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Biomonitoring of Fresh Water of Loktak Lake, India

By

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Abstract
The fresh water Mollusca (Scientific name; *Pila globosa*) was collected from nine different stations of Loktak lake to investigate the elemental profile in the tissue of it as it can be used as biomonitor of the pollution of water. As the Gastropoda family is a feeder filter, it is very useful to examine the trace elements as well as toxic elements in the environment where it survives. The elements detected in the samples are Mg, Al, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, Sr and the elemental concentrations were compared with FAO limits to check their toxic levels. The elements detected so far are the time integral of their concentration so, that are several orders of magnitude above ambient water concentrations. The analysis of the elements was done with the help of energy dispersive X-ray fluorescence (EDXRF) for its quick and multi-elemental detection capability.

Keywords: Pila globosa, Biomonitoring, Loktak lake, Trace elements, EDXRF

1. Introduction

Biomonitor means the species that accumulate trace elements in their tissue and therefore can be used to monitor the availability of these elements in the particular environment where they survive. The ultimate aim in ecotoxicology is to predict the effects and to diagnose the causes of ecological/biological effects that resulted from exposure to chemicals (heavy metals) and environmental stresses [1]. The pollution levels of the aquatic environment by heavy metals can be estimated by analysing water, sediments and marine organisms. Chemical analysis of the water does not provide information on the bioavailability of metals present in the environment [2]. Again, while it is possible to utilize direct chemical analyses of water and sediment which are usually very sensitive and accurate, monitoring systems which employ direct analysis do not necessarily reflect the true ecological state, for several reasons [3]. First, the number and

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range of pollutants known to be discharged to the environment far exceeds the capability of available chemical tests, not to mention unknown pollutants. Second, pollutants may act synergistically, which limits the value of direct measurements dramatically. Finally, the true threat to the environment and its inhabitants also depends on the biological availability of suspected agents, which cannot be accounted for by chemical means. Moreover, biomonitors, exclusively provide time-integrated measures of the bioavailable levels of substance [4]. One of the criteria when an organism is proposed as a biomonitoring agent is a simple correlation between pollutant levels present in the organism and those in its environment [5]. Molluscas are filter-feeders and thus obtain heavy metals not only from food and water but also from ingestion of inorganic particulate materials. Gastropoda mollusks [6-8] were employed as bio-indicator to determine the effect of marine pollution. These organisms were considered as appropriate bio-indicators since they are available all year long and easily collected. In addition to it, they are found in all types of surface water systems [9].
Loktak lake is considered as the lifeline of Manipur, due to its importance in the socioeconomic and cultural life of the people. It is the largest natural fresh water lake in the north-eastern region of India with an area of 236.21 km^2 and plays an important role in the ecological and economic security of the region. The sewage and other waste products from industries in the Imphal town are drained into Nambul river and help directly to dump into the Loktak lake. The quality of water of this river is badly affected while it enters the city as the sewage, waste products from industries are dumped into it. Population of 0.28 million living within Nambul river catchments generates 31,207 cubic metre of sewage daily. With rapid urbanization, industrialization and increasing population of the city the river gets polluted day by day leading to the increase of pollution levels of Loktak lake. The pollution of the lake is also being increased day by day due to the use of excessive amount of fertilizers, pesticides, insecticides and fungicides by the farmers, cultivators and people around the lake. Trace metals and their compounds are the non-degradable pollutants and they cannot be destroyed. Hence, the pollution of the lake is monitored with the help of bio-indicator viz. *Pila globosa,* Family: Gastropoda which is also used as a food item. The EDXRF technique was used for trace elemental analysis of the bioindicator samples for its quick, powerful, reliable and multi-elemental detection capability. Here, the term 'trace metal' suggests occurrence at low (trace) concentrations (sometimes defined as 0.01% dry weight) in the environment, in both physical and biotic components, yet all of these metals do occur in raised concentrations in ores and some spectacularly so in organisms, as will be demonstrated. Moreover, the comparative study of the elements present in *Pila globosa* with that of FAO limits, a valuable conclusion can be made as the deficit or excess of trace elements may lead to metabolic disorder and health hazard.

2. Experimental

2.1 Sample Collection

Fig. 1 shows the map of Loktak lake (not scaled) and the central geographical position of the lake in terms of latitude and longitude is $24^0 32'$ and $93^0 49'$ respectively with an altitude 2516 ft above sea level. The specimens having approximate same size are collected from nine different sites around the lake and coded it as Moll 1, Moll 2 and so on. The muscular part excluding the intestinal portion which is inside the hard shell of *Pila globosa* was taken out and washed with distilled water several times to avoid any soil and foreign contamination.

2.2 Sample treatment

The tissue samples were dried in an oven at 65^0 C for 24 h and grounded into powder. Grinding of the samples is necessary to have homogeneity in three dimensions. 150 mg of the sample powder were pelletised into a thin pellet of uniform thickness having 13 mm in diameter under a pressure of 130 kg/cm^2 for two minutes.

2.3 Instrumental

The elemental analysis of the samples was carried out using a Jordan Valley EX-3600 Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer, which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV). The measurements were carried out in vacuum using different filters (between the source and sample for optimum detection of elements. The anode voltage of the X-ray tube was kept at 6 kV, 14 kV and 23 kV with no filter, Ti and Fe filter respectively. All measurements were carried out for 200 s. The X-rays were detected using a liquid-nitrogen-cooled 12.5 mm^2 Si (Li) semiconductor detector (resolution 150 eV at 5.9 keV) which was seen in the monitor fitted with the spectrometer. The spectra were analysed by the software called ExWIN integrated with the system [10]. The NIST bovine liver standard (SRM 1577c), apple leaf standard (SRM 1515), blood (IAEA A-13) were used as external standard reference materials.

3. Results and discussion

The EDXRF analysis was done for three replicates in each sample and altogether, fifteen elements viz. Mg, Al, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb and Sr were analysed. The mean concentrations with the standard deviations of the detected elements are shown in Table 1 and 2. The great variations occurring within one population, expressed in high standard deviations, are not unexpected reflecting the complex relationship between environmental concentrations and bioaccumulation [11]. As evident from Table 1 and 2, concentration of calcium was found to be highest in the range 12021.4 to 49420.6 ppm and that of the selenium as least concentration with the range 0.2 to 3.2 ppm. Moreover, highly toxic elements like Cd, As and Pb which was reported by earlier worker [12], could not be observed signifying that the water of the lake is not polluted with these elements. However, the elements detected as shown in the two tables, enable us to make conclusions about the idea of bioavailable element and pollution sources in the respective aquatic environment. It is appropriate to mention that the concentration of the dissolved metals in water gives the total, not bioavailable metal concentrations.

The comparison of concentrations of some elements viz. Cu, Zn and Se with that of the Food and Agricultural Organisation (FAO) of the United Nations are shown in Table 3 [13]. The concentrations of Cu and Zn are a bit less and that of Se is a bit higher as compare to FAO limits. Again, the essential elements take different roles in the proper functioning of life process. A deficit or excess in the supply of the elements in our food item will lead to metabolic disorder. Some of the daily intake requirements of elements are given as follows: P -700 mg, Cl – 750 mg, Ca – 1000 to 1200 mg, Cr – 0.05 to 0.2 mg, Cu – 1.5 to 3 mg [14]. The richness in Ca is significant for using it in Ca deficient persons. Each element is used for the smooth functioning of our body except for Rb, Sr and Al whose functions are not clear. The Se containing in the samples is remarkable as this element has anti-oxidant property so that it can be used for cancer treatment.

The coefficient of correlation among the elements in molluca tissue samples is shown in Table 4 and it was found to be highest in between Mn and Br followed by Mn and Cl with the coefficients 0.941 and 0.844 respectively. This signifies that when the Mn concentration is increased, the concentrations of Br and Cl are also increased showing the strong correlation between this two elements whose coefficient 0.797 as evident from the table. The highest negative coefficient of 0.715 is found between Ca and P suggesting for not formation of $CaPO_4$ or other calcium and phosphorus containing compounds in the tissue of *Pila globosa.* The factor analysis of the different elements was done with the help of principal component analysis extraction method. Factor analysis aims at obtaining the prominent interpretable factors hidden in the data set that explain as much of the total variability as possible. A total of four components were extracted from the analysis. The component score coefficient matrix is shown in Table 5. As evident from the table, it is observed that four components can be extracted out of which P, Cl, Mn, Br take main role for loading in the first component as compared to remaining components and similarly, Al, Fe, Se, Rb in the second component; Mg, K, Ca, Sr in the third component; S, Cu, Zn in the fourth component respectively.

Different metals dissolved in water may be accumulated by direct adsorption onto the body surface of mollusca, while particulate metals can be accumulated by organisms following the ingestion and digestion of food [15] as aquatic molluscs possess very diverse strategies in the handling and storage of accumulated metals. In addition to it, environmental and biological factors including dissolved metal interactions have been considered for their influence on trace metal uptake and bioaccumulation [16]. So, the food habit of *Pila globosa* as well as concentration of the various dissolved metals of the water environment where they survive is also important to know. The relatively insoluble compounds in the soil containing essential iron, manganese, copper and zinc are solubilized by chelating agents as the citrate anion. For manganese the microorganism is bacteria to dissolve the compounds of it in the soil and rocks by the anaerobic action. In most soils, several of these effective chelating agents are produced naturally from soil organic matter by soil microorganisms [17]. Thus the dissolved metals in water must have taken some roles in the bioavailability of the said metals.

Conclusion

The elements present in the muscular tissue of *Pila globosa* have been observed with their concentrations by using EDXRF technique. The elements detected in the samples are Mg, Al, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, Sr. Some elements were detected with high standard deviations while taking the mean from three replicates for the samples each leading to the fact that there is some complex relationship between environmental concentrations and bioaccumulation. The highest positive and negative coefficient of correlations were observed in between Mn and Br; Ca and P respectively among the elements. From the comparative study of concentrations of some elements with FAO limits and non detection of toxic elements like As, Cd, Hg, and Pb, it is concluded that the water of the lake is fresh in terms of trace elemental pollutants. Lastly, our observation shows that *Pila globosa can* also be used for biomonitoring of polluted environment.

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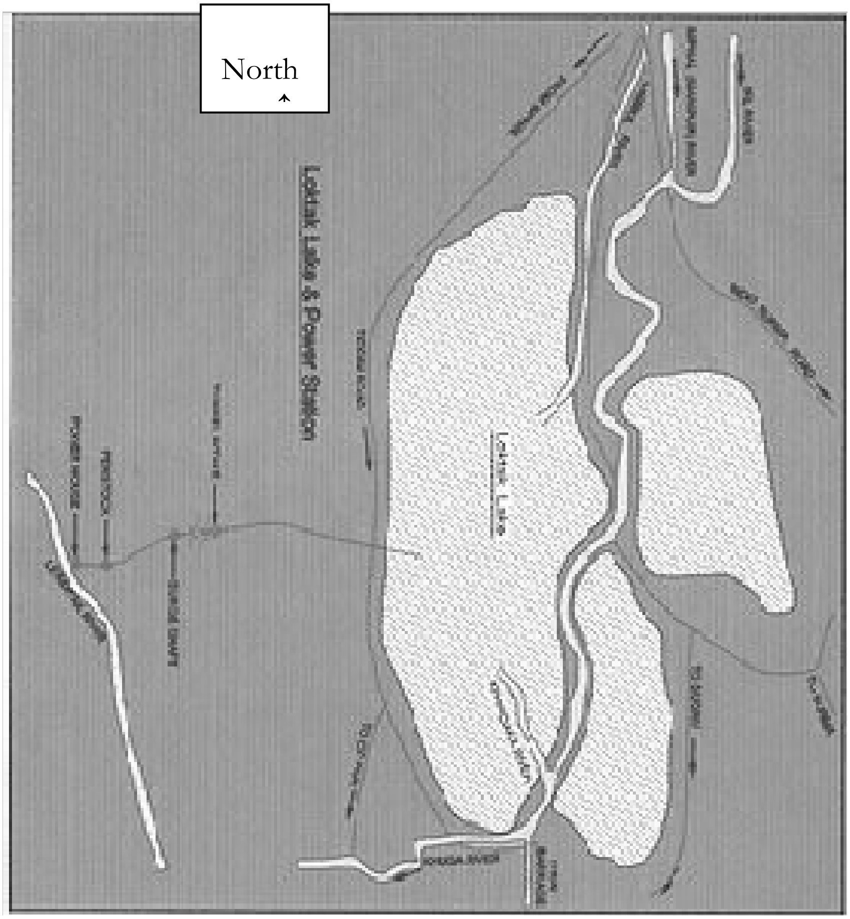

Fig. 1 Map of Loktak lake

Table 1. *Concentration (ppm) of the major elements in Pila globosa for three replicates in each sample*

Sample Id	Mg	P	S	K	Ca	Fe
Moll-1	3547.0 ±415.1	4869.3 ±513.4	4874.8 ±500.3	5811.9 ±397.5	39143 ±3261.2	1376.9±154.2
Moll-2	4139.6±149.9	5639.7±372.4	5757±103.8	6406.1±254.3	43878.5±3368.9	610.2±131.85
Moll-3	2496.7±249.7	7494.5±658.4	6631.4±564.4	4857.3±336.1	25953.7±2296.9	202.7±9.4
Moll-4	3955.8±294.8	5077.7±370.8	4886.6±493.8	5993.8±418.2	35858.6±18749	2602.7±287.6
Moll-5	3818.1±284.9	9839.3±286.7	5882.7±264.4	6725.9±205.5	12021.4±387.0	2778.5±145.6
Moll-6	1881.1±221.3	6475.2±688.3	5966.1±568.6	5241.8±440.3	16057.3±1806.6	407.2±8.5
Moll-7	3554.6±254.3	4838.5±223.3	4688.7±290.5	4695.4±373.2	49420.6±1278.6	181.2±8.8
Moll-8	3631.2±243.2	6389.0±409.8	6067.4±546.3	5790.2±390.6	35139.8±2792.6	1072.7±127.8
Moll-9	3601.8±259.0	6366.5±589.6	5806.9±322.1	5760.4±546.5	42784.7±4291.8	1570.4±97.2

Table 2. *Concentration (ppm) of the minor and trace elements in Pila globosa for three replicates in each sample.*

Sample ID	Al	Cl	Mn	Cu	Zn	Se	Br	Rb	Sr
Moll-1	1474.3 ±231.5	928.2 ±79.9	148.6±13.9	7.2±0.1	155.9±3.5	3.2±0.5	6.7±0.3	13.2±3.0	69.5±4.2
Moll-2	301.5±115.1	1043.2±55.3	33.7±1.3	23.6±1.0	159.0±3.6	2.3±1.8	10.0±0.6	7.6±1.4	69.7±5.7
Moll-3	133.1±12.8	838.5±76.3	57.1±10.6	37.8±2.4	136.1±7.4	1.2±1.1	12.0±0.3	3.7±0.4	38.9±5.1
Moll-4	4527.3±683.9	832.2±231.1	141.6±9.0	54.2±3.5	179.9±10.5	2.9±2.1	11.7±0.6	21.4±1.5	69.5±4.9
Moll-5	244.8±57.0	1719.2±101.4	2392.9±123.2	8.2±0.6	93.6±2.3	0.2±0.3	34.2±0.7	14.1±0.9	48.2±2.4
Moll-6	53.7±5.3	977.7±112.4	12.4±1.3	5.3±0.1	138.3±4.7	1.6±1.4	8.5±0.8	5.4±2.4	33.8±1.5
Moll-7	39.3±11.8	579.0±0.0	12.4±0.4	21.8±1.1	166.5±6.6	2.9±1.9	6.1±0.8	6.7±2.1	97.5±3.5
Moll-8	614.7±115.3	953.0±96.4	95.6±10.9	23.4±2.6	228.5±25.3	2.2±0.7	6.8±1.5	9.2±0.9	80.4±5.0
Moll-9	247.4±43.1	839.4±228.4	405.7±22.6	9.2±1.0	383.0±30.7	2.2±1.6	17.9±3.2	10.0±3.9	55.4±11.0

Table 3. *Comparison of metal concentrations of Pila globosa in Loktak lake with international standards in molluscas/shellfish(dry weight) compiled by the Food and Agricultural Organisation (FAO)*

Element	FAO limit (ppm) dry weight	*Pila globosa* (ppm) dry weight
Cu	50 – 150	5.3 – 54.2
Zn	200 -500	93.6 – 383.0
Se	1.5	0.2 – 3.2

Table 4. Karl Pearson's Coefficient of correlation

	Mg	Al	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
Mg	1	0.357	-0.084	-0.268	0.229	0.646	0.521	0.248	0.513	0.196	0.200	0.250	0.214	0.542	0.624
Al		1	-0.368	-0.447	-0.113	0.227	0.134	-0.129	0.586	0.649	-0.014	0.327	-0.108	0.799	0.189
P			1	0.665	0.804	0.406	-0.715	0.799	0.322	-0.235	-0.233	-0.598	0.827	-0.064	-0.539
S				1	0.365	0.198	-0.398	0.132	-0.201	-0.054	0.042	-0.480	0.254	-0.395	-0.529
Cl					1	0.768	-0.609	0.844	0.565	-0.338	-0.345	-0.361	0.797	0.249	-0.341
K						1	-0.118	0.549	0.667	-0.108	-0.008	-0.142	0.550	0.533	-0.008
Ca							1	-0.573	-0.267	0.211	0.505	0.459	-0.541	0.040	0.780
Mn								1	0.668	-0.319	-0.258	-0.415	0.941	0.306	-0.265
Fe									1	0.091	0.044	-0.040	0.660	0.843	-0.056
Cu										1	-0.081	0.102	-0.215	0.321	0.203
Zn											1	0.213	-0.083	0.041	0.169
Se												1	-0.442	0.084	0.431
Br													1	0.301	-0.388
Rb														1	0.224
Sr															1

Table 5. *Component Score Coefficient Matrix*

	Component			
	1	2	3	4
Mg	.005	.206	.261	.091
Al	-.031	.191	-.338	.111
P	.163	-.055	.039	.133
S	.082	-.129	.065	.552
Cl	.162	.040	.077	-.096
K	.106	.142	.161	.142
Ca	-.133	.086	.276	.143
Mn	.159	.053	.074	-.206
Fe	.098	.190	-.082	-.018
Cu	-.053	.089	-.334	.444
Zn	-.051	.036	.295	.414
Se	-.102	.084	.050	-.225
Br	.159	.047	.066	-.005
Sr	-.098	.127	.231	-.120
Rb	.034	.221	-.154	.021

*Extraction Method: Principal Component Analysis.