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Original Article

Bioaccumulation of heavy metals in anuran tadpoles: A study in Barak Valley, Assam

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Abstract: Heavy metal pollution plays an important role in global biodiversity decline. However, there is paucity of information concerning the effects of metals on amphibians. 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 six different anuran species, *Hoplobatrachus tigerinus*, *Leptobrachium smithi*, *Clinotarsus alticola*, *Fejarvarya* sp., *Sylvirana leptoglossa* and *Euphlyctis cyanophlyctis* were selected for this purpose. Heavy metal concentrations were determined in intestine, liver and tail of tadpole samples of these species. 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}) resulting in possibilities of higher accumulation of the metal in tadpoles and decline of amphibians' population. Total concentration of Cu in the tadpoles of different species of amphibians followed the order: *H. tigerinus* > *S. leptoglossa* > *E. cyanophlyctis* > *C. alticola* > *Fejarvarya* sp. > *L. smithi*, while concentration of lead followed the order: *E. cyanophlyctis* > *C. alticola* > *S. leptoglossa* > *Fejarvarya* sp.> *H. tigerinus* > *L. smithi.*

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Introduction

Amphibians are good bio-indicators of environmental pollution due to their susceptibility to chemicals and are well known for accumulating metals during their freshwater cycles (Loumbourdis and Wray, 1998). 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 and Mullherm, 1984). Their biology and habitat selection makes them candidates for heavy metal accumulation (Hayes et al., 2010). Decline in amphibian populations, throughout the world, in recent years have caused concern among the scientific community (Ezemonye and Enuneku, 2006). The reason of decline has been attributed to habitat destruction, introduction of invasive species, over-exploitation, emerging diseases, pathogen, climate change and environmental contamination (Hayes et al., 2010). Aquatic systems become polluted with heavy metals discharged from industries and agricultural sectors. Among environmental pollutants heavy metals are of particular concern due to their potential toxic effects and ability to bioaccumulate in aquatic ecosystems (Censi et al., 2006). Bioaccumulation is the building up of a chemical to a toxic level in 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 water, sediment and aquatic animals causing heavy metal pollution in water bodies (Mallick et al., 2010).

Toxicological research on amphibians is scarce compared to other vertebrates. Metals have the tendency to accumulate in various organs of aquatic

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organisms especially in fish (Karadede et al., 2004). Frogs are opportunistic breeders and breed in wide variety of aquatic systems like ephemeral pools, small and large ponds, streams, ditch, drains, man-made tanks etc. Barak valley region has ideal habitats for breeding of anurans. The objectives of this study were to assess susceptibility of anuran tadpoles to heavy metals, Cu and Pb in Barak valley. Accordingly, six different anuran species, *Hoplobatrachus tigerinus*, *Leptobrachium smithi*, *Clinotarsus alticola*, *Fejarvarya* sp., *Sylvirana leptoglossa* and *Euphlyctis cyanophlyctis* were selected from urban, rural and industrial area and the level of Copper (Cu) and Lead (Pb) in water, sediment and selected organs of tadpoles were determined.

Materials and Methods

Barak Valley is situated in the southern part of the Assam, between 24º8'N and 25ºN latitudes and 92º15'E and 93º15'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 HPC/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 of 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 (FCO_2) and total alkalinity (TA) were analyzed using standard methods (Trivedy and Goel 1984; APHA, 2005).

Tadpoles were collected by dip net and were washed properly with double distilled water in the laboratory. Selected organs like intestine, tail and liver were removed from the tadpole samples and dried until reaching a constant weight. Digestion of all tadpole samples was done according to FAO/SIDA (1983). To each sample (0.1 g), 10 ml of perchloric acid: conc. HNO_3 (3:2 v/v) was added and the mixture was heated at 60ºC 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 and Enuneku, 2012).

The water collected in sampling bottles were pre-conditioned with dilute nitric acid (HNO_3) 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 (HNO_3) 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 45ºC 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. HNO_3). The mixture was digested on a hot plate in open beakers at 95ºC 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.

Abbreviation used: HT-*Hoplobatrachus tigerinus*, LS-*Leptobrachium smithi*, CA-*Clinotarsus alticola*, Fsp.-*Fejarvarya* sp., SL-*Sylvirana leptoglossa* and EC- *Euphlyctis cyanophlyctis.*

Results and Discussion

Table 1 presents the distribution of different species of tadpoles in different habitats. The physicochemical parameters of water are an important indication of its quality. Table 2 presents the physicochemical variables of the study sites. Air temperature ranged between 27.1ºC to 31.1ºC. Surface water temperature ranged from 22.7 to 26ºC.

Table 1. Habitat used by seven species of anuran tadpoles from Barak Valley, Assam.

Stations	**Habitat types**	**Tadpole sp.**	**Month of Collection in 2013**
1. Panchgram (IA)	Pond	*H. tigerinus*	May
2. Rosekandy (TG)	Stream	*L. smithi*	March
3. Rosekandy (TG)	Pond	*C. alticola*	September
4. Rosekandy (TG)	Temporary water bodies	*Fejervarya sp.*	March
5. Rosekandy (TG)		*S. leptoglossa*	May
6. Sildoobi (BK)		*E. cyanophlyctis*	September

IA- Industrial area, TG- Tea garden, BK- Brick kiln

Table 2. Physicochemical properties of water in different sampling sites.

Sites	**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.Panchgram (IA)	31.1±1.9	25.1±1	7.1±0.6	95±1	1.2±0.1	12.1±0.5	35.9±1.3
2.Rosekandy (TG)	28.5±0.4	23±0.9	5.4±0.3	24±1.5	3.2±0.7	6.2±0.4	45.4±3.1
3.Rosekandy (TG)	27.1±0.3	24.1±0.3	6.5±0.3	46.6±1.5	3±0.1	10.5±0.3	30.6±1.5
4.Rosekandy (TG)	28.2±0.9	22.7±0.9	5.4±0.3	31.3±1.5	2.9±0.4	6.3±0.5	50.2±2.3
5.Rosekandy (TG)	29.5±0.7	24.2±0.3	5.6±0.1	24.3±1.5	2.3±0.3	6.8±0.2	30.1±1.6
6.Sildoobi (BK)	28.2±0.3	26±0.2	7±0.1	69.6±0.5	1.2±0.1	11.1±0.2	42.6±2.1

Table 3. Heavy metal concentrations in different species of tadpole, sediment and water in different sampling sites (IA- Industrial area, TG- Tea garden, BK- Brick kiln).

Species	**Sites**	**Heavy metal concentration**					
		Tadpoles (µg gm^{-1})		**Sediment (µg gm^{-1})**		**Water (mg L^{-1})**	
		Copper	**Lead**	**Copper**	**Lead**	**Copper**	**Lead**
1. *H. tigerinus*	IA	2.599	0.114	0.342	0.245	1.83	1.42
2. *L. smithi*	TG	0.0052	0.084	0.0009	0.01	0.003	0.017
3. *C. alticola*	TG	0.064	1.77	0.73	0.91	1.09	0.325
4. *Fejervarya sp.*	TG	0.037	0.288	0.0009	0.007	0.002	0.013
5. *S. leptoglossa*	TG	2.456	1.476	3.135	0.445	0.815	0.341
6. *E.cyanophlyctis*	BK	1.91	4.632	0.345	3.335	1.675	1.085

The pH varied between 5.4-7.1 and conductivity ranged between 24 µs cm^{-1} to 95 µs cm^{-1}. Dissolved O_2 ranged between 1.2 mg L^{-1} to 3.2 mg L^{-1} and free CO_2 ranged between 6.2 to 12.1 mg L^{-1}. Total Alkalinity ranged between 30.1 mg L^{-1} to 50.2 mg L^{-1}. pH in the water bodies inside the Rosekandy tea estate were found to be acidic while the ponds in the industrial areas had pH ranging between 7-7.1.

Concentrations of Cu and Pb in water, sediment and tadpoles from different sites are presented in Table 3. Metal concentration in water depends in part on water quality and watershed influxes (Mason, 2002). Metal uptake and their toxicity in aquatic fauna are influenced by many factors such as pH, hardness, alkalinity and temperature of water (Osman and Kloas, 2010). A few studies have correlated aqueous concentrations of metals with other chemical parameters (Stephenson and Mackie, 1988, Watras et al., 1998). For instance, aqueous Cd, Pb, and Zn have been related to pH, dissolved oxygen, carbonate, and nutrients (Prahalad and Seenayya, 1989).

Correlation coefficients (r) computed between physicochemical properties and the concentration of Cu and Pb (Table 4) showed that the metal concentrations were significantly and positively correlated with pH, conductivity, free CO_2 and water temperature. The concentration of Cu and Pb were significantly and negatively correlated with dissolved oxygen which is similar to the findings of Singh et al. (2008) and insignificantly negatively correlated with alkalinity of water. Therefore, water quality

Table 4. Correlation coefficients between physico-chemical variables and concentration of heavy metals in water from sampling sites.

Water parameters	Copper (Cu)	Lead (Pb)
pH	**0.954****	**0.911***
Conductivity	**0.874***	**0.951****
Dissolved Oxygen	**-0.866***	**-0.949****
Free CO_2	**0.935****	**0.875***
Total alkalinity	-0.515	-0.267
Water temperature	**0.943****	**0.883***

Significant level at P values: * <0.05, ** <0.01

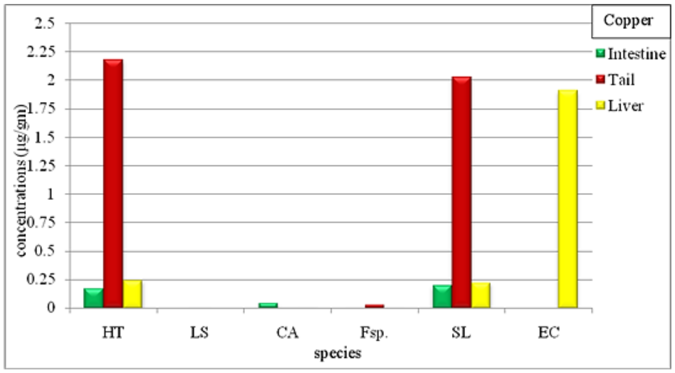

Figure 1. Copper concentrations (µg gm^{-1}) in intestine, tail and liver in six different species collected from six different sites.

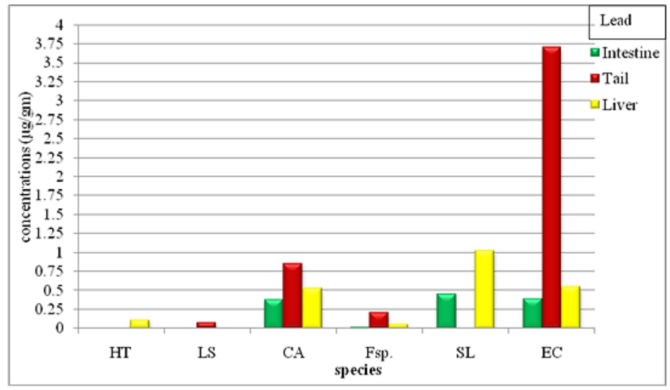

Figure 2. Lead concentrations (µg gm^{-1}) in intestine, tail and liver in six different species collected from six different sites.

parameters that affected metal concentration in water were pH, conductivity, free CO_2, DO and water temperature.

Among different species of tadpoles, the total concentration of copper was in the order of *H. tigerinus* > *S. leptoglossa* > *E. cyanophlyctis* > *C. alticola* > *Fejarvarya* sp. > *L. smithi*, while concentration of lead was in the order of *E. cyanophlyctis* > *C. alticola* > *S. leptoglossa* > *Fejarvayra* sp. > *H. tigerinus* > *L. smithi*. In *H. tigerinus* and *S. leptoglossa*, copper concentration was more in comparison to lead concentration. In tadpoles of *L. smithi*, *C. alticola* and *Fejarvayra* sp. and in sediment and water samples collected from the habitat, lead concentration was higher than copper concentration. In *E. cyanophlyctis* and in sediment sample lead concentration was more than the copper concentration but in water samples copper concentration was higher than the lead concentration.

In the sampled tadpoles, concentration of copper in intestine was in the order of *S. leptoglossa* > *H. tigerinus* > *C. alticola* > *Fejarvayra* sp. > *L. smithi* > *E. cyanophlyctis* and lead (Pb) in the order of *S. leptoglossa* > *E. cyanophlyctis* > *C. alticola* > *Fejarvarya* sp. > *L. smithi* > *H. tigerinus*. Among different species of tadpoles *E. cyanophlyctis* accumulated highest concentration of copper in tail while least accumulation was recorded in the tail of *L. smithi*. *Sylvirana leptoglossa* accumulated highest concentration of lead in tail. Copper concentration was high in tail and liver in *E. cyanophlyctis* in comparison to other tadpoles. Lead concentration was also high in liver of *E. cyanophlyctis*. (Fig. 1 and 2).

Highest copper concentration was found in the sediment sample collected from the habitat of *S. leptoglossa*, while highest concentration of lead was recorded in the sediment sample collected from the habitat of *E. cyanophlyctis*. In water samples highest copper and lead concentrations were recorded in the habitat of *H. tigerinus* which is an industrial area (Fig. 3).

Copper concentration in tadpoles were positively and significantly correlated with the concentration copper in water (r=0.725; Fig. 4), while concentration of lead in tadpole was positively and significantly correlated with concentration of lead in

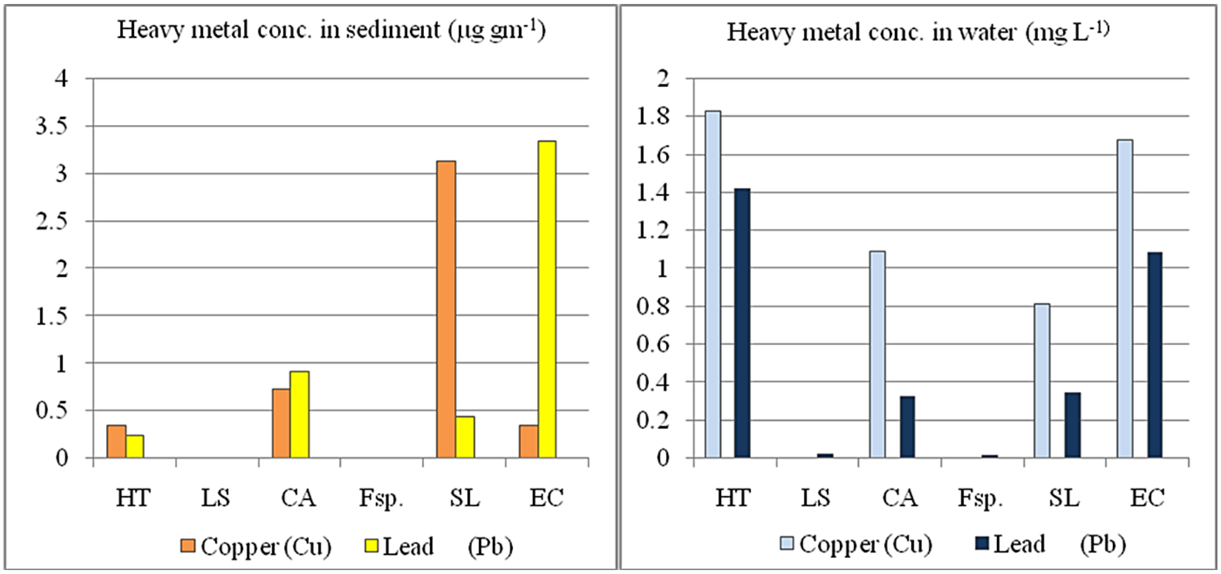

Figure 3. Heavy metal concentrations in sediment (left) and water (right) collected from the habitat of tadpole species.

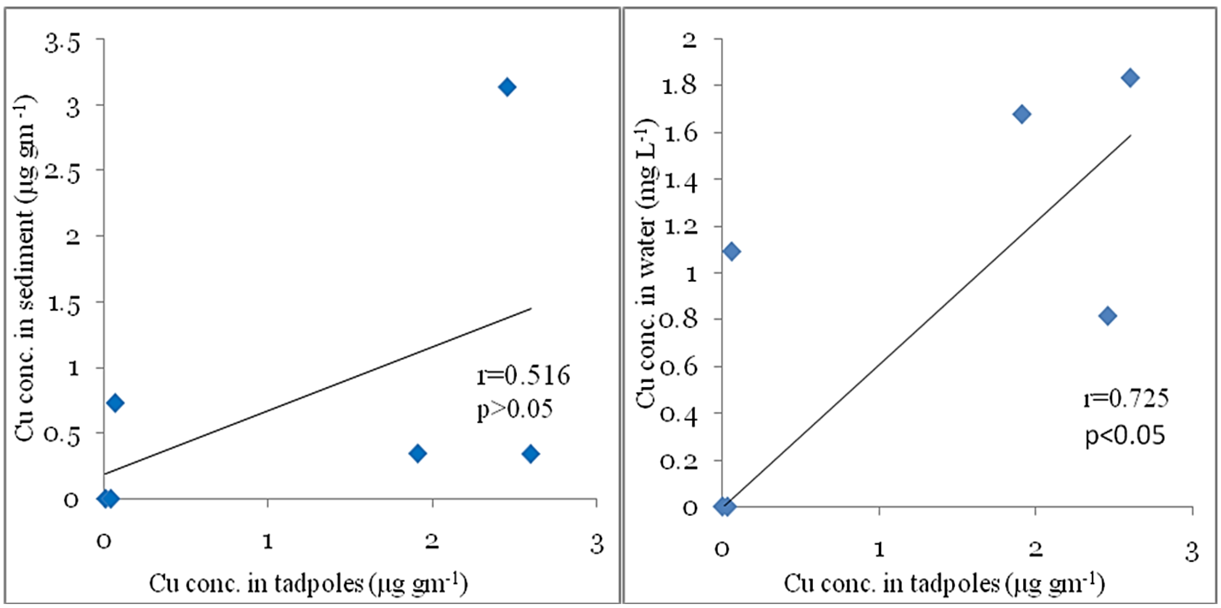

Figure 4. Correlation between copper concentrations in tadpoles and the corresponding concentrations in sediment (left) and water (right).

sediment (r=0.944; Fig. 5). Different species of tadpoles exhibit different metals accumulation rates. All the tadpoles collected for heavy metal analysis belongs to the stage 26-35 (Gosner, 1960). The result revealed that the concentration of lead in water samples was higher than the permissible limit of 0.01 mg L^{-1} but copper concentration was within the maximum permissible limit of 2 mg L^{-1} (WHO, 2011). The analysis for heavy metals (Cu and Pb) confirmed that the sediment concentrated more heavy metals than water. High concentration of heavy metal accumulation recorded in the study sites may be due to the excessive use of herbicides, chemicals, industrial effluents and other wastes in tea gardens. Shaapera et al. (2013) studied the concentrations of seven heavy metals (Pb, Cu, Zn, Cr, Fe, Cd and Mn) in the organs of adult *Rana esculenta* obtained from River Guma, Benue State of Nigeria and found that the trend of the heavy metals concentration in the organs were Fe > Mn > Pb > Zn > Cu > Cr > Cd. The present study also reveals that in most of the samples Pb was higher than the Cu. Sparling et al. (2000) reported that in amphibians, Pb exposure resulted in a range of effects including decrease of erythrocytes and leucocytes, neutrophils and monocytes, sloughing of the skin, excessive bile

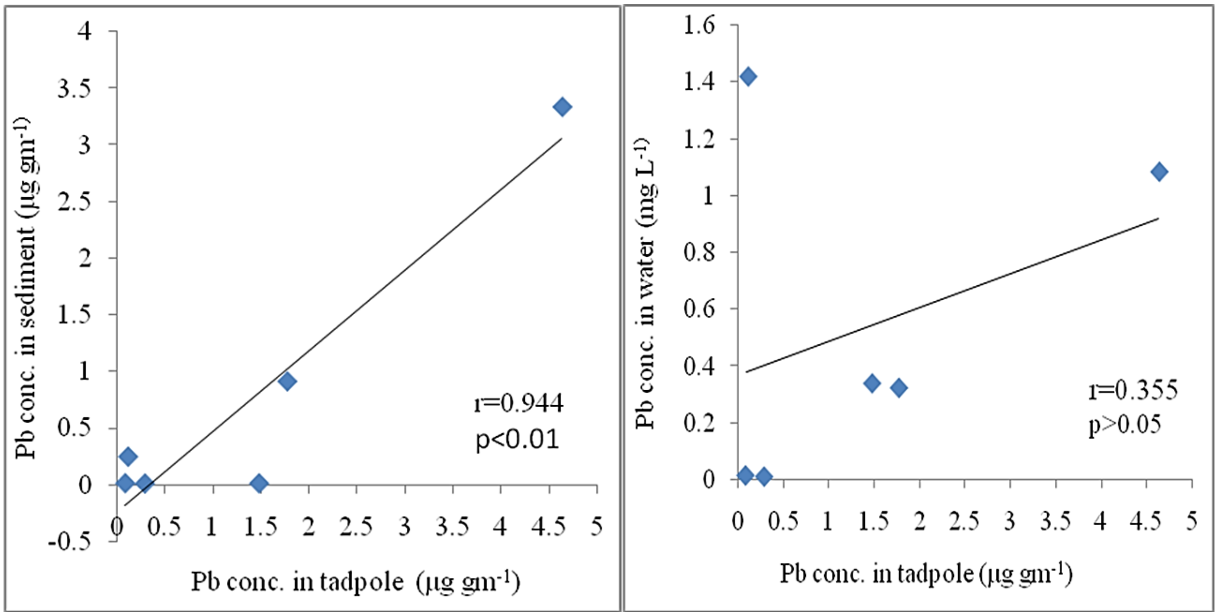

Figure 5. Correlation between lead concentrations in tadpoles and the corresponding concentrations in sediment (left) and water (right).

secretion, hypertrophy of liver, spleen and stomach, decreased muscle tone and loss of normal semi-erect posture, salivation, excitement and muscular twitching; and delayed metamorphosis. Copper is an essential element that serves as a cofactor in a number of enzymes systems and necessary for the synthesis of hemoglobin (Sivaperumal et al., 2007) but very high intake of Cu can cause adverse health effect problems for most living organism.

Various researchers have reported strong positive correlations between Pb concentrations in sediments and those in tadpoles (Sparling and Lowe, 1996). Karasov et al. (2005) exposed tadpoles along a pollution gradient in the Fox River and Green bay ecosystem in Wisconsin and found a positive correlation between levels in sediments and tadpoles for Cd, Cr, and Pb. In the present study, positive correlations was found between Pb and Cu in tadpoles and the respective levels in the sediment and water. Kelepertzis et al. (2012) reported that the significant correlation found between Pb and Cu in tadpoles and the respective levels in the sediments and water indicate that sediments provided the bulk of these metals for tadpoles. They also reported that differences in tadpole concentrations for the majority of analyzed metals were due to different metal levels in the sediments where they develop and breed. Amphibian larvae accumulated the highest concentration of most trace elements, possibly due to their feeding ecology. Since trace metals bioaccumulate in the food web, monitoring the quantities entering the trophic dynamics of the food chain can provide early warning systems on the potential environmental health consequences of current anthropogenic activities in the agricultural and industrial sectors. The edible frogs are an important link in the trophic transfer to higher vertebrates including birds, mammals, reptiles, predatory fish and man (Tyokumbur and Okorie, 2011). Several problems, such as delayed development, morphological deformities, decreased growth and size at metamorphosis are related to heavy metals (Sparling et al., 2006) and further investigation and monitoring of heavy metal residues in the environment is seriously necessary.

It was concluded from this study that environment of Barak valley is now becoming polluted by heavy metals and anuran tadpoles have become susceptible to this heavy metal pollution.

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References

APHA. (2005). Standard methods for the examination of water and waste water analysis, 21st ed. Washington, DC. 964 p.

ASTM. (1998)American society for testing of materials. Standard practice for conducting toxicity tests with fishes, Macroinvertebrates and Amphibians. ASTM, Philadelphia. Publication No. E729-80.

Censi P., Spoto S.E., Saiano F., Sprovieri M., Mazzola S., Nardone G., Di Geronimo S.I., Puntu R., Ottonello D. (2006). Heavy metals in coastal water system. A case study from the north western Gulf of Thailand. Chemosphere, 64: 1167-1176.

Ezemonye L.I., Enuneku A.A. (2006). Stage-dependent acute toxicity of exposure of *Bufo maculatus* and Ptychadena bibroni tadpoles to cadmium (Cd^{2+}). Journal of Applied Science and Technology, 11(1&2): 78-82.

Ezemonye L.I., Enuneku A.A. (2012). Hepatic bioaccumulation of cadmium in the crowned bullfrog, *Hoplobatrachus occipitalis* and flat backed toad, *Bufo maculates*. International Journal of Aquatic Science, 3(1): 15-22.

FAO/SIDA (1983). Manual of methods in aquatic environmental research part 9. Analysis of metals and organochlorines in fish. FAO fisheries/Technical paper 212.

Gosner K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica, 16: 183-190.

Hall R.J., Mulhern, B.M. (1984). Are anuran amphibians heavy metal accumulators?. In: R.A. Seigel, L.E. Hunt, J.L. Knight, L. Malaret, N.L. Zuschlag (Eds.). Vertebrate ecology and systematics: a tribute to Henry S. Fitch. The University of Kansas Museum of Natural History, Lawrence, KS, USA. pp. 123–133.

Hayes T.B., Falso P., Gallipeau S., Stice M. (2010). The cause of global amphibian declines: a developmental endocrinologist's perspective. Journal of Experimental Biology, 213: 921-933.

Karadede H., Oymak S.A., Unlu E. (2004). Heavy metals in mullet, *Liza Abu*, and cat fish, *Silurus triotegus*, from the Ataturk Dam Lake (Euphrates), Turkey. Environment International, 30: 183-188.

Karasov W.H., Jung R.E., Langenberg S.V., Bergeson T.L.E. (2005). Field exposure of frog embryos and tadpoles along a pollution gradient in the Fox River and Green Bay ecosystem in Wisconsin, USA. Environmental Toxicology and Chemistry, 24: 942-953.

Kelepertzis E. Argyraki A., Daftsis E. (2012). Geochemical signature of surface water and stream sediments of a mineralized drainage basin at NE Chalkidiki, Greece: a pre-mining survey. Journal of Geochemical Exploration, 114: 70-81.

Loumbourdis N.S., Wray D. (1998). Heavy metal concentration in the frog *Rana ridibunda* from a small river of Macedonia, Northern Greece. Environment International, 24: 427-431.

Malik N., Biswas A.K., Qureshi T.A., Borana K., Virha R. (2010). Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. Environmental Monitoring and Assessment, 160: 267-267.

Mason C.F. (2002). Biology of freshwater pollution. 4th ed. Essex Univ. England. 387 p.

Osman A.G., Kloas W. (2010). Water quality and heavy metal monitoring in water, sediment, and tissues of the African catfish *Clarias gariepinus* (Burchell, 1822) from the River Nile, Egypt. Journal of Environmental Protection, 1: 389-400.

Prahalad A.K., Seenayya G. (1989). Physico-chemical interactions and bioconcentration of zinc and lead in the industrially, polluted Husainsagar Lake, Hyderabad, India. Environmental Pollution, 58: 139-154.

Shaapera S., Nnamonu L.A., Eneji I.S. (2013). Assessment of heavy metals in *Rana esculenta* organs from River Guma, Benue State Nigeria. American Journal of Analytical Chemistry, 4: 496-500.

Singh A.P., Srivastava P.C., Srivastava P. (2008). Relationships of heavy metals in natural lake waters with physico-chemical characteristics of waters and different chemical fractions of metals in sediments. Water, Air and Soil Pollution, 188: 181-193.

Sivaperumal P., Sankar T.V., Nair V. (2007). Heavy metals concentrations in fish, shellfish and fish products from internal markets of India vis-à-vis international standards. Food Chemistry, 102: 612-620.

Sparling D.W., Krest S., Ortiz-Santaliestra M. (2006).

Effects of lead contaminated sediment on *Rana sphenocephata* tadpoles. Archives of Environmental Contamination and Toxicology, 51: 458-466.

Sparling D.W., Linder G.L., Bishop C.A. (2000). Eco-toxicology of Amphibians and Reptiles. Society of Environmental Toxicology and Chemistry, 2nd Edition, Par- sacola, Fla, 877 p.

Sparling D.W., Lowe T.P. (1996). Metal concentrations of tadpoles in experimental ponds. Environmental Pollution, 91: 149-159.

Stephenson M., Mackie G.L. (1988). Total cadmium concentrations in the water and littoral sediments of central Ontario Lakes. Water, Air and Soil Pollution, 38: 121-136.

Trivedy, R.K., Goel, P.K. (1984). Chemical and Biological Methods of Water Pollution Studies. Environmental Publications, Karad, India. 215 p.

Tyokumbur E.T., Okorie T.G. (2011). Macro and trace element accumulation in edible crabs and frogs in Alaro stream ecosystem, Ibadan. Journal of Research in National Development, 9(2): 439-446.

Watras C.J., Back R.C., Halvorsen S., Hudson R.J.M., Morrison K.A., Wente S.P. (1998). Bioaccumulation of mercury in pelagic freshwater food webs. Science of the Total Environment, 219: 183-208.

World Health Organization WHO. (2011). Iron, Zinc, Copper, Manganese, Cadmium and Lead in drinking-water. Guidelines for drinking-water quality, Geneva, World Health Organization. 4th ed.