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Combined effects of water pH and alkalinity on the accumulation of lead, cadmium and chromium to *Labeo rohita* (Hamilton)

[1*]S. Adhikari, [1]L. Ghosh and [2]S. Ayyappan

[1,2]Central Institute of Freshwater Aquaculture, P.O. Kausalyaganga, Bhubaneswar, India
[2]Indian Council of Agricultural Research, Krishi Anusandhan Bhawan,
Pusa, New Delhi, India

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ABSTRACT: Influence of pH (5.5, 7.0 and 8.5) and alkalinity (40 to 200 mg/L as $CaCO_3$) on the accumulation of $Pb(NO_3)_2$, $CdCl_2$, H_2O and $K_2Cr_2O_7$ to *Labeo rohita* (Hamilton) was investigated in the laboratory. Highest accumulation of Pb and Cr in whole fish occurred at pH 5.5 and at alkalinity level of 40 to 46 mg/L as $CaCO_3$ compared to 7.0 and 8.5. In case of Cd maximum accumulation occurred at pH 7.0 and at alkalinity of 100 mg/L as $CaCO_3$ than that of pH 5.5 and 8.5 and alkalinity 42 and 156 mg/L as $CaCO_3$. Maximum accumulation of lead and chromium occurred at total alkalinity level of 40 and 46 mg/L as $CaCO_3$, respectively while maximum accumulation of cadmium occurred at an alkalinity level of 200 mg/L as $CaCO_3$. A significant ($p<0.05$) linear relationship was demonstrated between increasing pH/ alkalinity and decreasing accumulation in Pb and Cr treatment at all exposure period while for Cd there was no significant linear relationship established.

Key words: *Lead, cadmium, chromium, Labeo rohita, accumulation, pH, alkalinity*

INTRODUCTION

In spite of a good progress made in environmental waste management, heavy metals still pose immense health hazards to aquatic organisms. Unlike other classes of pollutants, which can be biodegraded and destroyed completely, metals are non biodegradable (Wepener *et al.*, 2001) and can neither be created nor destroyed. However, these metals might be altered into more toxic forms or complexed to more stable and less toxic compounds (Viljoen, 1999). In an aquatic environment metal toxicity can be influenced by various abiotic environmental factors such as oxygen, calcium/ water hardness (Skidmore, 1964; Cairns and Mount, 1990 and Ghillebaert *et al.*, 1995), pH, and temperature (Cairns and Mount, 1990 and Kotze *et al.*, 1999). Other factors that can play a role are organic matter (Ghillebaert *et al.*, 1995), carbon dioxide (Skidmore, 1964), metabolic activity, biological half-life of the metal (Kargin and Cogun, 1999), suspended total organic carbon (TOC) (Cairns and Mount, 1990), interactions between pollutants, developmental stage of the organisms, and introspecific variations in susceptibility to metals (Hellawell, 1986; seymore, 1994 and Nussey, 1998). These factors determine metal chemical speciation and eventually the bioavailability to aquatic organisms (Abel, 1989; Welsh *et al.*, 1993; Seymore, 1994 and Wade *et al.*, 1995), having direct and simultaneous and/or interactive effects on living organisms (Ghillebaert *et al.*, 1995). Metals are naturally found in aquatic ecosystems by a wide range of natural and anthropogenic sources (Wepener *et al.*, 2001) and with anthropogenic being either domestic or industrial (Biney *et al.*, 1994). Some are essential to living organisms in trace amounts (for example copper and zinc) while others (for example lead, cadmium and chromium) have no significant biological roles (Seymore, 1994). The availability and toxicity of these metals are immensely influenced by the physicochemical factors of the specific environment. For example, the bioavailability of lead was strongly enhanced at decreasing water pH (Brown, 1979), increasing the toxicity of lead for fish (Stouthart *et al.*, 1994). Whitley (1968) also demonstrated that lead was more toxic at pH 8.6 than at pH 6.5 in tubificid worms. Lead toxicity to fungi was potentiated under acidic conditions at pH 5 and 6 (Babich and Stotzky, 1979) while a significant increase of Pb uptake at lower pH values by bluegill *Lepomis macrochirus* and isopod *Asellus communis* was also reported (Merlini and Pozzi, 1977 and Lewis and McIntosh, 1984). Hodson *et al.*

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*Corresponding author, Email: sadhikari66@indiatimes.com
Tel.: +91674-246 5446; Fax: +91674-246 5446

(1978b) also reported that blood lead concentrations of rainbow trout exposed to water borne lead increase as the pH of the test water decreases. According to Dave (1985) cadmium was most toxic at pH 6-7 and least toxic at pH 9 to Zebrafish egg, hatchlings and larvae survival. Vander *et al.* (1981) observed that the acute toxicity of hexavalent chromium, Cr (VI) to rainbow trout increased with decreasing pH in the range from 7.8 to 6.5. However, literature regarding the physico-chemical variables of water that affect the process of uptake of these metals in *Labeo rohita* is nonexistant. Therefore, the present study has been made to investigate the combined effects of water pH and alkalinity on lead, cadmium and chromium accumulation in Indian major carp, *Labeo rohtia* (Hamilton).

MATERIALS AND METHODS

The present investigation has been carried out in 2000 at the Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, Orissa.

Test fish and water characteristics

The freshwater teleost *Labeo rohita* (Hamilton) fingerlings were reared in the laboratory after collection from the Farm of Central Institute of Freshwater Aquaculture. They were kept in 500 L fibre glass tank in dechlorinated tap water with constant water characteristics averaging 120 mg/L hardness as $CaCO_3$, 130 mg/L total alkalinity as $CaCO_{3,}$ electrical conductivity 0.42 dS/m and pH 6.9. Water temperature was 27 °C. During acclimatisation for two weeks under natural temperature water was continuously aerated and the fish were fed daily with a commercial food (Crude protein 30%, crude lipid 8.0%, ash 8.5%, carbohydrate 45.7% and moisture 7.8%) at a rate of 2% body wt. per day. Tests were performed in identical test chambers unit, each including one control and three treatment chambers. The test chambers were glass jar with the water volume adjusted to 40 L. Stock concentrations of lead, cadmium and chromium were prepared from lead nitrate, cadmium chloride and potassiumdichromate, respectively for performing of the experiments using reagent grade chemicals and deionised water. Dechlorinated tap water was used in the experiments to reduce precipitation and adsorption of Pb into jar wall and also as its original pH was 7.0 ± 0.10 and at a decreased pH of 5.5 ± 0.05 and increased pH of 8.5 ± 0.10 were maintained by adding 6(N) HCl and 6(N) NaOH, respectively. The change in alkalinity of the experimental waters was simply a result of the HCl or NaOH added to change the pH. *Labeo rohita* fingerlings used in experiments had weights of 5 ± 0.5 g (mean ± SD) and total lengths of 6 ± 0.4 cm.

Experimental procedure

Labeo rohita fingerlings were acclimated for at least one week to the different pH levels. In each glass jar, six numbers of fish were added. The treatment consisted of three levels of pH (5.5 ± 0.05, 7.0 ± 0.10 and 8.5 ± 0.10) with three replications for each heavy metal. The jar receiving no metal served as controls. For controls, three replications were also made. Static renewable bioassay method was adopted for the determination of 96 h median lethal concentration and probit analysis was followed for the calculation of 96 h LC_{50} values of lead nitrate, cadmium chloride and potassium dichromate for *Labeo rohita* fingerling (average weight 5 ± 0.5 g and average length 6 ± 0.4 cm) (Finney, 1971). The 96 h LC_{50} value of Labeo rohita for Pb, Cd and Cr were 10.0, 5.0 and 15.0 mg/L, respectively (Ghosh, 2002). Adequate amounts of (10% i.e. 0.1 of 96 h LC_{50} values of *Labeo rohita* fingerling of Pb (1.0 mg/L), Cd (0.5 mg/L) and Cr (1.5 mg/L)) solutions were added to respective jars which were earlier lavelled. During exposure period specific amount of feed was provided to the fish in each jar to reduce loss of Pb, Cd and Cr from solution owing to adsorption to the uneaten food. After feeding, faeces and uneaten food were siphoned off the bottom of the jar. Water and exposure solutions were renewed every 96 h of the exposure to reduce the build up of metabolites. The metals were added $Pb(NO_3)_2$, $CdCl_2$ and $K_2Cr_2O_7$ (Merck standard solutions) in initial concentrations of 1.0, 0.5 and 1.5 mg/L, respectively. As indicated by coloured precipitates on the tank surfaces a considerable change of the metals did not stay in solution. In order to provide high metal levels over the entire 14 days period we changed the water every 4 days and added the metals again to a calculated concentrations of 1.0, 0.5 and 1.5 mg/L. The metal concentrations were also measured using atomic absorption spectrophotometer. During the experimental period, the concentration of lead, cadmium and chromium were 1.0 ± 0.05, 0.5 ± 0.03 and 1.5 ± 0.1 mg/L, respectively. Every 24 h interval pH was checked by Systronic pH meter. During exposure period chemical parameters of water such as total alkalinity, total hardness, electrical conductivity, dissolved oxygen and ammonia concentration were analysed by APHA (1992). The ammonia concentrations remained low (<0.05mg/L NH_4^+); dissolved oxygen was always above 90% saturation. The test solutions in the jar were continuously aerated with air diffuser stones to achieve sufficient oxygen concentrations for removing excess CO_2 released by the lowering of pH. Every 96 h interval Pb,Cd and Cr concentration were measured by Atomic Absorption Spectrophotometer (Black, 1965). Experiment was continued upto 14 days. Fish mortality percentage were observed during 14 days exposure period. To ensure reliable tissue metal concentration estimates, food was not supplied to fish from 24 h before sampling of total fish. After 7 days, three fish were removed from each experimental group for estimation of metals in the fish. At the same time three fish from the stocking tanks were added to each experimental group to maintain an equal density. The fish freshly added to each experimental group were marked by fin clipping for proper identification. Fish from each experimental jar were also sampled after 14 days for estimation of lead, cadmium and chromium accumulation in the fish. For accumulation studies, fish samples were collected at 7 days as well as 14 days exposure period from all respective jars.

Preparation of samples for heavy metal analysis

Fish samples were dried at 60 °C over night to a constant weight. Dried tissues were ground, transferred to a porcelain basin (1-2 g finely ground sample) and kept 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 transferred to 125 mL Erlenmeyer flask. Fish samples were digested with triacid mixture (HNO_3:$HClO_4$:H_2SO_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 whatman 42 filter paper and diluted to 25 mL with distilled water. They were stored in acid rinsed polyethylene bottle at 4 ^oC prior to analysis.

Measurement of heavy metals by Atomic Absorption Spectrophotometer

All heavy metals were measured with a Perkin-Elmer Atomic Absorption Spectrophotometer (Model No. 1025) by specific cathode lamp. The wavelength used for lead, cadmium and chromium measurement were 283.3, 228.8 and 357.9 nm, respectively. The limits of detection for lead, cadmium and chromium were 1.00, 0.08 and 1.50 µg/g, respectively.

Statistical analysis

All the data were subjected to statistical evaluation. One way analysis of variance (ANOVA) with the Duncan multiple range test (DMRT) was applied to find the significant difference among different accumulation means at various combined levels of pH and alkalinity using SPSS software.

RESULT

The water temperature reported for all treatment were stable for the duration of experiment. Dissolved oxygen concentrations ranged from 5.2 to 5.8 mg/L and were above 90% of saturation in each jar for all experiments. In all treatments total hardness ranged from 146 to 166 mg/L as $CaCO_3$ and total alkalinity varied from 40 to 200 mg/L. There were no mortality occurred in control group at three different combination of pH and alkalinity and control fish metal concentrations are reported in relevant Tables. In lead treatment, 100% survivability was there at both pH 7.0 and 8.5 and at alkalinity level of 118 to 200 mg/L as $CaCO_3$ while 80% survivability was recorded at pH 5.5 and at alkalinity of 40 mg/L as $CaCO_3$. There were significant differences observed among three different pH groups with respect to accumulation and pH/ alkalinity (Table 1) during experimental period. The whole fish Pb concentration was decreased significantly ($p<0.05$) with increase in water pH from 5.5 to 8.5 and also with an increase in water alkalinity from 40 to 200 mg/L as $CaCO_3$. For Pb, highest accumulation in whole fish was occurred at pH 5.5/ alkalinity 40 mg/L as $CaCO_3$ followed by pH 7.0/ alkalinity 118 mg/L as $CaCO_3$ and pH 8.5/ alkalinity 200 mg/L as $CaCO_3$ during 14 days exposure period. For cadmium exposure, 100% survivability was observed at pH 5.5 and at alkalinity of 42 mg/L as $CaCO_3$ while 80% and 90% survivability occurred at pH 7.0 and at alkalinity of 100 mg/ L as $CaCO_3$ and that of 8.5 and 156 mg/L as $CaCO_3$, respectively during 14 days experimental period (Table 2). Mean accumulation of cadmium in *Labeo rohita* at combined pH 5.5/alkalinity 42 mg/L as $CaCO_3$ and combined pH 8.5/alkalinity 156 mg/L as $CaCO_3$ were not statistically different from each other ($P>0.05$). However, the accumulation was significant ($P<0.05$) at pH 7.0/ alkalinity 100 mg/L as $CaCO_3$ when compared with low pH/ alkalinity and high pH/alkalinity.

Table 1: Accumulation and water quality data for *Labeo rohita* fingerlings exposed to lead at different pH and alkalinity. Values are (Mean±S.E.), N = 3

pH	Total alkalinity (as $CaCO_3$) mg/L	Total hardness (as $CaCO_3$) mg/L	Dissolved oxygen (mg/L)	Mean accumulation in whole body ^{a} (µg/g dw)			
				Control		Metal treated fish	
				7 days	14 days	7 days	14 days
5.5	40	164	5.5	1.05±0.03	1.10±0.04	7.92 ±0.04^{x}	12.93±0.09^{x}
7.0	118	166	5.6	1.00±0.02	1.06±0.04	3.86±0.03^{y}	5.20±0.08^{y}
8.5	200	162	5.7	1.00±0.03	1.05±0.03	1.53±0.08^{z}	3.95±0.11^{y}

Values within a specific time period (7, 14 days) and followed by the same superscripts in a column were not significantly different at 0.05 level.

Table 2: Accumulation and water quality data for *Labeo rohita* fingerlings exposed to Cadmium at different pH and alkalinity. Values are (Mean±S.E.), N = 3

pH	Total alkalinity (as $CaCO_3$) mg/L	Total hardness (as $CaCO_3$) mg/L	Dissolved oxygen (mg/L)	Mean accumulation in whole body ^{a} (µg/g dw)			
				Control		Metal treated fish	
				7 days	14 days	7 days	14 days
5.5	42	146	5.2	0.65±0.05	0.70±0.03	2.65 ±0.07^{x}	3.95±0.12^{x}
7.0	100	140	5.4	0.64±0.02	0.68±0.02	7.83±0.06^{y}	9.62±0.07^{y}
8.5	156	150	5.5	0.66±0.03	0.69±0.05	3.28±0.03^{x}	5.07±0.09^{x}

Values within a specific time period (7, 14 days) and followed by the same superscript in a column were not significantly different at 0.05 level.

Table 3: Accumulation and water quality data for *Labeo rohita* fingerlings exposed to Chromium at different pH and alkalinity. Values are (Mean±S.E.), N = 3

pH	Total alkalinity (as $CaCO_3$) mg/L	Total hardness (as $CaCO_3$) mg/L	Dissolved oxygen (mg/L)	Mean accumulation in whole body ^{a} (µg/g dw)			
				Control		Metal treated fish	
				7 days	14 days	7 days	14 days
5.5	46	155	5.6	0.36±0.05	0.37±0.03	2.26 ±0.06^{x}	6.31±0.08^{x}
7.0	100	160	5.8	0.35±0.03	0.36±0.04	1.09±0.05^{y}	3.00±0.09^{y}
8.5	175	160	5.7	0.34±0.03	0.36±0.02	0.86±0.07^{y}	2.70±0.07^{y}

Values within a specific time period (7, 14 days) and followed by the same superscripts in a column were not significantly different at 0.05 level.

Table 4: Correlation coefficients comparing 7 and 14 days accumulation and pH/ alkalinity for *Labeo rohita* fingerlings. Values are (Mean ± S.E.), N = 3

Heavy metals	Correlation coefficient (r)			
	pH		Alkalinity	
	7 days	14 days	7 days	14 days
Lead	-0.98^{S}	-0.92^{S}	-0.98^{S}	-0.91^{S}
Cadmium	0.11^{NS}	0.19^{NS}	0.12^{NS}	0.19^{NS}
Chromium	-0.93^{S}	-0.90^{S}	-0.89^{S}	-0.85^{S}

S: Values are significant ($p < 0.05$); NS: Not Significant

The accumulation of cadmium was maximum at intermediate pH/alkalinity followed by high pH/alkalinity and low pH/alkalinity during the experimental period. In chromium treatment, there was no mortality occurred during experimental period (Table 3). Mean accumulation of chromium in *Labeo rohita* at pH 7.0/alkalinity 100 mg/L as $CaCO_3$ and pH 8.5/ alkalinity 175 mg/L as $CaCO_3$ were not statistically different from each other ($P>0.05$). However, the accumulation was significant ($P<0.05$) at pH 5.5/ alkalinity 46 mg/L as $CaCO_3$ in comparison with pH 7.0/ alkalinity 100 mg/L as $CaCO_3$ and pH 8.5/ alkalinity 175 mg/L as $CaCO_3$. The accumulation of chromium was maximum at low pH/alkalinity followed by intermediate pH/alkalinity and high pH/alkalinity during experimental period. A significant ($p<0.05$) linear correlation was observed between increasing pH/alkalinity and decreasing accumulation in Pb and Cr treatment at all exposure period but in Cd treatment no statistically significant relationship could be established between accumulation and pH/alkalinity.

DISCUSSION AND CONCLUSION

Among the environmental factors critical to assessment of toxicity of heavy metals, pH, alkalinity and hardness play a major role due to their influence on solubility and element speciation and to their interactive effects with pH in low ionic strength waters. The alkalinity of water is dependent on the activity and proportion of acidic and basic ions and as such is governed by the full chemical composition of the water, as well as physical parameters such as temperature. In aquatic bodies, the proportions and concentrations of carbonate bicarbonate, form the primary buffering system. Hydrogen ions combine with carbonate (CO_3^{2-}) to form bicarbonate (HCO_3^-) and the further addition of H^+ to bicarbonate, forms carbonic acid (H_2CO_3), a weak acid. Dissociation of carbonic acid is only partial decreasing with decreasing pH and not contributing to the acidity of aqueous solutions less than pH 5. However, above pH 5, the acidity of carbonic acid potential has a large effect on total acidity because of the increasing solubility of carbon dioxide as pH increases. The pH is therefore regulated: increasing pH promotes the formation of acidic products, while decreasing pH promotes the dissociation of basic ions. Thus, the present investigation was made on the combined effects of pH and alkalinity on the toxicity of metals. In freshwater fish, metal uptake and toxicity are largely influenced by the pH, ca concentration and alkalinity of the water (Alabaster and Lioyd, 1980; Spry and Wiener, 1991 and Kock *et al.*, 1996). Under acidic conditions, the free divalent ion is the dominant form of many metals, readily absorbed by fish gills (Merlini and Pozzi, 1977 and Part *et al.*, 1985), while interference of H^+ ions with metal uptake at the gill surface has also been proposed as the dominant mechanism for decreasing metal toxicity at acidic pH (Part *et al.*, 1985 and Cuimano *et al.*, 1986). Kock *et al.*, (1995) also showed that Cd and Pb concentrations in the kidney of Arctic char (*Salvelinus alpinus*) from oligotrophic Austrian mountain lakes was negatively correlated with alkalinity.

Influence of pH on survivability of rohu

At acidic pH, fish mortality occurred but near neutral to alkaline pH protected fish mortality from Pb toxicity and this may be due to less availability of Pb at neutral to alkaline pH. Similarly, for Cd at neutral and acidic pH mortality occurred while at acidic pH, no mortality occurred. Hence Cd is more available at neutral and alkaline pH compared to acidic pH. For Cr, no mortality observed in the acidic (pH=5.5), intermediate (pH=7.0) and also in the alkaline pH (8.5) treatment.

Influence of pH and alkalinity on accumulation of Pb

In the present study, highest accumulation of Pb occurred at pH 5.5 compared to 7.0 and 8.5 which revealed that Pb was more available as free Pb^{2+} ion at lower pH compared to higher pH due to weakly bound form. The bioavailability of Pb is strongly enhanced at decreasing water pH (Brown, 1979), increasing the toxicity of Pb for fish. At water pH<6, Pb predominates as Pb^{2+}, a form which is bioavailable (Rickard and Nriague, 1978) whereas at water pH 6-10, $Pb(OH)_2$ and $PbCO_3$ are the prevailing forms in water (McComish and Ong, 1988). Thus at high alkalinity and pH, lead precipitate by forming complexation products and as a result the toxicity and accumulation of lead was least in the fish.

Influence of pH and alkalinity on accumulation of Cd

The pH effect on Cd uptake by fish may be attributed to a change in the concentrations of Cd complexes available to fish. The present study recorded the maximum Cd accumulation in whole fish occurred at pH 7.0 than at pH 5.5 and 8.5, which supported that Cd availability was more as free Cd^{2+} ion at neutral pH compared to acidic and alkaline pH. At alkaline pH, the solubility of Cd hydroxide complexes decreases as pH increases, owing to the formation of solid $Cd\ (OH)_2$. Gardiner (1974) reported about the depletion of Cd from the water column by precipitation of the sparingly soluble $Cd\ (OH)_2$. However, in neutral pH, it could be expected to favour the existence of Cd in one or more ionic forms (Cd^{2+}, $Cd\ (OH)^+$) which are presumably more readily absorbed by fish than insoluble complexes. Dave (1985) also reported that Cd was most toxic at pH 6-7 and least toxic at pH 9. A reduction in pH diminished Cd accumulation and toxicity of Cd in fish and other aquatic organisms exposed to a fixed Cd concentration (Campbell and Stokes, 1985; Cusimano *et al.*, 1986 and Spry and Weiner, 1991). McDonald *et al.,* (1989) suggested that competition between Cd and H^+ ions for the same cellular binding sites resulted in a decrease in cellular heavy metal uptake. Thus it is evident that bioavailability and thereby accumulation of cadmium was more at near neutral pH compared to acidic and alkaline pH. In neutral pH, cadmium was present in one or more ionic forms (Cd^{2+}, $Cd\ (OH)^+$) which are presumably more readily absorbed by fish than insoluble complexes such as $Cd\ (OH)_2$.

Influence of pH and alkalinity on accumulation of Cr

The result of Cr accumulation in whole fish has shown that at pH 5.5, considerably more Cr was accumulated with respect to pH 7.0 and 8.5. The pH effect on uptake and distribution of Cr (VI) may be attributed to the presence of different Cr (VI) species in the exposure solutions. Stumm and Morgan (1970) reported that at higher pH, the molar ratio of $CrO4^{2-}$ to $HCrO_4^-$ was 1:0.05, while at lower pH, this ratio was 1:1 indicating that the $HCrO_4^-$ concentration increased at the same total Cr (VI) concentration. Thus, the availability of Cr (VI) to the whole fish increased with an increasing $HCrO_4^-/CrO_4^{2-}$ ratio at lower pH levels because monovalent ion ($HCrO_4^-$) was more readily taken up by the gill tissue compared to divalent ions (Trama and Benoit, 1960). The oxidizing action of Cr (VI) also enhanced at a decreased pH (Silen and Martell, 1964), which was associated with an increased $HCrO_4^-/CrO4^{2-}$ ratio. Our results were also corroborates with Vander *et al.* (1981) who reported that higher Cr accumulation occurred at lower pH (6.5) than that of higher pH (7.8). As the present investigation was made with the 1/10th. of the LC_{50} values of metal concentrations, i.e. very low metal concentration. Therefore, their effect on fish survivability was not prominent. However, it is evident from the present investigation that that high pH and high alkalinity of the water could prohibit the bioavailability of lead and chromium and thereby bioaccumulation of these metals in the fish, though cadmium accumulation was least at near neutral pH and medium level of alkalinity (100 mg/L as $CaCO_3$) of water. In freshwater pond aquaculture, the growth of fish is optimum at slightly alkaline pH and at a alkalinity in the range of 75 to 200 mg/L as $CaCO_3$. Therefore, under higher pH and higher alkalinity, the chances of lead and chromium accumulation in the fish will be least. Acidic water could be limed to increase water pH and alkalinity which could prohibit the accumulation of these heavy metals in the fish. However, in acidic environment of any river or water bodies in mining areas, there is a chance for higher accumulation of heavy metals particularly lead and chromium in the fish. From the present investigation, it could be concluded that accumulation of lead and chromium to L. rohita was minimum at high pH/ alkalinity, while accumulation of cadmium was minimum at low pH/alkalinity and also at high pH/alkalinity regime. The growth of *Labeo rohita* is optimum at high pH/alkalinity regime. Therefore, the culture of *Labeo rohita* should be made always at high pH/alkalinity regime which could prohibit the accumulation of these metals in *labeo rohita.*

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AUTHOR (S) BIOSKETCHES

Adhikari, S., Ph.D., is a Sr. scientist in the Department of Soil and Water Chemistry, Central Institute of Freshwater Aquaculture (ICAR), Kausalyaganga, Bhubaneswar-751002, India. Email: *subhendu66@rediffmail.com.*

Ghosh, L., Ph.D., is a research scholar of Central Institute of Freshwater Aquaculture (ICAR), Kausalyaganga, Bhubaneswar-751002, India. Email: *lopagsarkar@yahoo.com.*

Ayyappan, S., Ph.D., is deputy director general (Fisheries), Indian Council of Agricultural Research, Krishi Anusandhan Bhawan, Pusa, New Delhi – 110012. Email: *ayyappans@icar1.nic.in.*

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