

Heavy metal pollution of aquatic systems and its possible impact on anuran tadpoles: a study in Barak Valley, Assam, India

Abstract

In the present study, investigations were made on the accumulation of heavy metals- copper (Cu) and lead (Pb) in water, sediment and tadpoles inhabiting the water bodies of Barak Valley, Assam. Tadpoles of *Microhyla ornata*, *Duttaphrynus melanostictus*, *Hoplobatrachus tigerinus* and *Polypedates teraiensis* were collected from eight different sites and heavy metal concentrations of Copper and Lead were determined in the whole body of tadpoles. Heavy metal concentrations in water and sediment collected from the habitat of tadpoles were also analyzed. The results revealed that the copper concentration in water samples was within the maximum permissible limit of WHO (2 mg L⁻¹), but the concentration of lead in water samples increased beyond the permissible limit of WHO (0.01 mg L⁻¹). The result of heavy metal analysis in tadpoles also revealed that tadpoles of *Microhyla ornata* collected from an industrial area showed highest concentrations of copper and lead whereas tadpoles of *Duttaphrynus melanostictus* collected from AUS campus showed the lowest concentration of metals. From the present study it can be concluded that the tadpoles can be considered as a good bio-indicators of metals contamination in streams, wetlands and other aquatic bodies.

Introduction

Amphibians are good bio- indicators of ecosystem health and can indicate pollution level. (Kelepertziset al, 2012, Loumbourdis & Wray, 1998). According to IUCN Red list nearly 41% of amphibian species are globally threatened (Hoffman, 2010). The dramatic decline in amphibian population worldwide is considered to be one of most critical threats to global biodiversity and measures of conservation for amphibians need to be designed which include creating nature reserves and national parks, monitoring of habitats as well as carrying out public awareness and educational outreach programmes. Various studies have opined that more than 76% of the world's organisms will face extinction in the next 300 years plunging the world into another mass extinction (Barnosky et al. 2011).

Amphibians are affected by environmental pollution due to their susceptibility to chemicals and high sensitivity to perturbations in the environment. They are

well known for accumulating metals during their freshwater cycles (Loumbourdis & Wray, 1998). Bioaccumulation is the building up of a chemical upto a toxic level in the body of an organism. It is the net accumulation of a substance by an organism as a result of uptake directly from all environmental sources and from all routes of exposure (ASTM, 1998). As heavy metals cannot be degraded, they are deposited, assimilated or incorporated in

Key words:

Anuran tadpoles, Heavy metal, pollution, Copper, Lead, South Assam.

Microhyla sp. tadpoles
Photo Credit: Vishal Prasad

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water, sediment and aquatic animals causing heavy metal pollution of water bodies (Mallick et al. 2010). Larval amphibians accumulate metals more readily than adults, possibly due to their semi permeable and highly vascularised skin which allows cutaneous respiration and high accumulation of environmental pollutants in the tissue from water and soil directly (Hall & Mullherm, 1984). In addition tadpoles during the development period are microphagous in feeding habit and ingest sediment in which heavy metals accumulate (Hopkins & Rowe, 2010).

One important reason that has been implicated as a cause of decline of amphibian and fish population in aquatic systems is contamination with heavy metals which is mainly from anthropogenic sources (Greiget et al. 2010; Hopkins & Rowe, 2010). The anthropogenic sources include industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbor activities, geological weathering of the earth crust and atmospheric deposition (Yilmaz, 2009). Heavy metals are also known as trace elements because they occur in minute concentrations in biological systems. Some metals are potentially toxic (As, Cd, Pb, Hg, etc.), while others are probably essential (Ni, V, Co), and many are essential (Cu, Zn, Fe, Mn) (Biswas et al. 2012). These essential metals can also produce toxic effects when the metal intake is excessively elevated (Tekin- Ozan, 2008). The heavy metals are hazardous because of their toxicity, persistence, and bio accumulation in the food chains. Frogs are opportunistic breeders and breed in wide variety of aquatic systems like ephemeral pools, small and large ponds, streams, ditch, drains, manmade tanks etc. The present study was conducted in Barak Valley region of Assam, India and heavy metal accumulation of Cu and Pb was analysed in water, sediment and whole body of anuran tadpoles collected from different types of habitat. Considering the body size of the tadpoles, heavy metal analysis was done in the whole body of the tadpoles (Gosner stage 26-35, tadpole size range: 10.3- 27.1 mm). Tadpoles of *Microhyla ornata* (MO), *Duttaphrynus melanostictus* (DM), *Hoplobatrachus tigerinus* (HT) and

Polypedates teraiensis (PT) were collected from eight different sites and heavy metal concentrations were determined in the whole body of tadpoles. The heavy metal concentrations of Copper (Cu) and Lead (Pb) was analyzed. Heavy metal concentrations of Cu and Pb in water and sediment collected from the habitat of tadpoles were also analyzed.

Materials and Methods

Barak Valley is situated in the southern part of the Assam, between 24°8'N and 25°N latitudes and 92°15'E and 93°15'E longitudes. The region abounds in wetlands, streams, pools, marshes, ponds etc. of various shapes, sizes and have small hillocks. The valley has urban areas, brick kilns, industrial area like Cachar Paper Mill at Panchgram situated at a distance of 25 Km from Silchar town and large number of tea gardens. The study was carried out in different selected habitats of Barak Valley which included tea estates, urban and industrial area and brick kilns. For physicochemical analysis and determination of Cu and Pb in water, three replicates of water samples were collected from the sites where tadpoles were present. Physicochemical parameters like pH, electrical conductivity (EC), dissolved oxygen (DO), free carbon dioxide (FCO₂) and total alkalinity (TA) were analyzed using standard methods (APHA, 2005; Trivedy & Goel, 1984). Tadpoles were collected by dip net and were washed properly with double distilled water in the laboratory. Drying of the whole body of tadpole was done until a constant weight was obtained. Digestion of all tadpole samples was done according to FAO/SIDA (Swedish International Development Authority Cooperative Programme, 1983). To each sample (0.1 g), 10 ml of perchloric acid: conc. HNO₃ (3:2 v/v) was added and the mixture was 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 bioaccumulated (Ezemonye & Enuneku, 2012). The water collected in sampling bottles were preconditioned with dilute nitric acid (HNO₃) and later rinsed thoroughly with double distilled water.

Precleaned 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 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 in the digested samples of tadpoles, water and sediment were determined in a Graphite Furnace-Atomic Absorption spectrophotometer (GF-AAS), Model Analytik Jena Vario-6.

Abbreviations used:MO: *Microhyla ornata*, DM: *Duttaphrynus melanostictus*, HT: *Hoplobatrachus tigerinus* and PT: *Polypedates teraiensis*.

Results

Heavy metal concentrations and bioaccumulation in whole body of tadpoles

For heavy metal analysis tadpoles were collected from different habitats of Barak Valley which included tea estates, rural, urban and industrial area and paddy fields during 2013 and 2014. Approximately 50-55 nos. of tadpoles of each species was sacrificed. Table 1 shows the habitats used by anuran tadpoles. Concentrations of two heavy metals-Copper and Lead in the whole body of tadpoles are given in Table 2. Copper concentration in whole body of different tadpole species ranged between $0.411 \pm 0.06 \mu\text{g gm}^{-1}$ to $2.07 \pm 0.01 \mu\text{g gm}^{-1}$. Tadpoles of *Microhyla ornata* collected from Panchgram accumulated the highest concentration of Cu ($2.07 \pm 0.01 \mu\text{g gm}^{-1}$); while *Duttaphrynus melanostictus* collected from Assam University Silchar (AUS) campus showed the lowest values of Cu ($0.411 \pm 0.06 \mu\text{g gm}^{-1}$). The order of Cu concentration was *Microhyla ornata* (Panchgram) > *Microhyla ornata* (Karimganj) > *H. tigerinus* (Karimganj) > *H. tigerinus*

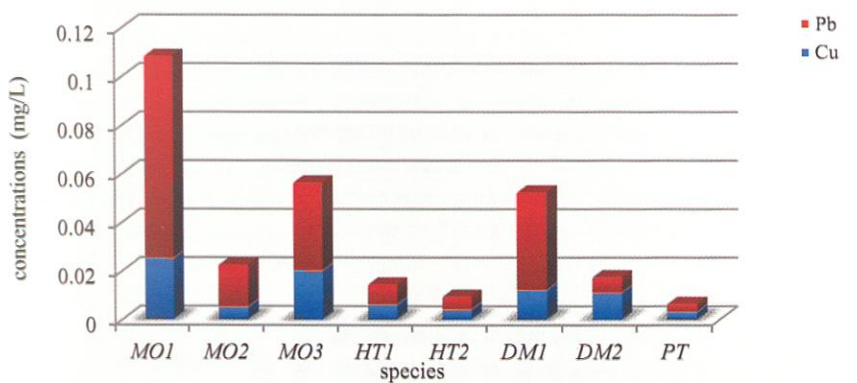


Figure 1: Heavy metal concentrations (mean) in whole body of different species of tadpoles

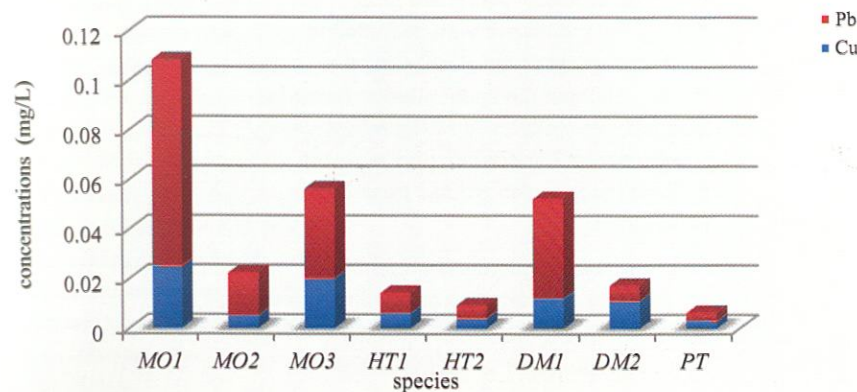


Figure 2: Heavy metal concentrations (mean) in water of different species of tadpole habitats

(Panchgram) > *P. teraiensis* (AUS campus) > *Microhyla ornata* (Rosekandy) > *D. melanostictus* (Panchgram) > *D. melanostictus* (AUS campus) (Fig. 1).

Copper (Cu) concentration in water (Fig. 2) and sediment (Fig. 3) samples ranged between $0.003 \pm 0.02 \text{ mg L}^{-1}$ to $0.025 \pm 0.04 \text{ mg L}^{-1}$ and $0.634 \pm 0.1 \mu\text{g gm}^{-1}$ to $4.27 \pm 0.06 \mu\text{g gm}^{-1}$ respectively. Water sample collected from the habitat of *Microhyla ornata* (Panchgram) showed the highest concentration of Cu ($0.025 \pm 0.04 \text{ mg L}^{-1}$) while site of *Polypedates teraiensis* (AUS campus) showed the lowest concentration ($0.003 \pm 0.02 \text{ mg L}^{-1}$). Considering sediment samples, habitat of *Duttaphrynus melanostictus* (Panchgram) showed highest conc. of Cu ($4.27 \pm 0.06 \mu\text{g gm}^{-1}$) and lowest was recorded in the habitat of *Polypedates teraiensis* collected from AUS campus ($0.634 \pm 0.1 \mu\text{g gm}^{-1}$).

Lead (Pb) levels in whole body of tadpoles ranged from $0.024 \pm 0.01 \mu\text{g gm}^{-1}$ (*Duttaphrynus melanostictus*, AUS campus) to $2.42 \pm 0.03 \mu\text{g gm}^{-1}$ (*Microhyla ornata*, Panchgram). The order of Pb concentration was *Microhyla ornata* (Panchgram) > *Microhyla ornata* (Karimganj) > *H. tigerinus* (Panchgram) > *D. melanostictus* (Panchgram) > *Microhyla ornata* (Rosekandy) > *H. tigerinus* (Karimganj) > *P. teraiensis* (AUS campus) > *D. melanostictus* (AUS campus) (Fig. 1).

Concentrations of Pb in water (Fig. 2) samples ranged from $0.003 \pm 0.03 \text{ mg L}^{-1}$ (*Polypedates teraiensis*, AUS campus) to $0.083 \pm 0.03 \text{ mg L}^{-1}$ (*Microhyla ornata*, Panchgram). Pb concentration in sediment (Fig. 3) samples ranged from $0.893 \pm 0.21 \mu\text{g gm}^{-1}$ (*Polypedates teraiensis*, AUS campus) to $5.55 \pm 0.08 \mu\text{g gm}^{-1}$ (*Duttaphrynus melanostictus*, Panchgram).

One-way ANOVA was applied to test for differences in trace elements concentration in the whole body of tadpoles among the different sites. All the tadpole species varied significantly ($p < 0.01$) in terms of metal accumulation as revealed by One-way ANOVA (Table 3). One-way ANOVA was also done to determine differences of metal accumulation in different species and the result showed that there was significant variations in terms of Cu ($F = 129.7$, $p < 0.01$) and Pb ($F = 163.1$, $p < 0.01$) accumulation in different species of tadpoles. One-way

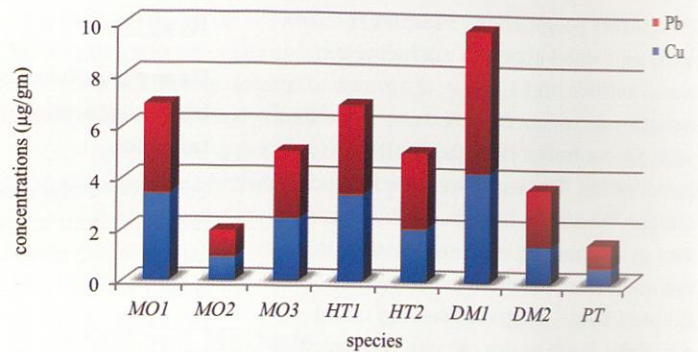


Figure 3: Heavy metal concentrations (mean) in sediment of diff. species of tadpole

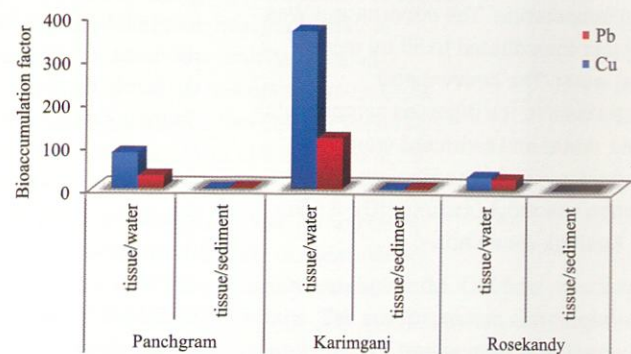


Figure 4: Bioaccumulation factor of heavy metals in whole body of *M. ornata*

ANOVA was done to determine differences of metal accumulation in water and sediment samples at different study sites. The result showed that statistically there was significant variations in metal concentrations in water ($F = 1.9$, $p < 0.05$) and sediment samples ($F = 1.03$, $p < 0.05$) collected from the different sites.

Bioaccumulation factors between metal concentration in tadpoles and water are presented in Fig. 4 to Fig. 7. The highest bioaccumulation factor of copper (Cu) were observed in *Microhyla ornata* from Karimganj and lowest were recorded in *Microhyla ornata* from Rosekandy tea garden. Highest bio accumulation factor for lead (Pb) were observed in *Hoplobatrachus tigerinus* from Panchgram and lowest in *Duttaphrynus melanostictus* from Panchgram.

Bioaccumulation factors between metal concentration in tadpoles and sediment are presented in Fig. 4 to Fig. 7. The highest bioaccumulation factor of copper (Cu) were

observed in *Microhyla ornata* from Karimganj and lowest were recorded in *Duttaphrynus melanostictus* from Panchgram. Highest bio accumulation factor for lead (Pb) were observed in *Microhyla ornata* from Karimganj and lowest were observed in *Duttaphrynus melanostictus* from Assam University campus. The results of Bioaccumulation factor showed that Cu and Pb in tadpoles from water were greater than that from sediment and this implies that the tadpoles bioaccumulated these metals from the water.

Physico-chemical characteristics of water samples

The physico-chemical characteristics of water from different sampling ponds are presented in Table 4. Surface water temperature (WT) ranged between 23 to 25.6°C. Air temperature (AT) ranged between 24.8°C to 28.6°C. pH was low in university campus having value of 5.2 while the highest value of 6.9 was recorded at Panchgram. Ponds in the industrial area had slightly higher pH towards alkalinity. Electrical conductivity showed lowest value at university campus ($40 \mu\text{s cm}^{-1}$) and the highest values were recorded at Panchgram ($95 \mu\text{s cm}^{-1}$, $1,90 \mu\text{s cm}^{-1}$ and $85 \mu\text{s cm}^{-1}$) followed by Rosekandy tea garden ($56 \mu\text{s cm}^{-1}$). Dissolved oxygen level was generally low and remained at the $<5 \text{ mg L}^{-1}$ in all cases and free CO_2 ranged between 8.2 to 13.2 mg L^{-1} . In different study sites total alkalinity ranged between 30 mg L^{-1} to 50 mg L^{-1} . All physiochemical characteristics of water in the tadpole habitats, varied significantly ($p < 0.01$) as revealed by One-Way ANOVA. (Table 5).

Correlation coefficients (r) computed between physico-chemical properties and concentrations of two different heavy metals (Cu and Pb) in water from different sites are presented in Table 6. The concentration of Cu and Pb in water samples was significantly positively correlated with water temperature, pH, electrical conductivity and significantly negatively correlated with dissolved oxygen. Air temperature was insignificantly negatively correlated and free CO_2 and total alkalinity

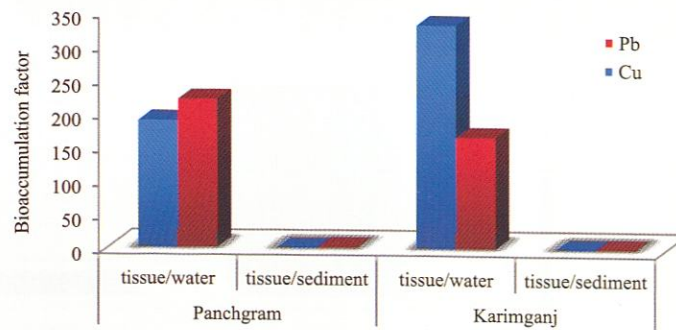


Figure 5: Bioaccumulation factor of heavy metals in whole body of *H. tigerinus*

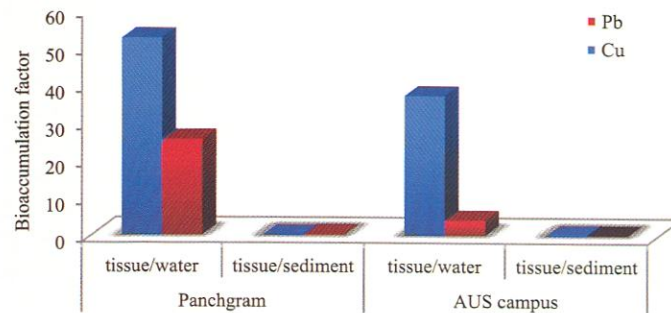


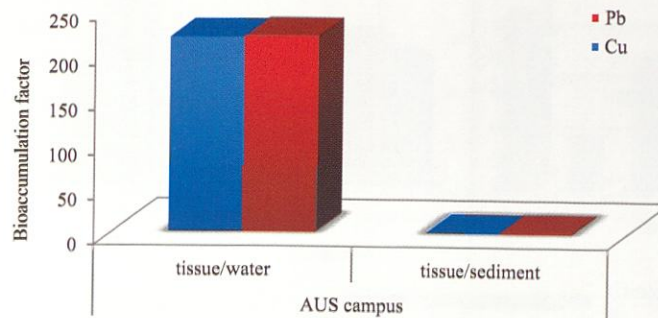
Figure 6: Bioaccumulation factor of heavy metals in whole body of *D. melanostictus*

were insignificantly positively correlated with the concentrations of copper and lead.

Discussion

Different species of tadpoles exhibit different metals accumulation rates which may be due to differences in their physiological roles. Lead concentrations were found to be highest in all the samples (whole body, sediment and water) when compared to copper. The samples collected from industrial area showed the highest concentration of lead. Urban and industrial sewage may be the source of lead in the area. Karasovet al. (2005) reported copper concentrations in whole body of *Rana clamitans melanota* and *Rana pipiens* tadpoles as 4-6.9 mg kg^{-1} and lead concentrations were below detection limit. Karasovet al. (2005) also reported that anuran species richness declined with

Figure 7: Bioaccumulation factor of heavy metals in whole body of *P. teraiensis*



increasing concentrations of cadmium, chromium and lead in pond. Birdsall et al. (1986) and Burger & Snodgrass (1998) studied lead concentration in whole body of *Rana catesbeiana* tadpoles and reported that whole body of tadpoles accumulated 20-250 mg kg⁻¹ and 5.43 mg kg⁻¹ of lead concentration respectively. Sparling & Lowe (1996) reported 9.8-15.7 mg kg⁻¹ of copper concentration and 6.7-19.7 mg kg⁻¹ of lead concentration in whole body of *Acris screpitans* tadpoles. In the present study among all the studied tadpoles highest concentrations of copper was found to be 2.07 µg g⁻¹ and lead concentration was found to be 2.42 µg g⁻¹ in whole body of *Microhyla ornata* tadpoles collected from industrial area (Panchgram). It is possible that this species being a filter feeder accumulated more from water and water of the Panchgram site contained higher concentration of lead. Barron (1995) and Singh et al. (2016a) also made similar observations that the tadpoles bioaccumulated these metals more from the water in comparison to sediment. The copper and lead concentrations recorded in the present study were lower than the value given by earlier workers (Kelepertzis et al. 2012). Lead can damage physiological processes and also accumulate in tissue (Sparling et al. 2000). It may lead to malformation of organs, deformities and prolong the development period. Heavy metals in water and sediment was found to affect occurrence of anuran species (Ficken & Byrne, 2013) and negatively correlated to species richness. They also opined that toxic heavy metal contamination is responsible for localised extinction of anuran species in Merri Creek corridor of

Victoria of south east Australia.

As evident from the present study, aquatic bodies of Barak valley are now becoming polluted by heavy metals and other pollutants. Anuran tadpoles may become susceptible to this heavy metal pollution. This is also evident from the study of Kelepertzis et al. (2012) where in it has been suggested that tadpoles be included in monitoring programs as good bioindicators of environmental pollution. From the present study also 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 were 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 management of aquatic bodies (both natural and man-made) which serve as breeding sites of anurans should be maintained properly to enable their successful breeding. 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. Creating general awareness regarding the role of anurans in the ecosystems and sensitizing the local people about the importance of conservation of aquatic habitats is needed. Future studies may be taken up on impact of specific heavy metals on different anuran tadpoles to understand acute and chronic effects.

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Duttaphrynus melanostictus.
Photo Credit: Abhijit Das



Table 1: Habitats used by anuran tadpoles from Barak Valley

Sites	Habitat types	Tadpole species
1. Panchgram (IA)	temporary water body	<i>Microhyla ornata</i>
2. Karimganj (RA)	temporary water body	<i>Microhyla ornata</i>
3. Rosekandy (TG)	temporary water body	<i>Microhyla ornata</i>
4. Panchgram (IA)	temporary water body	<i>Hoplobatrachus tigerinus</i>
5. Karimganj (UA)	paddy field	<i>Hoplobatrachus tigerinus</i>
6. Panchgram (IA)	temporary water body	<i>Duttaphrynus melanostictus</i>
7. AUS campus	temporary water body	<i>Duttaphrynus melanostictus</i>
8. AUS campus	temporary water body	<i>Polypedates teraiensis</i>

IA- Industrial area, RA-Rural area, TG- Tea garden, UA-Urban area

Table 2: Heavy metals concentrations (mean±SD) in whole body of tadpoles/water/sediment

Heavy metal concentrations ($\mu\text{g gm}^{-1}$ / mg L^{-1})*			
Species and sites		Copper (Cu)	Lead (Pb)
<i>Microhyla ornata</i> (MO1) (Panchgram)	Whole body	2.07±0.01	2.42±0.03
	Water	0.025±0.04	0.083±0.02
	Sediment	3.4±0.19	3.46±0.02
<i>Microhyla ornata</i> (MO2) (Karimganj)	Whole body	1.83±0.03	2±0.13
	Water	0.005±0.01	0.017±0.05
	Sediment	0.917±0.04	1.08±0.26
<i>Microhyla ornata</i> (MO3) (Rosekandy)	Whole body	0.6±0.03	0.868±0.03
	Water	0.2±0.08	0.036±0.27
	Sediment	2.43±0.13	2.6±1.54
<i>H. tigerinus</i> (HT1) (Panchgram)	Whole body	1.13±0.01	1.76±0.02
	Water	0.006±0.04	0.008±0.02
	Sediment	3.4±0.19	3.46±0.01
<i>H. tigerinus</i> (HT2) (Karimganj)	Whole body	1.33±0.008	0.83±0.002
	Water	0.004±0.05	0.005±0.04
	Sediment	2.07±0.10	2.96±0.07
<i>D. melanostictus</i> (D1) (Panchgram)	Whole body	0.632±0.005	1.02±0.14
	Water	0.012±0.12	0.04±0.09
	Sediment	4.27±0.06	5.55±0.08
<i>D. melanostictus</i> (DM2) (AUS campus)	Whole body	0.411±0.06	0.024±0.01
	Water	0.011±0.01	0.006±0.03
	Sediment	1.425±0.4	2.21±0.25
<i>P. teraiensis</i> (PT) (AUS campus)	Whole body	0.652±0.001	0.654±0.004
	Water	0.003±0.02	0.003±0.03
	Sediment	0.634±0.1	0.893±0.21

Table 3: Significance of differences between species among eight sites as revealed by one-way ANOVA

Species	F-value
<i>Microhyla ornata</i> (Panchgram)	42.49 _{2,14} **
<i>Microhyla ornata</i> (Karimganj)	192.8 _{2,14} **
<i>Microhyla ornata</i> (Rosekandy)	630.7 _{2,14} **
<i>Hoplobatrachus tigerinus</i> (Panchgram)	231.1 _{2,14} **
<i>Hoplobatrachus tigerinus</i> (Karimganj)	253.4 _{2,14} **
<i>Duttaphrynus melanostictus</i> (Panchgram)	234.7 _{2,14} **
<i>Duttaphrynus melanostictus</i> (AUS campus)	550.3 _{2,14} **
<i>Polypedates teraiensis</i> (AUS campus)	11.76 _{2,14} **

**Significant at the 0.01 level

Table 4: Physico-chemical properties of water in different sampling sites.

Site	WT (°C)	AT (°C)	pH	EC (µs/cm)	DO (mg/L)	FCO2 (mg/L)	TA (mg/L)
1.	25.1±0.6	28.6±0.2	6.8±0.9	95±1.8	1.52±0.2	9.4±0.2	45±2.1
2.	25.6±0.5	28.2±0.7	5.8±0.2	48±1.3	3.5±0.1	8.2±0.3	30±1.3
3.	25.3±0.2	24.8±0.9	6.4±0.6	56±1.2	2±0.3	13.2±0.5	50±1.43
4.	25.3±0.4	28.8±1.6	6.9±0.1	90±0.55	1.55±0.2	9.6±0.7	42.6±2.6
5.	25.6±0.8	28.2±1.3	5.8±0.6	48±1.6	3.5±0.6	8.2±0.4	45±2.6
6.	24.9±0.5	27±0.5	6.2±0.35	85±1.1	1.9±0.4	8.8±0.8	40±2.7
7.	23.5±0.2	28.1±1.1	5.6±0.38	40±0.6	3.7±1.1	9.6±0.2	45±0.8
8.	23.4±1.3	28.4±1.5	5.2±0.2	45±0.59	4.1±1.5	11.6±0.5	40±1.6

Table 5: Significance of differences between physico-chemical parameters (2013-2014) among eight sites as revealed by one-way ANOVA

Parameters	F-value
Water Temperature	63.24 _{7,16} **
Air Temperature	11.15 _{7,16} **
pH	659.2 _{7,16} **
Electrical Conductivity	109.7 _{7,16} **
Dissolved Oxygen	887.04 _{7,16} **
Free Carbon-dioxide	171.05 _{7,16} **
Total Alkalinity	12.25 _{7,16} **

**Significant at the 0.01 level

Table 6: Correlation coefficients between physico-chemical variables and concentration of heavy metals in water from sampling sites

Water parameters	Copper (Cu)	Lead (Pb)
Water temperature	0.165*	0.311*
Air temperature	-0.451	-0.172
pH	0.623*	0.642*
Electrical Conductivity	0.443*	0.622*
Dissolved Oxygen	-0.648*	-0.674*
Free CO ₂	0.331	0.045
Total alkalinity	0.650	0.357