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Heavy metal accumulations in three different species of anurans from five sites of Western Ghats, India

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ABSTRACT

Toxic manifestation of heavy metals namely cobalt, copper, manganese, nickel and zinc are well known. Accumulation of these metals in the body shows adverse effect in living organisms, its growth, health, life span and reproductive performance. As amphibians are very sensitive to environmental change present study was conducted. In order to assess the concentration of cobalt, copper, manganese, nickel and zinc in the liver and muscle of *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis* and *Duttaphrynus melanostictus* five stations from the Western Ghats were selected. Heavy metal concentrations in the tissues tended to vary significantly among stations, and one station thought to be contaminated by industrial effluent showed particularly high metal concentration. All heavy metals were higher in concentrations in the liver than muscle except for zinc. The results provide some evidence for the potential of the anurans as a biomonitors for heavy metal pollution. The results of this study indicated that the metals present in the environment were taken by the anurans through food, water and soil.

KEYWORDS

Biomonitoring;
Heavy metals accumulation;
Western ghats;
Anurans.

INTRODUCTION

The metals are intrinsic components of earth crust. The environmental contamination of these metals is due to the rapid development and the evolution of metal-based industries. Heavy metals have accumulated in the environment, and amphibians can be quite susceptible to them[18]. Frogs have been reported to be sensitive to a range of environmental pollutants including agricultural pesticides[12], herbicides[14], fungicides[4] and heavy metals[7]. The contaminants in the amphibians may indicate the possible presence of toxins in other animals that share the same habitat[16]. Anthropogenic activities, mainly industry, agriculture and transport are sources of the occurrence of heavy metals in the environment. Metals are persistent pollutants that can be biomagnified in the food chain, becoming increasingly dangerous to human and wildlife[8]. Heavy metals are major problem because they are toxic and tend to accumulate in living organisms[13]. It was reported that accumulation of heavy metal like Cd, Pb, Hg and Ar shown the adverse effect on life span. This has led to the development of monitoring schemes aimed at directly measuring levels of contaminants in various organisms, and biomonitoring schemes that use indicator species to estimate the levels in other parts of the ecosystem[17]. Direct and indirect methods have been developed to detect heavy metals quantitatively and qualitatively and to evaluate the level of pollution in an area. Direct methods are applied to soil and water; indirect methods use plant and animals as bioindicators[9,10,13]. Analysing pollutants in living organisms is more attractive and promising than analysing pollutants of the abiotic environment, as living organisms provide precise information about the bioavailability of pollutants and the magnification and biotransformation of pollutants[15].

According to Hecnar and M'Closkey[5,6] the amphibian fauna may provide an important indicator of the impact of anthropogenic disturbance to wetland ecosystem. Amphibians are known to be very specific as far as their environmental requirements are concerned. Because of their biphasic lifecycle, permeable eggs, skin and gills; amphibians are often considered to be sensitive to environmental contaminants[1,2,18]. Frogs have been used as bio-indicators for some time but their use is not widespread. It is known that frogs and especially tadpoles are sensitive to a range of water borne substances making them suitable candidates as bio-indicators. Frogs have been used in some places to measure environmental pollution. Anuran amphibians are expected to be well suited as indicators of certain types of environmental pollution, as their life cycles generally include both an aquatic and terrestrial phase. Their diets are also known, herbivorous as tadpoles, insectivorous as adult, so that sources of uptake of pollutants may be discernible[7].

MATERIAL AND METHODS

Study sites are located in the Western Ghats, which runs continuously north south between 8 and 21° N latitude and parallel to India's western coast. The study sites are covering two coastal districts Sindhudurg and Ratnagiri in Maharashtra having excellent mosaic of habitats, which range from almost untouched to highly degrade. The habitats include wetlands, forests, hill slopes, agricultural lands, plantations, human settlements, and industrial lands.

The Amboli (15° 57' N 73° 59'E)(site 1) site is considered as control site over all other study sites. The Amboli is almost undisturbed site as there is no industrial pollution and very less agricultural practices. Malvan (16° 03' N 73° 46' E)(site 2) and Vengurla (15° 87' N 73° 63' E)(site 3) are two coastal sites having paddy fields, mango, cashew and coconut plantations. These are also developing towns in the Konkan area Lanja (16° 85' N 73° 65' E)(site 4) has good number of commercial plantations on the hill slopes where there is use of fertilisers and pest control chemicals which get in to the natural water bodies. Lote (17° 57' N 73° 63' E) (site 5) is the industrial area where all the effluent coming from the industries like chemical and fertilisers directly get mixed in to the natural water bodies.

Sample collection and analysis

The adult individuals of *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis* and *Duttaphrynus melanostictus* were collected from five study sites in the monsoon period of 2005 and 2006. Each selected species represent a diverse habitat. The *Duttaphrynus melanostictus* is terrestrial, *Hoplobatrachus tigerinus* is semi aquatic or dwelling near water bodies and *Euphlyctis cyanophlyctis* is exclusively aquatic.

The collected live individuals of the target species were brought to the laboratory. These individuals were sacrificed and liver and muscles were removed immediately and used for the heavy metal accumulation determination. The tissues were weighed and cut into small pieces, dried in an oven at 80°C for 48 hours and powdered with pestle and mortar. Dried tissue powder was digested with 5ml HNO_3 and 5ml Perchloric acid over a hot plate until all material gets dissolved. Digests were further diluted with distilled water[3]. All the heavy metals (Cu, Ni, Zn, Co and Mn) were analysed using a Perkin-Elmer 300 A Analyst Atomic Absorption Spectrophotometer. The concentration of heavy metal in the tissues was expressed as mean and Standard Deviation in μ gm/gm of dry tissue weight.

RESULT AND DISCUSSION

Mean concentration and associated standard de-

TABLE 1 : Mean and standard deviation metal concentrations in the liver of three species from the five study site. (Heavy metal accumulation in μgm/gm of dry tissue weight)

Liver						
Species	**Sites**	**Co**	**Cu**	**Mn**	**Ni**	**Zn**
	1	0.26 ± 0.03	4.31 ± 0.37	2.23 ± 0.09	0.35 ± 0.04	2.12 ± 0.04
	2	0.72 ± 0.03	12.24 ± 0.22	4.06 ± 0.08	0.62 ± 0.03	4.11 ± 0.06
Hoplobatrachus tigerinus	3	0.75 ± 0.02	13.39 ± 0.30	4.40 ± 0.09	0.71 ± 0.02	4.20 ± 0.05
	4	0.76 ± 0.04	14.07 ± 0.24	4.59 ± 0.13	0.71 ± 0.03	4.55 ± 0.17
	5	0.90 ± 0.04	20.34 ± 0.30	6.26 ± 0.13	0.91 ± 0.04	6.34 ± 0.12
	1	0.41 ± 0.02	13.21 ± 0.50	1.60 ± 0.16	0.33 ± 0.01	1.96 ± 0.17
	2	0.73 ± 0.02	17.69 ± 0.56	4.47 ± 0.23	0.53 ± 0.02	2.15 ± 0.01
Euphlyctis cyanophlyctis	3	0.76 ± 0.02	18.26 ± 0.48	4.78 ± 0.19	0.59 ± 0.01	2.18 ± 0.02
	4	0.60 ± 0.03	18.12 ± 0.43	5.01 ± 0.10	0.98 ± 0.02	4.14 ± 0.21
	5	0.94 ± 0.06	22.21 ± 0.59	6.96 ± 0.09	1.00 ± 0.07	5.40 ± 0.31
	1	0.35 ± 0.06	40.76 ± 1.54	2.11 ± 0.14	0.10 ± 0.01	3.09 ± 0.41
	2	0.41 ± 0.04	45.59 ± 2.04	0.44 ± 0.02	0.97 ± 0.12	8.16 ± 0.37
Duttaphrynus melanostictus	3	0.20 ± 0.01	40.51 ± 1.38	2.52 ± 0.43	0.11 ± 0.02	4.16 ± 0.30
	4	0.25 ± 0.04	42.66 ± 1.47	1.16 ± 0.17	0.94 ± 0.05	6.59 ± 0.37
	5	0.45 ± 0.04	47.33 ± 1.99	2.78 ± 0.35	1.05 ± 0.26	7.90 ± 0.78

TABLE 2 : Mean and standard deviation metal concentrations in the muscle of three species from the five study site. (Heavy metal accumulation in μgm/gm of dry tissue weight)

Muscle						
Species	**Sites**	**Co**	**Cu**	**Mn**	**Ni**	**Zn**
	1	0.04 ± 0.01	1.36 ± 0.06	1.23 ± 006	0.20 ± 0.01	5.51 ± 0.22
	2	0.13 ± 0.01	0.44 ± 0.02	2.36 ± 0.03	0.41 ± 0.01	10.14 ± 0.05
Hoplobatrachus tigerinus	3	0.17 ± 0.01	2.52 ± 0.43	2.51 ± 0.03	0.46 ± 0.01	11.10 ± 0.08
	4	0.20 ± 0.01	4.64 ± 0.10	3.26 ± 0.05	0.46 ± 0.01	10.75 ± 0.27
	5	0.26 ± 0.02	7.65 ± 0.09	5.54 ± 0.04	0.66 ± 0.03	13.53 ± 0.30
	1	0.05 ± 0.01	2.37 ± 0.23	1.70 ± 0.06	0.11 ± 0.01	6.68 ± 0.12
	2	0.11 ± 0.01	3.08 ± 0.04	2.63 ± 0.04	0.29 ± 0.02	10.20 ± 0.09
Euphlyctis cyanophlyctis	3	0.15 ± 0.01	3.26 ± 0.05	2.74 ± 0.03	0.39 ± 0.02	10.60 ± 0.07
	4	0.22 ± 0.03	4.02 ± 0.14	3.06 ± 0.12	0.42 ± 0.02	12.51 ± 0.19
	5	0.44 ± 0.02	6.02 ± 0.14	4.49 ± 0.21	0.62 ± 0.03	14.76 ± 0.08
	1	0.19 ± 0.01	2.31 ± 0.13	1.13 ± 0.04	0.38 ± 0.06	3.38 ± 0.09
	2	0.25 ± 0.02	3.30 ± 0.14	2.30 ± 0.04	0.55 ± 0.05	4.62 ± 0.06
Duttaphrynus melanostictus	3	0.28 ± 0.02	3.26 ± 0.08	2.40 ± 0.01	0.58 ± 0.04	4.67 ± 0.06
	4	0.27 ± 0.02	3.34 ± 0.19	4.15 ± 0.17	0.68 ± 0.03	4.67 ± 0.09
	5	0.31 ± 0.02	3.87 ± 0.21	5.20 ± 0.20	0.86 ± 0.05	5.03 ± 0.10

viation of cobalt, copper, manganese, nickel and zinc in the liver and muscle of *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, and *Duttaphrynus melanostictus* in five study sites selected from the Western Ghats were given in TABLE 1 and 2.

The highest cobalt content in the liver was reported from the individuals of *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis* 0.90 ± 0.04 and 0.94 ± 0.06μgm / gm of dry tissue weight respectively from the Lote (site 5). The lowest concentration was recorded from the Vengurla (site 3) in *Duttaphrynus melanostictus* (0.20 ± 0.01μgm). In muscles the highest content was 0.44 ± 0.02μgm at Lote (site 5) in *E. cyanophlyctis* while lowest at Amboli (site 1) in *H.*

tigerinus (0.04 ± 0.01 μgm). For copper content in the liver the maximum content was from the Lote (site 5) in *Duttaphrynus melanostictus* (47.33 ± 1.99μgm) and lowest content was in *Hoplobatrachus tigerinus* (4.31 ± 0.37μgm) from Amboli (site 1). The highest and lowest values were reported in the *Hoplobatrachus tigerinus* (7.65 ± 0.09, Lote (site 5) and 0.44 ± 0.02μgm, Malvan (site 2)).

The highest manganese content was in the liver of *E. cyanophlyctis* and in the muscle of *H. tigerinus* from the Lote (site 5). The lowest content of manganese in both the tissues were reported lowest in *D. melanostictus*. Increased amount of heavy metal in Lote and Malvan could be due to contamination of water by industrial discharge and horticultural practices.

The highest and lowest Nickel content were found in liver of *D. melanostictus* from the Lote (site 5) and Amboli (site 1) respectively. The highest content in the muscle was recorded in *D. melanostictus* from Lote (site 5) while lowest content in *E. cyanophlyctis* from Amboli (site 1).

The liver of *D. melanostictus* contents highest amount of zinc from Malvan (site 2) while liver of *H. tigerinus* has lowest zinc content. The highest amount zinc was found in the muscle of *E. melanostictus* from Lote (site 5) while lowest content was in the muscle of *D. melanostictus* from Amboli (site 1).

It was convincingly clear that for all the three test species, in liver and muscle, the highest accumulation of the five heavy metals was at Lote (site 5) and the lowest was at Amboli (site 1). Except in the liver of *Duttaphrynus melanostictus* in Malvan (site 2) and Vengurla (site 3) for copper, manganese and zinc, where it did not follow the general trend.

The TABLE 1 shows that the *Duttaphrynus melanostictus* show very high accumulation of the Cu in all selected site as compared with the other species collected from the same site. Whereas same species had shown the low concentration of the Mn in the liver. Also the lowest concentration was observed in Amboli (site 1) in *Hoplobatrachus tigerinus* and highest concentration in the *Duttaphrynus melanostictus* at the Lote (site 5). It indicates that different species of the anurans have the different tendency of heavy metal accumulation. For example as mentioned above the *Duttaphrynus melanostictus* shown high accumulations of the Mn as compared to other species from same site. In same way *Euphlyctis cyanophlyctis* having the minimum capacity for the accumulation of the Zn.

In the studies by Loumbourdis and Wray (1998) the concentration of 14 heavy metals in the tissue of the frog *Rana ridibunda*, living in a small river of Macadonia, Northern Greece, high concentrations of copper, chromium, molybdenum, zinc, manganese, and aluminium, corresponding to the highly polluted areas, were detected from the liver of the frog. Also it has been reported by Misyura *et al*. (1998) that highlighted influence of iron mining industry on biochemical parameters of amphibians. According to their study, influence of heavy metals like Fe, Mn, Ni, Zn, Pb, Cd brings about changing level of carbohydrates, lipids and protein metabolism. There is also reduction in the comparative weigh of detoxification organs like liver, kidney, spleen, heart and lungs.

Environment act as a sink for deposition of different pollutants and therefore it can be concluded by this study i.e. metals were taken by the anurans through food, water and soil. The present studies on the heavy metal toxicity in the tissues of the test anurans confirm the levels of toxic metal concentrations at the study sites and their obvious impact on the accumulation levels of the metals by the amphibians.

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