

A Study on Bioaccumulation of Heavy Metals in two Anuran Tadpoles: *Clinotarsus alticola* and *Leptobrachium smithi* from Rosekandy Tea Estate, Cachar, Assam

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ABSTRACT

Considering the significance of heavy metal pollution in aquatic system bioaccumulation of heavy metals in two species of tadpoles namely *Clinotarsus alticola* and *Leptobrachium smithi* collected from tea gardens of Barak valley, Assam was studied. Aquatic life is affected by heavy metal pollutants present in water as well as in sediment. The result of the study revealed that the concentration of iron, chromium, cadmium and lead in water samples was higher than the permissible limit of 0.3, 0.05, 0.003, 0.01 mg/L respectively but that of copper and zinc concentration was within the maximum permissible limit of 2 mg/L and 3 mg/L (WHO, 2005). The accumulation pattern of different heavy metals in different organs viz., intestine, liver and tail was studied. Overall the metal burden in different organs of *Clinotarsus alticola* and *Leptobrachium smithi* was in the order of liver>tail>intestine. Liver had highest accumulation of metals while intestine accumulated the least. Iron (Fe) was highly and zinc (Zn) was the least accumulated metal in both the tadpoles. The accumulation of heavy metals might be due to tea plantation influx water, domestic and associated anthropogenic activities.

Key words: anurans, bioaccumulation, heavy metals, tea estate.

INTRODUCTION

Anurans belonging to class amphibians occupy important position in the ecosystem being both predators and prey and are good bio-indicators of environmental pollution. Amphibians are intimately tied to aquatic as well as terrestrial environment and the quality of water in which they live can affect their growth, development, and survival. The negative impact of anthropogenic activities on biodiversity is becoming increasingly conspicuous and amphibians are currently the most globally threatened group of vertebrates, approximately 41% of all species¹. Various causes for declining amphibian populations are habitat destruction, introduction of exotic species, pathogens, pollution, and global climate

change. A variety of pollutants occur in natural habitats including fertilizers, pesticides and heavy metals introduced into the environment by direct application, runoff from crop, forest applications or mines, urban and industrial sewage, and atmospheric deposition^{2,3,4}. Amphibians have permeable, exposed skin and eggs that may readily absorb toxic substances from the environment. Their biology and habitat selection makes them candidates for heavy metal accumulation⁵. Freshwater systems contaminates with heavy metals from industrial and agricultural sources^{6,7}. The objective of the present study was to estimate the levels of heavy metals; copper (Cu), iron (Fe), chromium (Cr), cadmium (Cd), lead (Pb) and zinc (Zn) in tadpoles of two different anuran species - *Clinotarsus alticola* and

Leptobranchium smithi belonging to two different families Ranidae and Megophryidae respectively and also the accumulation pattern in different organs viz., intestine, liver and tail. The study was conducted in aquatic systems of Rosekandy Tea Estate, Cachar, Assam.

MATERIALS AND METHODS

Study site

The study was conducted at Barjalenga (24°42'29.3" N, 92°41'25.23" E), Rosekandy Tea Estate, Cachar District, Assam, 25 kms from Silchar town. There are a number of permanent and temporary lentic and lotic water bodies (in the form of nullahs or drains) inside the tea gardens. The permanent standing water bodies are situated in disturbed as well as undisturbed areas and are adjacent to the forest and tea plantation area. The disturbed area contains human settlements, factories and tarred roads.

Collection of samples

Tadpoles of *Leptobranchium smithi* were collected from shallow water body and *Clinotarsus alticola* were collected from permanent lentic body with the help of dip net and were washed properly with double distilled water in the laboratory. The selected organs like intestine, tail and liver were removed and dried until reaching a constant weight. Digestion of samples was done according to⁹. To each sample of organs (0.1g), 10 ml of perchloric acid: conc. HNO₃ (3:2 v/v) was added and heated at 60°C until a clear solution was formed. The resulting solutions were cooled, and the volumes were made up to 50 ml using double distilled water. The samples were then stored in plastic bottles till analysis to determine the amount of heavy metal bioaccumulated⁹. The water collected in sampling bottles were pre-conditioned with dilute nitric acid (HNO₃) and later rinsed thoroughly with double distilled water. Pre-cleaned polyethylene sampling bottles were immersed about 10 cm below the water surface and 1 liter of the water sample was taken. Samples were acidified with concentrated nitric acid (HNO₃) for preservation. The samples were filtered through Whatman filter paper No. 1 and kept in refrigerator until analysis. The sediment samples were oven dried at 45°C followed by grinding and sieving using <2 mm sieve, 5 gm of dry sample was

poured into a beaker and mixed with 2 ml of aqua regia 1:3 (1 conc. HCL : 3 conc. HNO₃). The mixture was digested on a hot plate in open beakers at 95°C for 1 hr and allowed to cool to room temperature. The supernatant was filtered and then diluted to 50 ml using distilled water. The heavy metal concentrations were determined with a Graphite Furnace- Atomic Absorption spectrophotometer (GF-AAS), Model Analytik Jena Vario-6.

RESULTS AND DISCUSSION

The heavy metals including copper (Cu), iron (Fe), chromium (Cr), cadmium (Cd), lead (Pb) and zinc (Zn) were analyzed in the intestine, liver and tail of two different tadpole species, *Clinotarsus alticola* and *Leptobranchium smithi*. Some physico-chemical parameters of water were also determined as they are indicators of water quality. Table 1 presents the physico-chemical variables of the water in study sites.

The pH varied between 5.1-6.24 and conductivity ranged between 50.16 $\mu\text{s cm}^{-1}$ to 50.26 $\mu\text{s cm}^{-1}$. Dissolved O₂ ranged between 3.73 mg L⁻¹ to 4.23 mg L⁻¹ and Free CO₂ ranged between 21.8 to 41.6 mg L⁻¹. Total Alkalinity ranged between 29.7 mg L⁻¹ to 44 mg L⁻¹. Surface water temperature ranged from 22.63 to 24°C. Air temperature ranged between 29.16°C to 29.26°C. pH and alkalinity are important factors controlling metal fate, transport and bioavailability¹⁰. Metal uptake and their toxicity in aquatic fauna are influenced by many factors such as temperature, dissolved oxygen, hardness, alkalinity, pH, salinity, suspended particulate matter, pH, hardness, alkalinity and temperature of water^{11,12}.

The levels of heavy metals: Cu, Fe, Cr, Cd, Pb and Zn in two different anuran species - *Clinotarsus alticola* and *Leptobranchium smithi* was analyzed (Table 2). The sequence of metal accumulation in the intestine (Fig. 1a) and liver (Fig. 1b) of *Clinotarsus alticola* was Fe>Pb>Cr>Cu>Cd>Zn, while in the intestine and liver of *Leptobranchium smithi* sequence of metal accumulation was, Fe>Cu>Cr>Pb>Cd>Zn and Fe>Cu>Cr>Pb>Zn>Cd respectively. The order of metal bioaccumulation in the tail of both *Clinotarsus alticola* and *Leptobranchium smithi* was Fe>Cr>Pb>Cu>Cd>Zn (Fig. 2). Iron (Fe) was the highly and zinc (Zn) was the least accumulated

metal in both the tadpoles. Considering the metal burden in different organs of *Clinotarsus alticola* and *Leptobranchium smithi* it was in the order of liver>tail>intestine. Similar result was obtained in fish where maximum accumulation occurred in liver and least was in muscle¹³. In the present study also liver showed higher accumulation of metals than other organs.

The accumulation of Fe, Cr, Cd, Pb and Zn in intestine of *Clinotarsus alticola* was higher in comparison to *Leptobranchium smithi*, while Cu accumulation in intestine of *Leptobranchium smithi* was higher than *Clinotarsus alticola*. The concentration of Cu, Fe, Cr and Zn was found to be highest in the liver of *Leptobranchium smithi* while concentration of Cd and Pb was recorded highest in *Clinotarsus alticola*. Heavy metal concentration was higher in the tail of *Leptobranchium smithi* as compared to *Clinotarsus alticola*

The sequence of metal accumulation in the sediment samples collected from the habitat of *Clinotarsus alticola* was Pb>Fe>Cr>Cu>Cd>Zn and from the habitat of *Leptobranchium smithi* was

Pb>Fe>Cu>Cr >Cd>Zn. Lead (Pb) concentration in sediment samples collected from the habitat of *Leptobranchium smithi* and *Clinotarsus alticola* was found to be highest and zinc (Zn) concentration was least (Fig. 3a).

The order of metal concentration in water samples collected from the habitat of *Clinotarsus alticola* was Fe>Cu>Pb>Cr>Cd>Zn and from the habitat of *Leptobranchium smithi* was Fe>Pb>Cu>Cr>Cd>Zn. Iron (Fe) concentration was recorded highest and zinc (Zn) concentration was least (Fig. 3b). The sediment and water samples collected from the habitat of *Leptobranchium smithi* accumulated high concentration of metals in comparison to that of *Clinotarsus alticola*.

The Bioaccumulation factor was calculated according to formula¹⁴

$$\text{BAF} = \frac{\text{Conc. of heavy metal in tissue}}{\text{Conc. of heavy metal in abiotic media (water and sediment)}}$$

The results of Bioaccumulation factor (Table 3) showed that Cr, Pb in *C. alticola* tadpoles from

Table 1: Physiochemical properties of water in two different study sites

Species	Air Temp. (°C)	Surface Water Temp. (°C)	pH	Cond. (µS cm ⁻¹)	Dissolved Oxygen (mg L ⁻¹)	Free CO ₂ (mg L ⁻¹)	Total Alkalinity (mg L ⁻¹)
1. <i>C. alticola</i>	29.26±0.2	24±0.2	6.24±0.08	50.16±1.2	3.73±0.15	21.8±0.2	44±1
2. <i>L. smithi</i>	29.16±0.2	22.63±0.4	5.1±0.02	50.26±0.7	4.23±0.15	41.6±0.4	29.7±0.2

Table 2: Heavy metals concentrations in different organs of *Clinotarsus alticola* and *Leptobranchium smithi* (µg gm⁻¹).

Metals Organs	Copper(Cu)	Iron(Fe)	Heavy metal concentration (µg gm ⁻¹)			Zinc(Zn)
			Chromium(Cr)	Cadmium(Cd)	Lead(Pb)	
			<i>Clinotarsus alticola</i>			
Intestine	0.122±0.077	1.233±0.647	0.166±0.035	0.021±0.016	0.169±0.092	0.009±0.006
Liver	0.48±0.311	5.57±0.608	1.365±0.968	0.195±0.077	1.625±2.015	0.06±0.056
Tail	0.09±0.070	0.58±0.240	0.154±0.072	0.029±0.015	0.138±0.002	0.022±0.015
			<i>Leptobranchium smithi</i>			
Intestine	0.126±0.082	0.560±0.319	0.096±0.016	0.019±0.003	0.066±0.024	0.008±0.002
Liver	2.25±1.626	8.825±2.298	2.125±0.106	0.145±0.007	0.775±0.388	0.25±0.212
Tail	0.113±0.074	0.902±0.130	0.217±0.038	0.066±0.062	0.145±0.119	0.025±0.020

water were greater than that from sediment and this implies that the tadpoles bioaccumulated these metals from the water¹⁴. Also the accumulation of Cu, Cd and Zn in tadpoles of *C. alticola* from water were lower than that from sediment and this implies that the tadpoles bioaccumulate these heavy metals from the sediment. Similar report has been made where significant correlation was found between heavy metal accumulation in tadpoles and the respective levels in the sediments and water¹⁵. Tadpoles are omnivorous, macrophagous filter feeder and ingest anything from sediment. Hence they may accumulate

elements from sediment. Bioaccumulation factor (Table 4) of Cu, Cr, Pb in *L. smithi* tadpoles from water were greater than that from sediment and this implies that the tadpoles bioaccumulated these metals from the water. Also the accumulation of Fe, Cd, Zn in tadpoles of *L. smithi* from water were lower than that from sediment and this implies that the tadpoles bioaccumulated these heavy metals from the sediment. This might be due to the feeding behavior of tadpoles which feeds at the bottom of the habitat or sequester metal from the habitats.

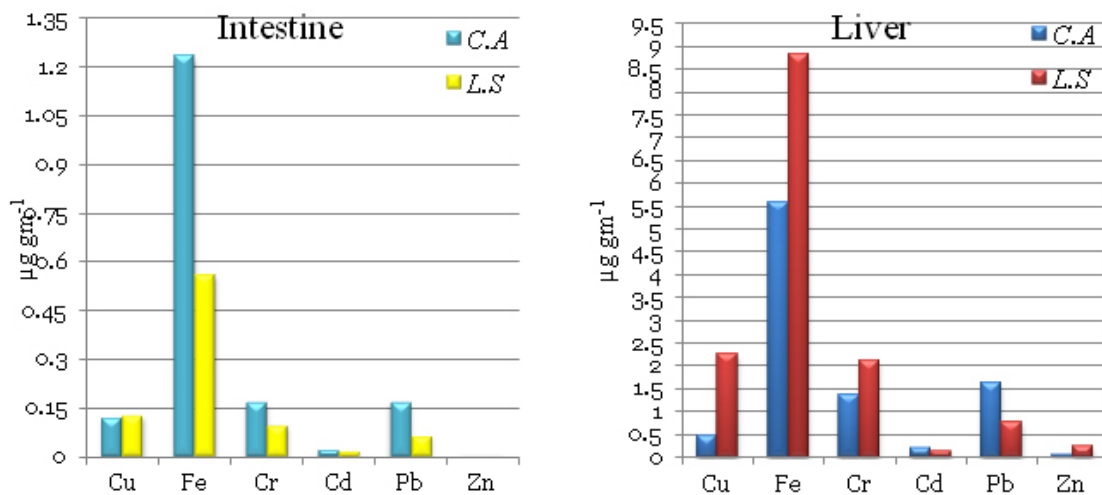


Fig.1 (a and b): Heavy metal bioaccumulation in intestine and liver of *C. alticola* and *L. smithi*

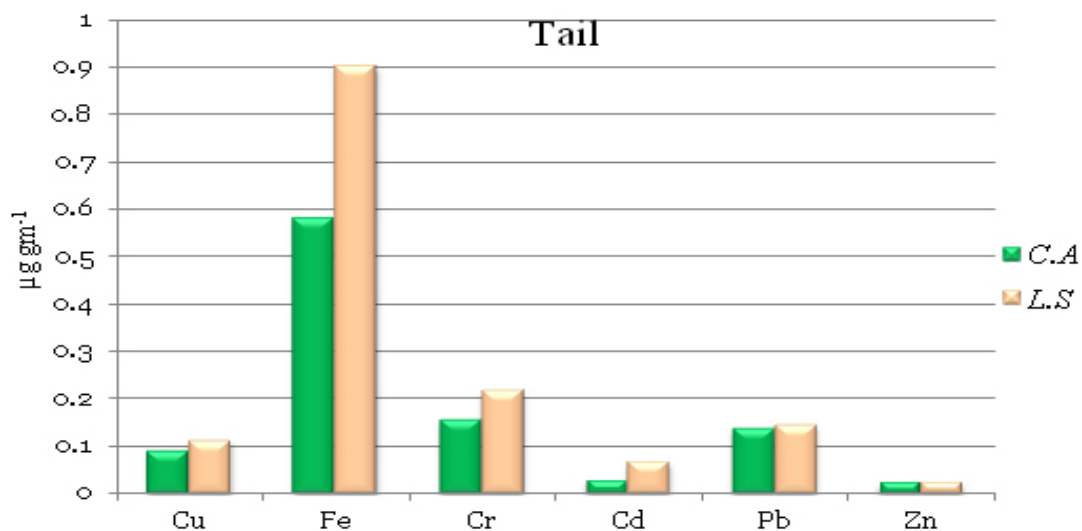


Fig. 2: Heavy metal bioaccumulation in tail of *Clinotarsus alticola* and *Leptobranchium smithi*

The present study reveals that liver of both the tadpoles accumulated more concentration of metals when compared to other organs. The liver plays an important role in accumulation and detoxification of heavy metals¹⁶. The differences in accumulation of metal in the liver, tail and intestine of the two tadpole species could be attributed to differences in their physiological roles. It has been reported that different organisms have different metabolic rates and different food requirements and amounts. Organisms with high food intake tend to accumulate more metals¹⁷. Concentration of iron (Fe) was highest in different organs of tadpole and in the water samples but metal concentrations in the different organs of tadpoles showed differences in accordance to the concentration of metals in sediment. Lead (Pb) was the highest accumulated metal in both the sediment samples. Zinc (Zn) was the least accumulated metal in the tadpole, sediment and water samples. The result revealed that the concentration of iron, chromium, cadmium and lead in water samples was higher than the permissible limit of 0.3, 0.05, 0.003, 0.01 mg/L respectively but copper and zinc concentration was within the maximum permissible limit of 2 mg/L and 3 mg/L (WHO, 2005). Heavy metals contamination may have a significant impact on aquatic organisms, disturbing the ecological balance and potentially contaminating the aquatic food chain as well as humans¹⁸. Accumulation of such metals by animal tissue may be transferred to next higher level of

organism through food chain. Such studies have been conducted in fish which is consumed as an important protein diet¹⁹.

There are many factors, physicochemical and biological factors, affecting the accumulation and toxicity of metals in aquatic organisms. Cd and Pb entering aquatic systems from industrial and consumer waste and can damage physiological processes or accumulate in tissues of aquatic organisms even at concentrations far below the lethal level²⁰. Cd and Pb increased the mortality and malformation ratio and prolonged the development and metamorphosis period in tadpoles²¹. Sediments accumulated more heavy metals than water²² and same was also observed in the present study. Given the static nature of sediments, they tend to accumulate more toxicant than water which may flow away, drain off or even evaporate²³. In the present study area influx of water from tea plantation and other anthropogenic activities may account for higher concentration of heavy metals in sediment which may lead to serious environmental problems and finally affect the anuran tadpole population and their life cycle. Heavy metal concentration in sediment as well as in water was found to affect occurrence of anuran and species richness in any system²⁴. From the present study it can be concluded that the tadpoles can be considered as a good bio-indicators of metals contamination in streams and other aquatic bodies. In different sampling sites there was

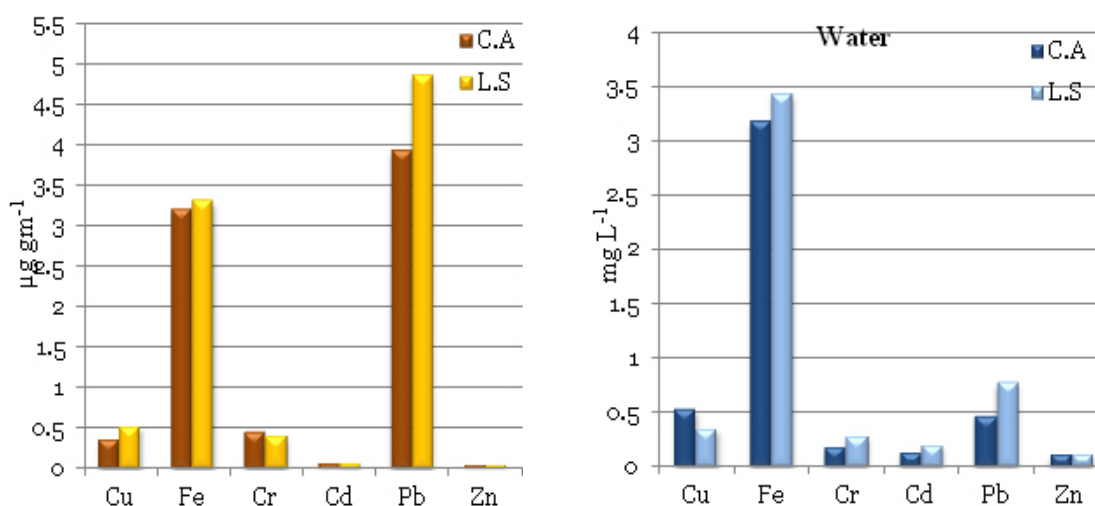


Fig. 3 (a and b): Heavy metal concentrations in sediment and water of *Clinotarsus alticola* and *Leptobranchium smithi* habitats

Table 3: Bioaccumulation factors of heavy metals in different organs of *Clinotarsus alticola*

<i>C. alticola</i>	Cu	Fe	Cr	Cd	Pb	Zn
Liver/water	0.91	1.75	7.89	1.44	3.53	0.56
Liver/sediment	1.40	1.74	3.13	4.14	0.14	1.62
Tail/water	0.17	0.18	0.89	0.21	0.3	0.20
Tail/sediment	0.26	0.19	0.34	0.61	0.03	0.59
Intestine/water	0.23	0.38	0.95	0.15	0.36	0.08
Intestine/sediment	0.35	0.40	0.36	0.44	0.04	0.24

Table 4: Bioaccumulation factors of heavy metals in different organs of *Leptobranchium smithi*

<i>L. smithi</i>	Cu	Fe	Cr	Cd	Pb	Zn
Liver/water	6.42	2.57	7.72	0.73	1.01	2.13
Liver/sediment	4.32	2.68	5.42	2.23	0.15	6.75
Tail/water	0.32	0.26	0.78	0.33	0.18	0.21
Tail/sediment	0.21	0.27	0.55	1.01	0.02	0.67
Intestine/water	0.36	0.16	0.34	0.09	0.08	0.06
Intestine/sediment	0.24	0.17	0.24	0.29	0.01	0.21

contamination by pollutants and long time exposure of anuran tadpoles to heavy metals may lead to decline of the anuran population in the region.

In view of the importance of anurans in ecosystems, it is recommended that monitoring of water and sediment in various habitats should be done regularly. Proper disposal techniques of industrial effluents, agricultural drainage water containing pesticides, fertilizers and domestic sewage should be practiced to avoid entry of these metals into environment which may lead to severe pollution of water bodies.

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