cd and 41 and 53% of Cr uptake in whole body at 28 days exposure period, respectively. There were marked significant differences observed in Pb and Cr uptake in whole body at 3 and 4 mM L^{-1} Ca exposed but in Cd uptake difference was not significant. For Cd, higher elimination occurred compared to Pb and Cr at 3 and 4 mM L^{-1} Ca. Muscle tissues showed 4.9 and 4% accumulation of Pb, 6.2 and 5.4% of Cd and 6.9 and 6.6% of Cr of whole body uptake at 28 days. Lead accumulation in muscle tissue did not show significant differences in 1 and 2 mM L^{-1} Ca with Pb alone while in Cd and Cr consistent differences were there with their respective metal concentration. Muscle tissue resulted significantly lower accumulation compared to whole body uptake. With increase in Ca concentration of approximately 3 and 4 mM L^{-1} showed higher decrease of Pb, Cd and Cr accumulation compared to whole body uptake. The metal transfer or concentration factor of Pb, Cd and Cr from water to the gills also showed an inverse relationship between metal uptake and Ca concentration (Fig.1 a-c). The greatest effect of Ca on Pb, Cd and Cr uptake was found between metal alone and 3 and 4 mM L^{-1} Ca group (Fig. 1 a-c). Figure 2a shows the variations of predicted and experimental values of Pb concentration in fish body treated with calcium. The correlation of coefficient and root mean square percent deviation were found 0.99 and 11.88% and showed fair agreement.

Table 1: Water quality parameters of experimental water exposed with Pb, Cd and Cr with and without calcium. Mean values are given

Metals	pH	Dissolved oxygen (mg L ⁻¹)	Total alkalinity (mg L ⁻¹)	Calcium (mM L ⁻¹)
Pb	7.7	5.6	120	0.45
Pb+ 1 mM L ⁻¹ Ca	7.7	5.6	120	1.0
Pb+ 2 mM L ⁻¹ Ca	7.8	5.7	122	2.0
Pb+ 3 mM L ⁻¹ Ca	7.8	5.6	123	3.0
Pb+ 4 mM L ⁻¹ Ca	7.9	5.5	124	4.0
Cd	7.8	5.6	122	0.45
Cd+ 1 mM L ⁻¹ Ca	7.8	5.6	122	1.0
Cd+ 2 mM L ⁻¹ Ca	7.9	5.5	123	2.0
Cd+ 3 mM L ⁻¹ Ca	7.9	5.4	124	3.0
Cd+ 4 mM L ⁻¹ Ca	7.9	5.6	124	4.0
Cr	7.9	5.4	126	0.45
Cr+ 1 mM L ⁻¹ Ca	8.0	5.5	128	1.0
Cr+ 2 mM L ⁻¹ Ca	8.1	5.6	132	2.0
Cr+ 3 mM L ⁻¹ Ca	8.2	5.6	134	3.0
Cr+ 4 mM L ⁻¹ Ca	8.2	5.5	136	4.0

Concentration of Pb= 0.15 mg L⁻¹; Cd= 0.05 mg L⁻¹ and Cr = 0.20 mg L⁻¹ in individual as well as with Ca treatment

Table 2: Variation of accumulation of lead in muscle and whole body to *Cirrhina mrigala* fingerlings exposed to Pb alone and Pb+ Ca mixture

Concentration	Accumulatin (µg g ⁻¹ dry wt)				Interactive effects
	14 days		28 days		
	Muscle	Whole body	Muscle	Whole body	
0.15 mg L ⁻¹ Pb	0.22±0.08 ^a	3.18±0.11 ^a	0.30±0.08 ^a	3.82±0.15 ^a	-
0.15 mg L ⁻¹ Pb+1.0 mM L ⁻¹ Ca	0.20±0.11 ^a	3.09±0.12 ^a	0.18±0.09 ^a	2.86±0.14 ^a	ant
0.15 mg L ⁻¹ Pb+2.0 mM L ⁻¹ Ca	0.17±0.07 ^a	2.42±0.16 ^b	0.14±0.09 ^a	2.24±0.18 ^b	ant
0.15 mg L ⁻¹ Pb+3.0 mM L ⁻¹ Ca	0.12±0.06 ^b	1.84±0.14 ^c	0.08±0.07 ^b	1.63±0.23 ^c	ant
0.15 mg L ⁻¹ Pb+4.0 mM L ⁻¹ Ca	0.09±0.04 ^b	1.43±0.09 ^d	0.06±0.04 ^b	1.26±0.17 ^d	ant

Different letters show significant differences between different treatments within specific time period. Data shown with different letters are statistically significant at (p<0.01) level. Values are mean±SD Ant- antagonistic effect

Table 3: Variation of accumulation of cadmium in muscle and whole body to *Cirrhina mrigala* fingerlings exposed to Cd alone and Cd+ Ca mixture

Concentration	Accumulatin (µg g ⁻¹ dry wt)				Interactive effects
	14 days		28 days		
	Muscle	Whole body	Muscle	Whole body	
0.05 mg L ⁻¹ Cd	0.26±0.05 ^a	3.65±0.16 ^a	0.39±0.12 ^d	4.22±0.13 ^d	-
0.05 mg L ⁻¹ Cd+1.0 mM L ⁻¹ Ca	0.25±0.06 ^b	3.57±0.22 ^a	0.20±0.09 ^a	3.42±0.18 ^a	ant
0.05 mg L ⁻¹ Cd+2.0 mM L ⁻¹ Ca	0.20±0.04 ^a	3.18±0.15 ^b	0.16±0.05 ^b	2.94±0.12 ^b	ant
0.05 mg L ⁻¹ Cd+3.0 mM L ⁻¹ Ca	0.13±0.04 ^c	1.74±0.12 ^c	0.10±0.07 ^c	1.58±0.16 ^c	ant
0.05 mg L ⁻¹ Cd+4.0 mM L ⁻¹ Ca	0.12±0.03 ^c	1.63±0.08 ^c	0.08±0.04 ^c	1.46±0.21 ^c	ant

Different letters show significant differences between different treatments within specific time period. Data shown with different letters are statistically significant at (p<0.01) level. Values are mean±SD Ant- antagonistic effect

Table 4: Variation of accumulation of chromium in muscle and whole body to *Cirrhina mrigala* fingerlings exposed to Cr alone and Cr+ Ca mixture

Concentration	Accumulatin (µg g ⁻¹ dry wt)				Interactive effects
	14 days		28 days		
	Muscle	Whole body	Muscle	Whole body	
0.20 mg L ⁻¹ Cr	0.34±0.13 ^a	4.26±0.15 ^a	0.52±0.12 ^d	4.58±0.26 ^d	-
0.20 mg L ⁻¹ Cr+1.0 mM L ⁻¹ Ca	0.31±0.12 ^a	4.17±0.18 ^a	0.29±0.09 ^a	4.02±0.14 ^a	ant
0.20 mg L ⁻¹ Cr+2.0 mM L ⁻¹ Ca	0.26±0.09 ^b	3.53±0.12 ^b	0.24±0.11 ^b	3.24±0.15 ^b	ant
0.20 mg L ⁻¹ Cr+3.0 mM L ⁻¹ Ca	0.19±0.06 ^c	2.68±0.16 ^c	0.17±0.07 ^c	2.46±0.12 ^c	ant
0.20 mg L ⁻¹ Cr+4.0 mM L ⁻¹ Ca	0.15±0.04 ^c	2.16±0.11 ^d	0.13±0.04 ^c	1.93±0.17 ^d	ant

Different letters show significant differences between different treatments within specific time period. Data shown with different letters are statistically significant at (p<0.01) level. Values are mean±SD Ant- antagonistic effect

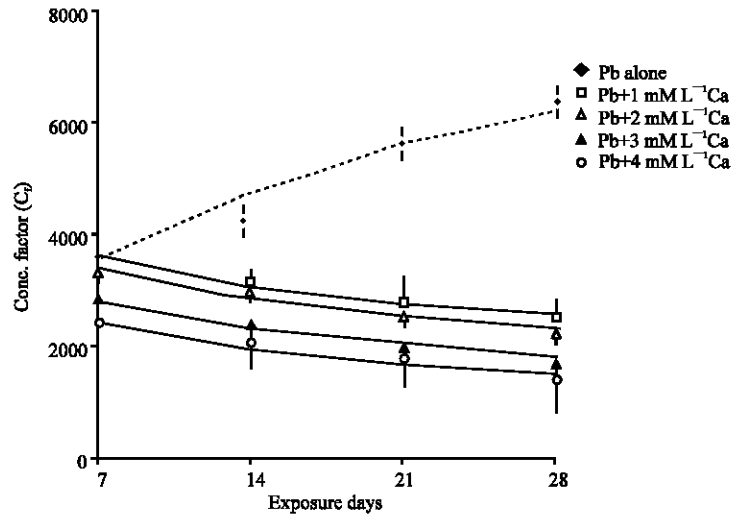


Fig 1a: Lead concentration factor (C_f , $\mu\text{L g}^{-1}$ wet wt., mean \pm SE) in gill tissue of *Cirrhina mrigala* against time exposed to lead alone and lead with four different calcium concentrations. Regression curves were fitted to all experimental C_f values

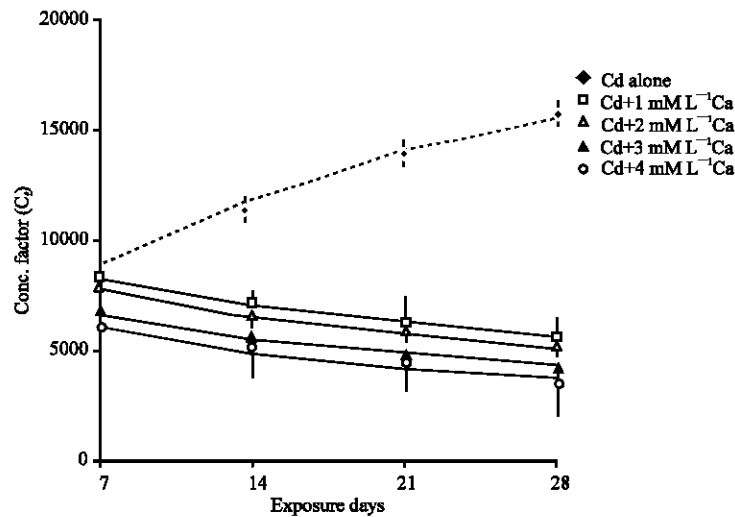


Fig. 1b: Cadmium concentration factor (C_f , $\mu\text{L g}^{-1}$ wet wt., mean \pm SE) in gill tissue of *Cirrhina mrigala* against time exposed to cadmium alone and cadmium with four different calcium concentrations. Regression curves were fitted to all experimental C_f values

Predicted and experimental values of Cd concentration in fish for different Cd+Ca mixture is presented in Fig 2b and verified in terms of correlation of coefficient ($r=0.99$) and root mean square percent deviation ($e=7.71$).

The effect of calcium on Cr concentration in fish was compared with the predicted and experimental values of Cr concentration in Cr+Ca mixture. The predicted values was highly correlated with the experimental values with $r = 0.99$ and $e = 1.14\%$, respectively (Fig 2c).

Discussion

The present study shows that there is an inverse relationship between calcium concentration in the water and lead, cadmium and chromium uptake in whole body,

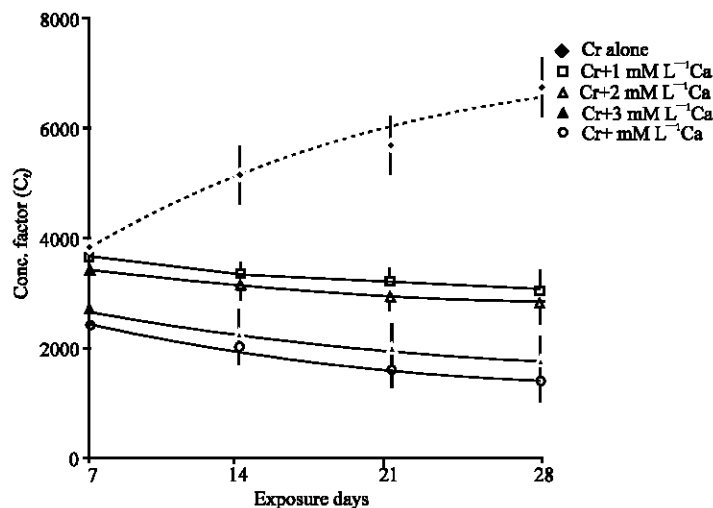


Fig. 1c: Chromium concentration factor (C_b , $\mu\text{L g}^{-1}$ wet wt., mean \pm SE) in gill tissue of *Cirrhina mrigala* against time exposed to chromium alone and chromium with four different calcium concentrations. Regression curves were fitted to all experimental C_b values

muscle and gills of *Cirrhina mrigala*. A similar findings have been found on whole body uptake of Cd (Wright *et al.*, 1985) and on gill uptake of lead (Varanasi and Gmur, 1978).

At higher calcium concentration, i.e., at above 3 mM L^{-1} least accumulation occurred due to $\text{Ca}^{2+}/\text{Pb}^{2+}$ concentration ratio which lead to decrease Pb^{2+} uptake through Ca^{2+} channel. Similar findings were observed by Bell (1976) and Varanasi and Gmur (1978) reported that the rate of waterborne Pb uptake decreased with increasing the hardness of water.

In this study, an antagonistic effect of calcium on Pb accumulation was determined in the *Cirrhina mrigala*, in other words, on the protective effect of calcium against toxicity and accumulation of Pb.

Significant lower accumulation occurred at 3 mM L^{-1} Ca and higher because $\text{Ca}^{2+}/\text{Cd}^{2+}$ concentration ratio increases which led to decrease Cd^{2+} uptake i.e. apparently block or minimize the effects of Cd at the sites of toxic action. Cadmium, a divalent cation, would have chemical activity and ionic form similar to the calcium ion. The present study correlates with Pagenkopf (1983) who reported that the reduced $\text{Ca}^{2+}/\text{Cd}^{2+}$ concentration ratio led to increased Cd uptake through a Ca^{2+} selective channel. Ionic radii of Ca^{2+} and Cd^{2+} almost same so, there is a competition between these two metals for binding sites on the gill surface. Similar findings were made by Khan *et al.* (1984) and Sauer and Watabe (1988) and reported Ca and Cd have almost same pattern of deposition in the cell walls. A similar trend has been found on whole body uptake of Cd in calcium enriched waters is attributable to the increased in ionic strength by reducing Cd^{2+} activity (Part *et al.*, 1985). Pascoe *et al.* (1986) concluded that Cd was less toxic to rainbow trout, *Salmo gairdneri*, in hard water than in soft water, but this effect could not be explained by a decreased whole body Cd uptake in hard water. Part (1984) found that the Cd transfer, from water to blood-side, through perfused rainbow trout gills decreased as the Ca^{2+} concentration in the water increased. Increased Ca levels resulted in a slower transfer of Cd from the gills to the blood and slower accumulation rates in liver and kidney.

At lower calcium concentration more Cr was dissolved in soluble form i.e. in ionic form. But at 4 mM L^{-1} Ca and higher concentration Cr accumulation was significantly ($p < 0.05$) lower than Cr alone treatment. At higher calcium concentration, lower accumulation caused due to increase of $\text{Ca}^{2+}/\text{Cr}^{6+}$ uptake through a calcium channel i.e., minimize the effects of Cr at the sites of toxic action.

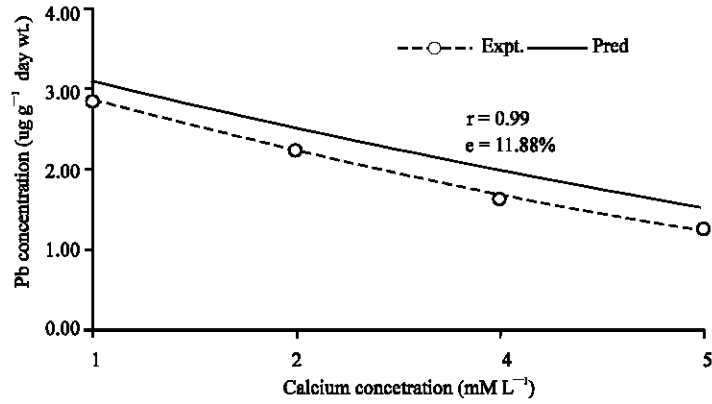


Fig. 2a: Predicted and experimental values of lead concentration in whole body of *Cirrhina mrigala* at various calcium treatments for 28 days period

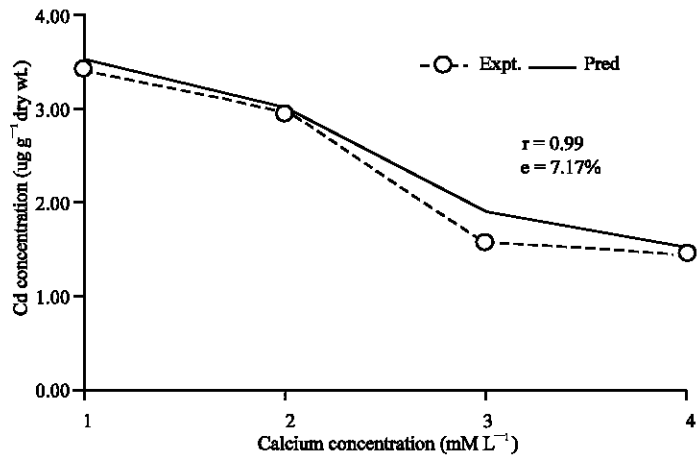


Fig. 2b: Predicted and experimental values of cadmium concentration in whole body of *Cirrhina mrigala* at various calcium treatments for 28 days period

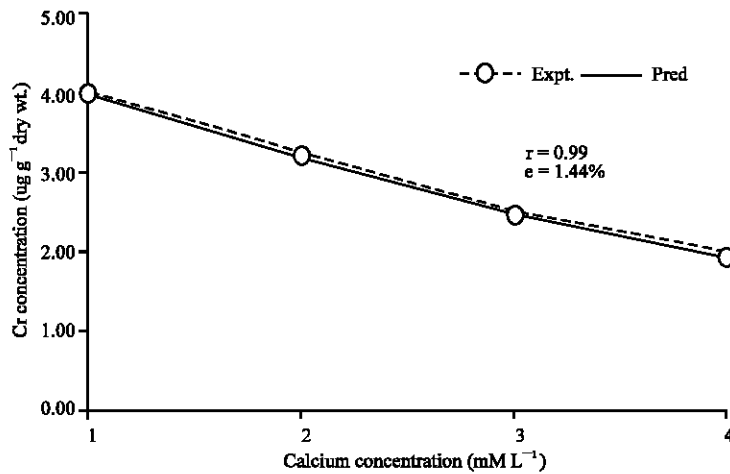


Fig. 2c: Predicted and experimental values of chromium concentration in whole body of *Cirrhina mrigala* at various calcium treatments for 28 days period

Similar findings was observed by Metelev *et al.* (1983) and reported that increased water Ca were antagonistic to Cr toxicity and precipitated metals as insoluble less toxic hydrates. Joshi and Patil (1992) revealed that low hardness enhanced the toxicity of Cr indicating synergistic effects whereas high hardness reduced the toxicity of Cr to a considerable extent showing antagonistic effect. It is to be expressed that the Ca has potential effect on Pb, Cd and Cr uptake and transfer could partly be a result of a concurrence between the ions for Ca uptake and transporting mechanisms in the fish gills. The site of Ca uptake in the gills of freshwater fish is probably in the chloride cell (Payan *et al.*, 1981; Flick *et al.*, 1985). Therefore, the interactive effects of Ca on Pb, Cd and Cr accumulation is antagonistic in nature. The presence of calcium reduces the uptake as well as accumulation of Pb, Cd and Cr in fish.

The toxic effects and accumulation of different combinations of various metals on organisms depend not only on the metals of mixture but also on the organism's uptake capacity, exposure dose, exposure period, metabolic activity and age of fish (Heath, 1987; Goyer, 1991). In the present study, all the three metals showed a similar trend of reduction in accumulation in fish body treated with different calcium concentration.

It is clear from the present study that toxicity of metals is affected by calcium which reduces the toxic effect of a metal through competitive inhibition at the gill surface. The non toxic calcium ion competes with the toxic metals for the same binding sites. If calcium occupies the sites, the lamellae are protected from deterioration. Calcium afforded protection by reducing ion loss and thereby reduced fish mortality.

Conclusions

From the present study it is evident that calcium has an antagonistic effect on Pb, Cd and Cr accumulation in *Cirrhina mrigala* compared to metals alone. Calcium causes a decreased uptake of the metal in gills and muscle tissue indirectly in whole fish as the Ca concentration in the water increases. Hence, this study concludes that at and above 3 mM L⁻¹ calcium concentration can effectively remove Pb, Cd and Cr from metal contaminated water and improve the physiological functions/activities of fish during 28 days exposure period. Though 28 days is not sufficient for complete removal of these metals from metal contaminated medium and fish body. Further, it requires longer duration.

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