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Comparative Metal Analysis of the Soft Tissues of Three Species of *Anodonta* (Class: Bivalvia) From Kubanni Lake in Zaria Nigeria

Gadzama Ibrahim Madu Katsallah[1], Muhammad Rukayya[2], Dariya Sambo[3], Tabita Gaba Sule[4]
[1,2] Department of Biological Sciences, Ahmadu Bello University Zaria Nigeria.
[3] College of Education Gidan Waya, Kaduna State, Nigeria.
[4]Umar Suleiman College of Education, Gashua Yobe State, Nigeria.

Abstract: *A comparative metal analysis was carried to determine the deposition and composition of metals in the soft tissues of freshwater bivalves; Anodonta anatina, A. marginata and A. implicata, and to establish their role as the possible routes to which metallic elements may get into the food chain. Collections of molluscs were made from four different sites along the bank and substratum of the lake, every two months from July 2011 to June 2012. Prepared soft tissues of the bivalves were digested by wet chemical method and subjected to multi-element analysis using Instrumental Neutron Activation Analysis. Nineteen metallic elements were analysed in the soft tissues of the bivalves, with some of the metals occurring below detectable limits. There was significantly variation ($P < 0.02$) in the concentrations of the 19 metals in the three species. Although A. implicata had the highest total concentrations of the metals, the difference did not differ statistically ($P > 0.05$) from the other two species. Chemically, the transition metals were higher in number of elements and concentrations with the metalloids having the least concentrations. Six of the metals from the bivalves (Mn, As, Cr, Fe, Co and Zn) are heavy metals. The concentrations of Mn, K, As, Fe, and Ba varied in the bivalves. High concentrations of Mn, K and Zn have been observed in this study. Highly significant positive and negative interactions were observed among the heavy metals. The significant variation in the concentrations of the metals shows that the different metals accumulate differently in the soft tissues of the bivalves. The high tolerance of metal load shown by these molluscs could be indicative of an adaptation to metal pollution. Such high concentrations of the heavy metals could be physiologically detrimental and impair their ecological roles in the Lake ecosystem. This study has shown that the bivalves showed similarity in the type of metals they accumulate and therefore could be used as reliable bio-indicators of metal pollution in the Lake and could serve as routes by which these metals could get to higher trophic levels of the food chain.*
Keywords: *Bivalves, Kubanni Lake, Metals, Soft tissues, Zaria.*

I. Introduction

The bivalves (also known as shellfish, clam or mussels) are in some ways the most highly modified in the phylum Mollusca. With few exceptions all bivalves are sedentary. Some of them remain anchored to the substratum or firmly cemented, as with oysters [1] in lakes and rivers. As part of the food web in an aquatic ecosystem, freshwater mussels feed on phytoplankton, diatoms, bacteria etc. Freshwater bivalves are consumed by muskrats, otters, and racoons and young bivalves are often eaten by ducks, herons, and fishes, as well as other invertebrates [2; 3; 4; 5]. Four species of freshwater molluscs namely: *Pila* sp., *Viviparus* sp., *Planorbis* sp. and *Bithynia* sp. were reported as part of the food items recovered from the gut of the silver catfish *Chrysichthys nigrodigitatus* that inhabits both freshwater and brackish water of West Africa [6]. Molluscs have been widely used for biological monitoring of trace metal variations in aquatic environment [7], and of past and present water quality conditions in rivers and lakes [5]. Notably are the works of [8] on marine mussels, *Perna perna*, in Sao Brazil, [9] on short-lived gastropods, *Viviparus georgianus* and *Ellioptio camplanata* in Quebec, Canada and [10] on bivalves *Anodonta* sp. and *Unio pictorum* and two gastropod species, *Radix ovata* and *Viviparus* sp. in Vienna, Australia. Marine bivalves reported suffered biological consequences on chronic and sublethal exposures to copper [11]. Varying concentrations of Cd, Pb, Cu, Zn, Ni, Co, Cr, Mn and Fe were reported in the soft tissue of bivalves *Paphia undulata* and *Gafrarium pectinatum* from Lake Timsah in Egypt [12]. Heavy metals such as Hg (Mercury), Cd, Cr, Mn, Pb, Cu and Ni were also reported in the tissues of *Etheria radiata* from creeks contaminated with heavy metals in Delta State Nigeria [13].

Quite varying amounts of inorganic elements such as Mg, Ca, Fe, Mn, Zn, Cr, Co, Ni, Cu, Cd and Pb have been reported to be present in Kubanni Lake. The concentrations of some of these elements exceeded the maximum permissible limit for drinking water [14]. Varying quantities of trace metals have also been reported in fish, water and sediment samples from Kubanni Lake [15; 16; 17]. The freshwater bivalves of the genus

Anodonta [18] constitute part of the malacofauna of the Lake. The sedentary way of life of freshwater bivalves has made them exceptionally vulnerable to water pollution. Metals are known to reduce the performance of bivalve molluscs [19]. The persistence of heavy metals in the environment may lead to contamination of aquatic organisms [20]. This could in turn have negative effects on the ecological balance of the aquatic ecosystem and may limit the diversity of aquatic organisms. More so heavy metals have the potential for human exposure and increased health risk. This study therefore compares the deposition of metals in the soft tissues of three species of bivalve of the genus *Anodonta* in Kubanni Lake, in order to establish their role as the possible route to which metallic elements may get into the food chain.

II. Materials and Methods

2.1 Study Lake

Kubanni Lake is located on N11°08.234′ and E007°39.345′ south of Ahmadu Bello University Zaria Samaru campus, with an elevation of 2111 ft above sea level and reservoir area of 57 Km^2 and mean depth of 6 meters. The lake receives runoff and domestic waste waters from within the campus, nearby irrigation farms and Samaru community. Kubanni River which empties into the lake is known to play a major role in the disposal of industrial wastes accumulated from industries cited in Zaria [21].

2.2 Collection of Bivalves

Four different sites were selected at random and marked along the bank of the lake for collection of bivalve molluscs. Collections were made on the same day after every two months from July 2011 to June 2012, covering rain and dry seasons. Collection of bivalves from the substratum of each site was done using improvised scoop net and sorted by hand-picking, with hands protected in disposable hand gloves. Samples collected were washed at collection sites and placed in labeled plastic containers. The bivalves were identified using standard identification guides.

2.3 Preparation of Soft Tissues for Analysis

The shells of the bivalves were parted open around the hinges and the soft tissues separated from the shell using sharp stainless-steel scalpel. The tissues were macerated using a sharp stainless-steel blade and placed in a crucible and dried at 60° C for 36 hours. The dried soft tissues of the bivalves were weighed and ground into powdery form (enough to pass 1mm sieve) using porcelain pestle and mortar, and dissolved by wet chemical digestion in prepared 1 volume to 4 of 62% Perchloric acid and 70% Nitric acid [22].

2.4 Metal Analysis of Soft Tissues

The triplicate samples of the prepared soft tissues of each species of bivalves were subjected to multi-element analysis using Instrumental Neutron Activation Analysis (INAA). This was carried out at the Nuclear Science and Technology Section of Center for Energy Research and Training, Ahmadu Bello University Zaria (Ref: NIRR-1/JC/12/03).

2.5 Statistical Analysis

A one-way analysis of variance (ANOVA) was used to determine the variation in concentrations of the metallic elements among the three species of bivalve. Where significant, the least significant difference (LSD) was used to separate the means. Pearson correlation coefficient was used to establish the relationships of the heavy metals. The student's t- test was employed to compare concentrations of metals in the bivalves in the dry and rainy seasons. Analyse-it v.2.14 [23] software was used.

III. Results

A total of 19 metals were present in the analysed soft tissues of the three species of bivalves studied in the Lake. The elements vary in concentrations in the three bivalve species, with some of the elements falling below detection limit (Table 1). The *t* – test shows that the concentrations of metals in the bivalves did not differ significantly ($P < 0.05$) between rain and dry seasons. All the metals except Sm, U, Cr and Th were found in the 3 species of bivalve with Sm and Cr occurring only in *A. implicata* and *A. marginata* respectively. The metals As, U, Sc, Eu, Lu, Yb, Th, Sb and Rb can be considered as trace metals as they occur in low concentrations and below detectable limit. Though *A. implicata* had the highest total concentrations of the metals, the difference did not differ statistically ($P > 0.05$) in the three species of bivalve (Fig. 1). The variation in concentrations of the 19 metals in the 3 species was significantly different ($P < 0.02$). The concentrations of Mn, K, As, Fe and Ba varied in the bivalves. Considering the chemical properties of the metals from the bivalves, the transition metals were higher in number of elements and concentrations with metalloids having the least concentration (Fig. 2). Six of the elements, Mn, Sc, Cr, Fe, Co and Zn are the d-block metals. The s-block metals are Na, K, Ba and Rb while As and Sb are the only p-block metals. The Lanthanides; La, Sm, Eu, Lu, Yb and Actinides; U and Th belong to the f-block metals. Six of the metals from the bivalves (Mn, As, Cr, Fe, Co and Zn) are heavy metals. High concentrations of Mn, K and Zn have been observed in this study (Fig. 3). The correlation matrix (Table 2) shows both positive and negative interactions among the heavy metals in the three species of bivalve. A Significant positive influence was observed between Mn and Cr. Zinc was positively correlated with Mn (r = 0.86) and Fe (r = 0.45). The Strong negative correlations of Mn with As and Co were highly significant, while Fe showed highly significant moderate negative relationships with As (r = - 0.66) and Co (r = - 0.65). There was a weak but significant negative correlation (r = - 0.24) between Fe and Cr (Table 2). The strong relationships of As with Co and Zn, Cr and Co, Mn with Fe and the moderate relationships of Cr with Co and As, were statistically not significant ($P < 0.05$).

Table 1: Mean (±S.E.) concentrations of metals in the three species of bivalve studied in Kubanni Lake.

Metal element	**Category of metal**	***A. anatina***	***A. marginata***	***A. implicata***
Manganese (Mn)	Transition Metal	24580 ± 123 ^{a}	28790 ± 202 ^{b}	29880 ± 209 ^{b}
Sodium (Na)	Alkali Metal	1456 ± 6 ^{a}	1195 ± 6 ^{a}	1059 ± 5 ^{a}
Potassium (K)	Alkali Metal	1523 ± 218 ^{a}	1370 ± 236 ^{b}	1278 ± 236 ^{c}
Arsenic (As)	Metalloid	0.12 ± 0.003 ^{a}	0.043 ± 0.003 ^{b}	0.05 ± 0.003 ^{b}
Lanthanum (La)	Rare Earth Metal	76 ± 0.23 ^{a}	65 ± 0.2 ^{a}	67.4 ± 0.2 ^{a}
Samarium (Sm)	Rare Earth Metal	BDL	BDL	3.95 ± 0.024
Uranium (U)	Rare Earth Metal	0.08 ± 0.004 ^{a}	0.07 ± 0.004 ^{a}	BDL
Scandium (Sc)	Transition Metal	0.39 ± 0.01 ^{a}	0.28 ± 0.01 ^{a}	0.16 ± 0.01 a
Chromium (Cr)	Transition Metal	BDL	1.5 ± 0.4	BDL
Iron (Fe)	Transition Metal	26280 ± 237 ^{a}	28140 ± 225 ^{a}	32770 ± 262 ^{b}
Cobalt (Co)	Transition Metal	1.13 ± 0.06 ^{a}	0.6 ± 0.04 ^{a}	0.66 ± 0.05 ^{a}
Zinc (Zn)	Transition Metal	594 ± 12 ^{a}	843 ± 14 ^{a}	758 ± 16 ^{a}
Barium (Ba)	Alkali Earth Metal	11410 ± 68 ^{a}	15440 ± 77 ^{b}	15340 ± 77 ^{b}
Europium (Eu)	Rare Earth Metal	0.57 ± 0.1 ^{a}	0.67 ± 0.1 ^{a}	0.8 ± 0.08 ^{a}
Lutetium (Lu)	Rare Earth Metal	0.39 ± 0.3 ^{a}	0.14 ± 0.03 ^{a}	0.1 ± 0.02 ^{a}
Yttrium (Yb)	Transition Metal	0.39 ± 0.3 ^{a}	0.65 ± 0.1 ^{a}	0.66 ± 0.04 ^{a}
Thorium (Th)	Rare Earth Metal	0.13 ± 0.03 ^{a}	0.13 ± 0.02 ^{a}	BDL
Antimony (Sb)	Metalloid	BDL	BDL	BDL
Rubidium (Rb)	Alkali Earth Metal	BDL	BDL	BDL

Key: BDL – Below Detectable Limit.
$^{a, b, c}$ – Means followed by the same superscript along the row are not significantly different ($P < 0.05$).

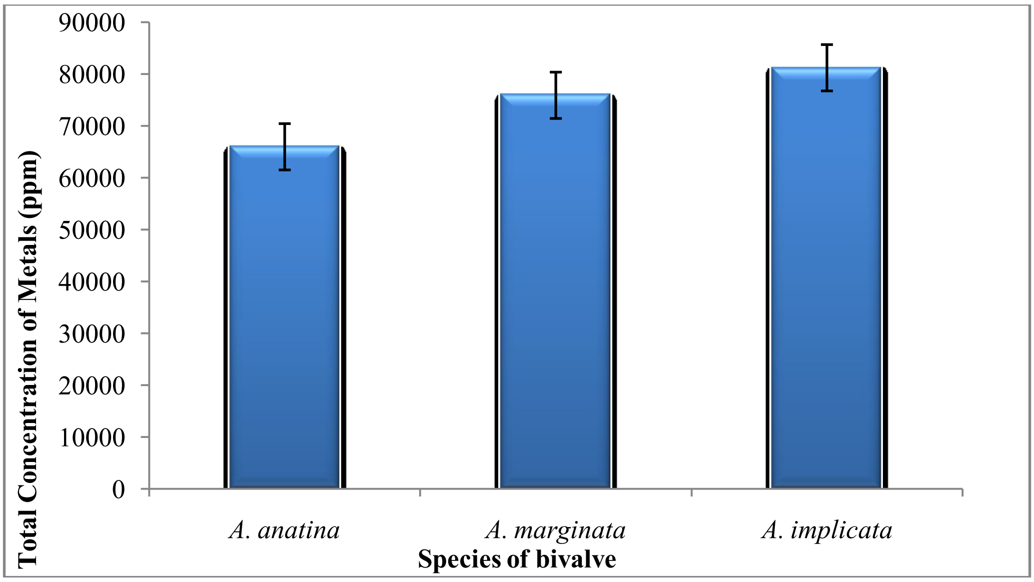

Figure 1: Total metal load of each species of bivalve studied in Kubanni Lake.

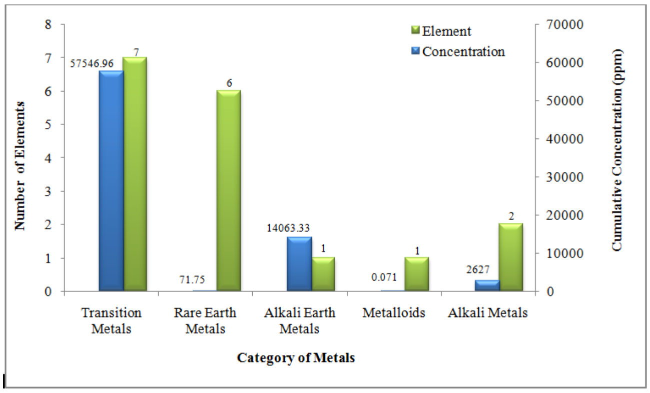

Figure 2: Total number of elements and concentrations in each metal category from the three species of bivalve.

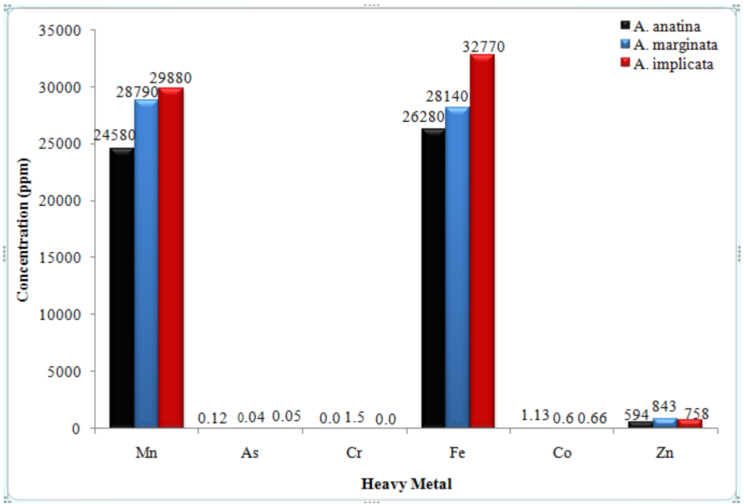

Figure 3: Variation in concentrations of heavy metals in the bivalves from Kubanni Lake.

Table 2: Correlation matrix of the heavy metals from the three bivalves in Kubanni Lake.

	Mn	As	Cr	Fe	Co	Zn
Mn	1.00					
As	-0.96**	1.00				
Cr	0.32**	-0.57	1.00			
Fe	0.84	-0.66**	-0.24**	1.00		
Co	-0.96**	1.00	-0.59	-0.65**	1.00	
Zn	0.86**	-0.97	0.76	0.45**	-0.97	1.00

Key: ** **-** Highly significant ($P < 0.01$).

IV. Discussion

The soft tissues of *Anodonta anatina*, *A. marginata* and *A. implicata* in this study contained wide range of metals at different concentrations. The significant variation in metal concentrations in the bivalves suggests that the different metals accumulate in different patterns and concentrations in the soft tissues of the bivalves. The different forms (colloidal, particulate, and dissolved forms) that metals exist in water have predisposed bivalves to continuous metal intake. The uptake from solution [24] through the gills, feeding on contaminated algae and coming in-contact with the metal- enriched sediments of Kubanni Lake could have exposed these three species of bivalves to such a wide range of metals. The report of [25] on partitioning of metals in burrowing bivalve *Scrobicularia plana* suggested uptake of metals was mainly through ingestion of intertidal sediments.

The variation in high concentrations of Mn, K, As, Fe, and Ba in the species of bivalves in this study may be due to differences in physiological ability to maintain internal metal concentrations or could be from species-specific capacity to regulate or accumulate trace metals [26; 27]. For the bivalves to tolerate such high metal load, it could be that some of the metals may not be bioavailable and therefore have little or no influence on the physiology and general body organ functions of the bivalves. This high level of metal tolerance shown by the bivalves in this study is indicative of an adaptation to metal pollution. This is contrary to the report of [28] that mussels to be sensitive but less adapted to metal pollution. Mussels were reported [29] to be more effective than oysters in the regulation of internal metal levels. The high concentrations of Mn, Na, K, Fe and Ba metals in this study is an indication that these metals are either of physiological significance as essential elements in the bivalves [30] or easily assimilated by the soft tissue of the organisms. Studies have shown that specified groups of metals have effects on biological processes in organisms. The alkali metal ions act as bulk electrolytes and have more specialized functional roles as structure promoters and enzyme activators [31]. The d-block metals play catalytic role in enzyme actions. Transition metals such as Fe, Cu, Co and Mn were reported to be essential in animals but may be toxic at high concentrations [32; 33]. The high atomic number of the p-block metals increases their tendency to bind strongly to sulphur; this is a major cause of their toxicity in organisms. The lanthanide and actinide elements are usually of no biological importance, but some of the actinide group may have biological significance [31]. The low concentrations of U observed in *A. anatina* and *A. anodonta* could be from water trapped in the enclosed shell of the bivalves as U naturally occurs in open waters and unlike to accumulate in aquatic animals. Yttrium and Sc are known to cause damage to cell membranes in aquatic animals and may have negative effects on their reproductive abilities. The accumulation of high amounts of La and Yb were reported in mussels [34].

The concentrations of heavy metals in the bivalves in this study are higher than the values reported by [8] in freshwater crab *Cardisoma guahumi* from the same lake. Also [35] reported that some benthic invertebrates and fish accumulate heavy metals from water and sediments and that molluscs and crustaceans have higher concentrations than other invertebrates. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Depending on the animal, low amount of Mn can be lethal. Iron is rapidly absorbed in the gastrointestinal tract and its corrosive nature can be toxic to the organism [34]. Despite its toxicity, As is an essential trace element for some animals. Arsenic is an essential element for some animals and accumulates in especially plant-eating freshwater organisms such as bivalves and is known to bio magnify in the food chain [34].

From the correlation value of the heavy metals, the concentration of Mn led to corresponding increase in Zn and Cr, and decrease in concentrations of As and Co. Increase in concentration of Fe resulted in the increase of Zn and decrease in As, Cr and Co. The strength of relationships is indicative that these interactions could mean that the metals may complement, influence uptake/accumulation or antagonise each other in the soft tissues of the bivalves.

Although bivalve molluscs show adaptive biochemical processes and can store toxic materials at cellular levels of the body tissues to reduce the metal toxicity [36], they are reported to respond to high metal concentrations in their surrounding by greening and thinning of shells [37], produce fewer byssus threads [38], abnormal developments in the adult and larvae [39], reduced heart and filtration rates [40], and closed their shells for a longer period of time [41]. The zebra mussel, *Dreissena polymorpha* was reported to exhibit a dose-dependent response by the by closing of valves and stoppage of filtration rate [19].

V. Conclusion

This study has shown that the presence of metal elements in Kubanni Lake can be said to have predisposed the three bivalve species found in the lake to accumulate wide-range concentrations of different metals of biological and public health importance. The similarity of the species in the type of metals they accumulate is an indication that any of the species could be used as reliable bio-indicators of metal pollution in a freshwater environment, especially in a lake ecosystem. Therefore any of the species could serve as a route by which these metals could get to higher trophic levels of the food chain. Aside the biological importance of some of these metals as micro- and macroelements, their high and even low concentrations (As and Cr) in the soft tissues of these bivalve species could impair their ecological roles in the aquatic ecosystem of Kubanni Lake.

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