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Research Note

Effect of Calcium Hardness on Toxicity and Accumulation of Water-borne Lead, Cadmium and Chromium to *Labeo rohita* (Hamilton)

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Abstract: Effect of calcium hardness (120, 250, 350 and 750 mg/L) on the toxicity and accumulation of lead, cadmium and chromium to *Labeo rohita* (Hamilton) was investigated in the laboratory. For lead treatment, no mortality occurred, whereas 50% and 20% mortality occurred at 120 and 250 mg/L calcium hardness, respectively for cadmium treatment. In chromium treatment, 10% mortality was recorded at 120 mg/L calcium hardness. The minimum accumulation of Pb, Cd and Cr in whole fish occurred at 350 mg/L hardness as compared to other treatments.

Key words: Lead, cadmium, chromium, *Labeo rohita*, accumulation, calcium hardness.

Introduction

Water hardness influences the toxicity of heavy metals by forming insoluble carbonates or by absorption on calcium carbonate (Dodge and Theis, 1979; Block, 1991). Calcium and magnesium ions compete with heavy metal ions for active sites in fish tissues and this may affect the toxicity of heavy metals (Part, 1983). Water hardness affects the gill permeability to water and ions such that the harder the ambient water, the less permeable is the tissue (McWilliams and Potts, 1978). The calcium ion, which is the major cation responsible for hardness, also causes the electrical charge on the outside of the gills to be more positive. Several studies have shown that increasing water hardness reduces heavy metal toxicity to fish (Bradley and Sprague, 1985; Pascoe et al., 1986). However, reports about the influence of calcium hardness on Pb, Cd and Cr toxicity to Indian major carps is non-existent. So, the aim of this present study was to investigate the toxicity and accumulation of Pb, Cd and Cr in fingerlings of *Labeo rohita* (Hamilton) acclimatized to different calcium levels.

Material and Methods

Four hundred fingerlings of Indian major carps, *Labeo rohita* were collected from the institute's farm. The fingerlings had an average weight 5.0±0.5 g and average length of 6.0±0.4 cm of the same stock. They were brought to the laboratory in buckets of well-oxygenated water and stocked in five tanks of 500 L of well-oxygenated water. The fingerlings were acclimatized for two weeks in the laboratory conditions (dissolved oxygen 5.8±0.5 mg/L, temperature 27±2°C, pH 6.9±0.2, electrical conductivity 0.42±0.08 dS/m, total alkalinity 130±8 mg/L as $CaCO_3$ and water hardness 120±7 mg/L as $CaCO_3$). During acclimatization water was changed on alternate days and the fish were provided pelleted feed of 2% body weight per day. Stock solutions of lead, cadmium and chromium were prepared from their respective reagent grade salts of $Pb(NO_3)_2$, $CdCl_2.H_2O$ and $K_2Cr_2O_7$ in deionised water for performing the

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experiments. One thousand ppm stock solution of calcium was prepared from calcium chloride (analytical grade, BDH). Dechlorinated tap water was used in the experiments as its original calcium hardness was 120 mg/L and other higher doses viz. 250, 350 and 750 mg/L were maintained by adding calculated amount from 1000 mg/L calcium stock solution. *Labeo rohita* fingerlings were acclimated for one week to the different calcium hardness levels. The experiment was conducted in rectangular glass jars of maximum capacity of 40 L. In each glass jar, ten fish were added and for each treatment, three replications were maintained. Control group was maintained side by side. For the present study, the concentrations of lead, cadmium and chromium were 1/10th of 96h LC_{50} values i.e., 1.0, 0.5 and 1.5 mg/L, respectively.

During exposure period, the same commercial feed was provided to the fish in each jar. Faeces and uneaten food were siphoned off the bottom of the jar at regular interval. Water and exposure solutions were renewed every 96h of the exposure to reduce the build up of metabolites. Every 24h interval, hardness was checked by EDTA titration method. Every 96h interval, Pb, Cd and Cr concentration were measured by Atomic Absorption Spectrophotometer. During exposure period, chemical parameters of water such as pH, alkalinity, dissolved oxygen and ammonia concentration were analysed using common methods. The ammonia concentrations remained low (< 0.05 mg/L NH_4^+); dissolved oxygen was always above 90% saturation. Experiment was continued upto 14 days. Fish mortality percentage was observed during 14 days exposure period. For accumulation studies, fish samples were collected at seven days as well as 14 days exposure period from all the respective jars.

Dried fish samples were ashed in muffle furnace at 550°C, then digested with triacid mixture (HNO_3 : $HClO_4$: H_2SO_4 = 10:4:1) on a hot plate at 100°C till the liquor was clear. All the digested liquors were filtered through Whatman 42 filter paper and diluted to 25 ml with distilled water for metal analysis using a Perkin-Elmer Atomic Absorption Spectrophotometer (Model No. 1025) by specific cathode lamp. The wavelength used for lead, cadmium and chromium were 283.3, 228.8 and 357.9 nm, respectively while the detection limit for the same were 1.00, 0.08 and 1.50 $\mu g g^{-1}$, respectively. The test of significance was analysed among the accumulation of a metal at various calcium hardness levels by Duncan's Multiple Range Test (DMRT).

Results

There were no mortality occurred in control group (without heavy metals) at four different calcium hardness and no heavy metals were detected in fish tissues during 14 days period. In lead treatment, there were no mortality occurred at four different hardness during 14 days period. There were only significant ($p<0.05$) differences observed between 120 and 250 mg/L calcium hardness but between 350 and 750 mg/L calcium hardness no significant differences were observed with respect to lead accumulation in fish (Table 1). For cadmium treated fish, 50% and 80% survivability were observed at 120 and 250 mg/L calcium hardness, respectively whereas 100% survivability was recorded at and above 350 mg/L hardness. There were significant ($p<0.05$) differences among four different hardness during 14 days period (Table 2) with respect to accumulation and hardness. For chromium treatment, 90% survivability was there at 120 mg/L hardness and 100% survivability was observed at and above 250 mg/L hardness. Significant ($p<0.05$) differences were observed among 250, 350 and 750 mg/L calcium hardness compared to 120 mg/L calcium hardness but among 250, 350 and 750 mg/L calcium

Table 1: Mean survival, accumulation and water quality data for *Labeo rohita* fingerlings exposed to lead at different calcium hardness

Calcium hardness (mg/L)	*Total alkalinity (as $CaCO_3$) mg/L*	*pH*	*Dissolved oxygen (mg/L)*	*Mean survival (%)*		*Mean accumulation in whole body[a] (µg/g dry wt)*	
				7 days	*14 days*	*7 days*	*14 days*
120	165	7.8	5.8	100	100	2.64^x	5.05^x
250	170	7.9	5.7	100	100	2.07^x	3.79^x
350	170	7.9	5.6	100	100	0.62^y	1.37^y
750	165	8.0	5.8	100	100	1.08^y	1.97^y

[a]Values in a column followed by the same superscript were not significantly different at the 0.05 level.

Table 2: Mean survival, accumulation and water quality data for *Labeo rohita* fingerlings exposed to cadmium at different calcium hardness

Calcium hardness (mg/L)	*Total alkalinity (as $CaCO_3$) mg/L*	*pH*	*Dissolved oxygen (mg/L)*	*Mean survival (%)*		*Mean accumulation in whole body[a] (µg/g dry wt)*	
				7 days	*14 days*	*7 days*	*14 days*
120	160	7.9	5.5	70	50	3.87^{x}	5.03^{x}
250	160	7.9	5.4	90	80	3.04^{x}	3.82^{x}
350	162	7.9	5.4	100	100	0.46^{y}	0.57^{y}
750	155	7.9	5.6	100	100	0.71^{y}	1.02^{y}

[a]Values in a column followed by the same superscript were not significantly different at the 0.05 level.

Table 3: Mean survival, accumulation and water quality data for *Labeo rohita* fingerlings exposed to chromium at different calcium hardness

Calcium hardness (mg/L)	*Total alkalinity (as $CaCO_3$) mg/L*	*pH*	*Dissolved oxygen (mg/L)*	*Mean survival (%)*		*Mean accumulation in whole body[a] (µg/g dry wt)*	
				7 days	*14 days*	*7 days*	*14 days*
120	145	8.3	5.5	90	90	4.44^{x}	8.05^{x}
250	145	8.25	5.3	100	100	1.75^{y}	3.12^{y}
350	143	8.28	5.5	100	100	1.36^{y}	1.83^{y}
750	146	8.3	5.4	100	100	1.63^{y}	2.68^{y}

[a]Values in a column followed by the same superscript were not significantly different at the 0.05 level.

hardness, there were no significant differences observed during experimental period with respect to chromium accumulation (Table 3).

Discussion

The present study demonstrated that there was an inverse relationship between calcium hardness of water and heavy metals (Pb, Cd and Cr) accumulation in whole body of rohu fingerlings. The present data indicated that lead accumulation was minimum at 350 mg/L calcium hardness in the fish and this may be due to Ca^{2+}/Pb^{2+} concentration ratio which led to decreased Pb^{2+} uptake through a Ca^{2+} channel. Similar observations were made by Bell (1976) and Varanasi and Gmur (1978) who reported the reduction of the toxicity of lead ion by increasing water hardness.

For Cd treatment, the data showed that a calcium hardness above 250 mg/L would reduce toxicity and also protect fish mortality. The minimum accumulation of Cd in the fish at 350 mg/L hardness may be due to block or minimize the effects of cadmium at the sites of toxic action. Cadmium, a divalent cation, would have chemical activity and ionic form similar to the calcium ion. Competition between Ca^{2+} and Cd^{2+} for binding sites on the gill surface would affect the uptake. The greater toxicity of Cd in soft water than in hard water (Pickering and Henderson, 1965) was probably due to a greater solubility of Cd in low hardness water. Part (1983) 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.

At lower hardness, the accumulation of more Cr was may be due to its higher availability. At hardness 350 mg/L, least Cr was accumulated and this was possibly due to the precipitation of chromium. Similar findings were also observed by Metelev et al. (1983) and Joshi and Patil (1992) who reported that increased Ca in water was antagonistic to Cr toxicity and precipitated metals as insoluble less toxic hydrates while low hardness enhanced the toxicity of Cr indicating synergistic effects. The accumulation of Cr has also been found to be greater at low hardness (EIFAC, 1983).Thus it is clear from the present investigation that toxicity of metals is affected by hardness (Ca) which reduces the toxic effect of a metal through competitive inhibition at the gill surface. The non-toxic Ca^{2+} ion competes with the toxic metals for the same binding sites. If Ca occupies the sites, the

lamellae are protected from deterioration. Calcium (Ca^{2+}) afforded protection by reducing ion loss and thereby reduced fish mortality.

Conclusion

The present study demonstrates that an optimum level of calcium hardness of 350 mg/L $CaCO_3$ could effectively reduce lead, cadmium and chromium accumulation in fish to a considerable extent.

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