African Scientist Vol. 17, No. 1 March 31, 2016
Printed in Nigeria

1595-6881/2016 $10.00 + 0.00

http://www.niseb.org/afs

AFS 2015002/17101

Bioaccumulation Profile of Selected Heavy Metals in Whole Tissue of *Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii* from Benin River in Delta State, Nigeria

A.A. Enuneku[1*], I.M. Ezenwa[2], S. Adeosun[3]
[1]Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Nigeria.
[2,3]Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Nigeria.
*Corresponding Author Email: alex.enuneku@uniben.edu. [1]Phone: +2348030773314

(Received February 15, 2015; Revised and accepted January 6, 2016)

ABSTRACT: Bioaccumulation of selected heavy metals in whole tissue of shrimps (*Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii*) inhabiting a stretch of Benin River in Delta State, Nigeria was studied. Water samples for heavy metal determination were also collected from three different locations within the sampled stretch from January to June, 2013. Heavy metal concentrations were quantified with Unicam Atomic Absorption Spectrometry (PG 550). The concentrations of heavy metals in the surface water samples (mg/l) were Fe(0.40),Zn(0.17), Mn(0.03), Cu(0.04), Cd (0.01), V (0.02), Cr (0.02), Pb (0.02) and Ni (0.06).The mean heavy metals concentrations in whole tissue of *M. macrobrachion* in mg/kg were: Fe (73.04), Zn (13.47), Mn (8.28), Cu (3.43), Cd (1.12), V (1.79), Cr (2.60),Pb(1.30), Ni (6.17) while in *M. vollenhovenii* the concentrations of these heavy metals in mg/kg were: Fe (8.01), Zn (5.55), Mn (2.74), Cu (1.42), Cd (0.46), V (0.73), Cr (1.27),Pb(0.54), Ni (2.54). The concentrations of these heavy metals in both shrimps (except Cu in *M. vollenhovenii*) exceeded the acceptable standards of WHO and USEPA. Bioaccumulation factor (BAF) for the heavy metals which in all cases were > 1, is an indication that these heavy metals were bioaccumulated and further evidence that *M. macrobrachion* and *M. vollenhovenii* can be used as bioindicators of heavy metal pollution.

Keywords: Bioaccumulation, Heavy Metals, Shrimps, Benin River, Bioaccumulation Factor.

Introduction

Bioaccumulation profile of heavy metals in the tissue of aquatic biota has received relatively huge attention from many researchers globally (Anetekhai *et al*., 2007; Falusi and Olanipekun 2007; Omoigberale and Eweka 2010; Jimoh*, et al.,* 2011; Nwabueze 2011; Babatunde *et al*., 2012). Heavy metals accumulate in the environment from natural sources and anthropogenic activities. Unsustainable management of our natural resources in recent times has enhanced the availability of these toxic substances in the environment. Some heavy metals including iron, cobalt, copper, manganese, molybdenum, strontium, vanadium and zinc are required by living organisms but in minute quantities. Excessive levels of these metals, however, can be detrimental to living organisms. Other heavy metals such as arsenic, cadmium, lead and mercury have no known beneficial effect on organisms, and their accumulation over time in the bodies of mammals can cause serious illness (Hawkes 1997). Over some decades, aquatic ecosystems especially freshwaters have been envisaged as receptacle of assorted waste types. The aquatic ecosystem receives influx of industrial waste arising from manufacturing, mining, agricultural, domestic waste water, metal finishing plants and atmospheric precipitation (Van den Broek *et al*., 2002) and heavy metals are inevitable constituents of these wastes. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and on the diversity of aquatic organisms (Ashraj 2005).

Shell-fishes are among animals utilized by man as source of protein. *M. macrobrachion* and *M vollenhovenii* are not exceptions to this. These shrimp species are among the economically important invertebrates highly valued by man as a source of food when cooked. Being aquatic organisms, their tissues may be susceptible to contamination by contaminants especially heavy metals within the medium. Illicit disposal of heavy metals containing effluents directly or indirectly into the aquatic ecosystem enhances the accumulation of heavy metals in both the biotic and abiotic components of the ecosystem.

The analysis of water and sediment samples does not allow for the estimation of the quality of the metal which is biologically available (Etim *et al*., 1991), hence the inclusion of *M. macrobrachion* and *M. vollenhovenii* as one of the major matrices to be evaluated. Bioaccumulation assessment of heavy metals offers an appealing tool for the estimation of metal pollution in aquatic ecosystem. Targeted groups for this include algae, macrophyte, zooplankton, insect, bivalve molluscs, gastropods, shell-fishes, fishes and amphibians (Qunfang *et al*., 2008). Shell-fishes have been widely used as bio-indicators to assess heavy metal concentrations in the environment due to their wide range of distribution and their importance in food chain (Asuquo and Udoh 2002) and slow mobility. Unlike the fin fishes that will quickly swim away from polluted environment, shell-fishes may remain in the polluted environment and this makes them a better bio-monitor than fin fishes (Kumolu-Johnson *et al*., 2010).

The aim of this study is to provide information on bioaccumulation of Fe, Zn, Mn, Cu, Cd, V, Cr, Pb and Ni in the whole tissue of *M. macrobrachion* and *M.vollenhovenii* sampled from the stretch of Benin River at Koko, Delta State and further to ascertain the risk of these heavy metals toxicity to the consumer.

Materials and Methods

The Benin River is located in the coastal belt of Southern Nigeria at the Western boundary of the upper Delta and the lowlands. This River drains the major rivers Ethiope, Ossiomo, Osse and Siluko into the Atlantic Ocean. It is approximately 93 km long with average width of 3.0 and 1.4 km in its downstream and upstream sections, respectively. It is an important channel for small ships and other watercrafts like speed boat, yacht and canoe. Three distinct longitudinal zones could be recognized in this river, the upper freshwater zone, the middle transitional zone with salinity fluctuations and the lower coastal zone which is predominately saline. The predominant vegetation at the shed include *Pandanus candelabrum; Elaeis guineensis; Nymphaea lotus; Salvinia nymphellula; Echinochloa pyramidalis; Pistia stratiotes; Azolla africana.*

Samples of water for characterization of heavy metal concentrations were obtained from three designated stations along stretch of Benin River at Koko town (Figure 1), north central part of Delta State (Latitudes $05^0$59'43.6" – $05^0$59'35.7"N; Longitude $005^0$28'06.7"- $005^0$25'56.2"E).Samples of *M. macrobrachion* and *M. vollenhovenii* were collected within these designated stations. Along this stretch is located bitumen blending plant belonging to Total Nig. Ltd, facilities of Optima Petroleum Company and watercraft maintenance workshop.

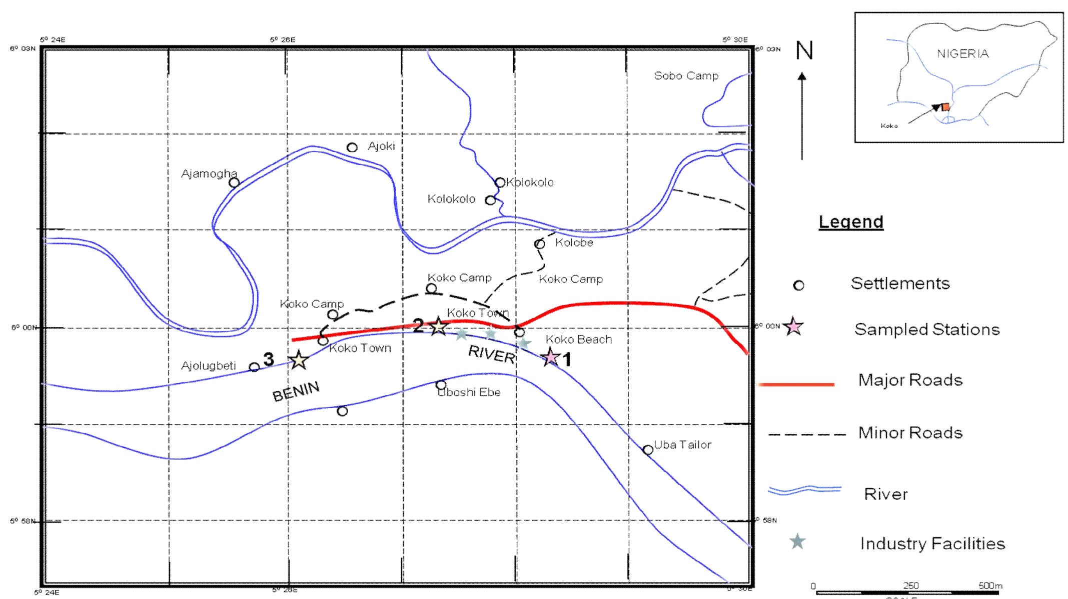

Figure 1: Map showing Sampled Stations

The sampling period spanned from January, 2012 to June, 2012. Shrimps were collected at low tide regime using woven cylindrical traps with non-return valve. The samples were preserved in ice chest at -4^{o}C and transferred immediately to the laboratory for analysis.

Water samples for heavy metal determination were collected in triplicates in acid washed polyethylene bottles at three different locations within the sampled stretch. The bottles were rinsed thoroughly with deionised water after washing with dilute nitric acid (HNO_3). In the field the bottles were rinsed several times with the river water and 1 litre sample was then collected at about 50 cm below the water surface. The water samples were acidified with concentrated nitric acid for preservation. In the laboratory, the samples were digested in concentrated nitric acid and analysed using Atomic Absorption Spectrometry-PG 550 (Radojevic and Bashkin 1999).
In the laboratory prior to drying in an oven at 105^{o}C, weights of *M. macrobrachion* and *M vollenhovenii* samples were measured with the aid of electronic balance. Two grams of dried homogenized sample of shrimp tissue was digested in 20 ml nitric acid, perchloric acid and hydrogen peroxide 1:1:4 solution and heated on an electric burner for 30 minutes till all fumes were expelled and the tissues dissolved completely. The beaker and its contents were allowed to cool and thereafter the digested sample made up to 50ml. The digested sample was analyzed for levels of heavy metals using the Atomic Absorption Spectrometer (PG 550).

Data Analysis

Inter station comparisons were carried out to test for significant differences in the concentration of the heavy metals in the water using parametric analysis of variance (ANOVA). If significant values ($P<0.05$) were obtained in the ANOVA, Duncan multiple range test was performed to determine the location of significant differences. Independent samples t-test was used to compare the concentrations of individual heavy metals in the whole tissue of *M. macrobrachion* and *M. vollenhovenii*. Bioaccumulation factor (BAF) determined according to Demina *et al.* (2009) as:

$$BAF=\frac{\text{Conc.of heavy metal in } \textit{shell fish} \text{ (mg/kg)}}{\text{Conc.of heavy metal in water (mg/l)}}$$

Results

Table 1 shows the summary of the various heavy metals characterized in the water samples obtained from the three stations studied.

Table 1: Summary of the various heavy metal concentrations in the raw water samples from the three designated stations in Benin River.

	Station 1	Station 2	Station 3	
Parameters	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SD	FMEnv. Permissible Limits (mg/l)
Fe	0.39±0.08	0.45±0.12	0.36±0.12	1.00
Zn	0.16±0.03	0.19±0.05	0.15±0.05	1.00
Mn	0.03±0.01	0.03±0.01	0.03±0.01	0.05
Cu	0.03±0.01	0.04±0.01	0.04±0.01	0.10
Cd	0.00±0.00*	0.02±0.00	0.01±0.01	0.01
V	0.02±0.00	0.02±0.01	0.02±0.01	0.01
Cr	0.02±0.00	0.02±0.00	0.02±0.01	0.05
Pb	0.00±0.00*	0.03±0.01	0.03±0.01	0.05
Ni	0.05±0.01	0.06±0.02	0.07±0.02	0.05

Heavy metal concentrations in the water were measured in mg/l; (*) - Significantly different ($p<0.05$)

With the exception of Cd and Pb, the concentrations of other heavy metals showed no significant difference ($p>0.05$) among the three stations where water samples were obtained. The mean concentrations of heavy metals in the surface water samples were Fe: 0.40±0.05 mg/l; Zn: 0.17±0.02 mg/l; Mn: 0.03±0.00 mg/l; Cu: 0.04 ±0.01 mg/l; Cd: 0.01±0.01 mg/l; V: 0.02±0.00 mg/l; Cr: 0.02±0.00 mg/l; Pb: 0.02±0.01 mg/l and Ni: 0.06±0.01 mg/l.

The weights of *M. macrobrachion* and *M. vollenhovenii* obtained throughout the study period ranged between 14.13 - 38.84 grams and 8.08 – 9.69 grams respectively.

Table 2 shows the summary of bioaccumulation of various heavy metals in the whole tissue of *M. macrobrachion* and *M.vollenhovenii* compared with acceptable standards.

Table 2: Summary of bioaccumulation of various heavy metals in the whole tissue of *Macrobrachium macrobrachion and Macrobrachium vollenhovenii* compared with acceptable standards.

	M. macrobrachion	*M. vollenhovenii*		
Heavy metals	$\bar{x}$±SD	$\bar{x}$±SD	WHO, 2003	USEPA, 1986
Fe	73.04±22.72*	8.01±3.91	0.3	0.5
Zn	13.47±4.19*	5.55±2.71	5	5
Mn	8.28±2.57*	2.74±1.34	0.5	0.02
Cu	3.43±1.07*	1.42±0.70	2.25	2.25
Cd	1.12±0.35*	0.46±0.23	0.01	0.01
V	1.79±0.56*	0.73±0.36	N/A	N/A
Cr	2.60±0.81*	1.27±0.62	N/A	N/A
Pb	1.30±0.41*	0.54±0.26	0.01	0.11
Ni	6.17±1.92*	2.54±1.24	N/A	N/A

Mean heavy metal concentrations in *M. macrobrachion* and *M. vollenhovenii*were measured in mg/kg; (*) - Significantly different ($p<0.05$)

Also the concentrations of the individual heavy metals analyzed in the whole tissue of these shell-fish showed significant difference ($p<0.05$) for both species.

Figure 2 depicts the bioaccumulation of various heavy metals in the whole tissue of *Macrobrachium macrobrachion and Macrobrachium vollenhovenii* samples from Benin River.

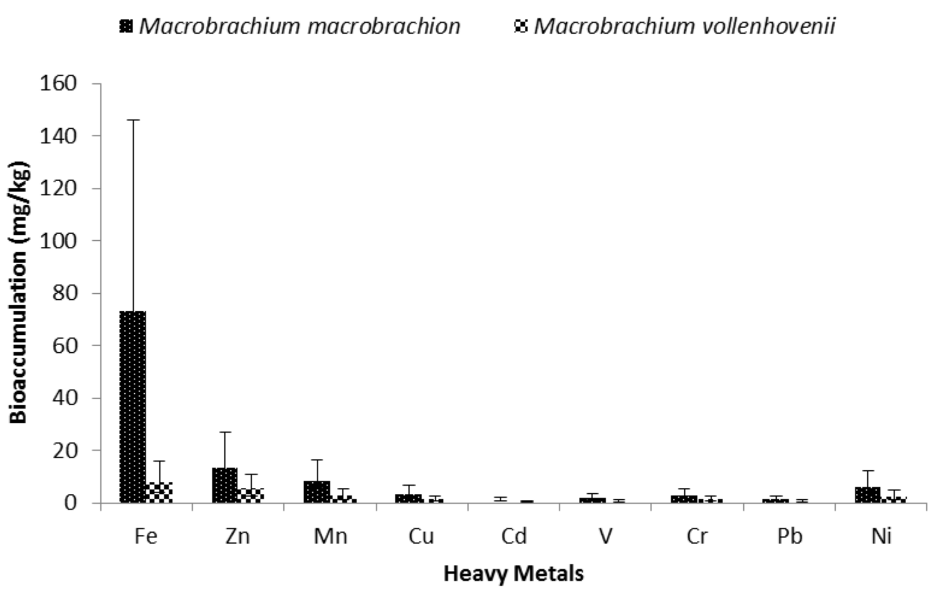

Fig. 2: Bioaccumulation of various heavy metals in the whole tissue of *Macrobrachium macrobrachion and Macrobrachium vollenhovenii* samples from Benin River.

Bioaccumulation Factors (BAF) of Heavy Metals in the Whole Tissue of *M. macrobrachion* and *M. vollenhovenii* are shown in Table 3.

Table 3: Bioaccumulation Factor (BAF) of Heavy Metals in the Whole Tissue of *M.macrobrachion* and *M. vollenhovenii*

Heavy Metals	Fe	Zn	Mn	Cu	Cd	V	Cr	Pb	Ni
M.macrobrachion	182.92	80.00	283.80	86.79	103.07	83.69	158.43	61.97	101.03
M. vollenhovenii	20.06	33.19	93.96	35.97	42.37	34.41	77.42	25.48	41.53

For *M. macrobrachion,* Fe (182.92) had the highest bioaccumulation factor while Pb had the lowest (61.97). For *M. vollenhovenii,* Cr (77.42) had the highest BAF while Fe (20.06) had the lowest.

Discussion

Results from this study have shown that iron was the most dominant metal recorded in the surface water of Benin River. Within this eco-region, the dominance of Fe in surface freshwater has been reported (Adefemi and Awokunmi 2010; Wogu and Okaka 2011; Omoigberale *et al*., 2013). Dominance of Fe in the water is in consonance with the report that iron occurs at high concentration in Nigeria soil (Nwajei and Gagophien 2000; Asaolu and Olaofe 2004).The observed concentrations of Cd, V and Ni in the raw water samples compared unfavourably with Federal Ministry of Environment permissible limit for surface water.

From Table 2 it can be observed that iron and cadmium were the most and least bioaccumulated heavy metals respectively in the whole tissue of both shell-fishes studied. The order of the magnitude of these heavy metals accumulation in the whole tissue of *M.macrobrachion* and *M.vollenhovenii* was Fe> Zn>Mn> Ni> Cu> Cr> V>Pb> Cd. Similar order of accumulation observed in these two species of shell-fish was probably due to the fact they inhabited the environment and were of the same genus.

The concentrations of Cu and Zn analysed in the whole tissue of these two species of shell-fish were lower than the range of Cu (860-1620 mg/kg); Zn (6910 – 10400 mg/kg) reported by Anetekhai *et al. (*2007) in *M. vollenhovenii* in Ologe Lagoon, Lagos, Nigeria. This difference might be due to greater metal load in Ologe Lagoon because of the presence of Agbara Industrial Estate, which discharges its waste into the lagoon. This further reflected that these species of shell-fish tend to accumulate individual heavy metals in relation to their prevailing environmental magnitude. The maximum accumulation of Fe in the tissue of *M. macrobrachion* and *M. vollenhovenii* recorded herein is in conformity with findings of Edema and Egborge (1999) and De Silva and Anderson (1995) in a related study on heavy metal content of *M. vollenhovenii* from Warri River (Delta State) and Ovia River (Edo State) respectively. Apart from the high concentration of Fe in the water, the high levels of Fe in *M. macrobrachion* and *M. vollenhovenii* can possibly be related to its presence in cytochromes and proteins.

It was observed in this study that the concentrations of all the heavy metals analyzed in the whole tissue of *M. macrobrachion* exceeded the international guideline limits of WHO (2003) and USEPA (1986). The same situation was observed in *M. vollenhovenii* except the concentration of Cu. This is of high health significance because this shell fish is one of the main sources of protein for some persons living within and outside this community. Some of these heavy metals have been identified to be of very high health risk: Cr and Ni have been identified as carcinogenic agents, Cd as nephrotoxic agent and Pb as neutrotoxic and enzyme inhibitor (Ernest 2010). High concentration of heavy metals in the whole tissue of these shell fish can enhance the bioavailability of these toxic substances to organisms including man which occupy the higher trophic level. Hence there is need for urgent action in order to curtail the possible source of these toxicants into this aquatic ecosystem.

The presence of relatively high concentrations of these heavy metals in the whole tissue of *M. macrobrachion* and *M. vollenhovenii* is indication that these shrimp species can serve as bioindicators. Bioaccumulation was further proven by positive difference values observed between the concentrations of these heavy metals in the shellfish and water. Thus *M. macrobrachion* and *M. vollenhovenii* bioaccumulated all the heavy metals studied. Relatively high concentration of manganese recorded in the whole tissue of these shell-fish may be attributed to a combination of factors such as metabolic co-factor (De Silva and Anderson 1995), low toxicity of Mn and high threshold of these organisms (Murphy *et al*., 1978). The relatively high levels of Cu in these shell-fish can at least be attributed partly to its function in the respiratory pigment haemocyanin (Van de Brock 1979) and metalloenzymes (De Silva and Anderson, 1995). High concentration of Zn analysed in the whole tissue of these shell-fish may not have resulted only from the fact that Zn is one of the micronutrients required by living organism (Solomon *et al*., 1999) and its function in the respiratory pigment haemocyanin (Van de Brock 1979) but also to the contamination of this aquatic ecosystem by substances containing Zn possible from the surrounding industries. According to Boyden *et al*., (1979) Zn concentration might exceed the range of regulation by the shrimp. Thus homeostasis of Zn by shrimp can be influenced by external input. Lead concentration in the whole tissue of these shell-fish was fairly high. This is possibly from wastes generated by industries on the watershed and activities of motorized water craft which dominate the means of transportation therein. Similar result was documented (Edema and Egborge, 1999) in the tissue of *M.vollenhovenii* from Warri River (an aquatic ecosystem with similar anthropogenic activities as Benin River).Cadmium is a common inorganic contaminant of coastal and inland water sediments due to anthropogenic pollution and natural sources. It can be accumulated in aquatic animals (e.g. crabs, shrimps, oysters and mussels) after entering through different ways such as respiratory tract, digestive tract and surface penetration (Sokolova *et al*., 2004; Ivanina *et.al*., 2008; Ivanina *et al*., 2010). Earlier studies by some researchers showed that like other non essential trace elements, cadmium is not regulated by decapods (Dethlefsen 1978; Jennings and Rainbow 1979), thus can be bioaccumulated by them. This was further proven by a positive difference of values between the concentrations of cadmium in these shellfish and water. Lead is a highly toxic heavy metal which occurs naturally in parts of the environment but is now ubiquitous because of human activities. Combustion of oil and gasoline alone accounts for 50% of all anthropogenic emissions (Palani S.K. and Sharadhamma 2012). Apart from the fact that lead is a trace element, its relatively low accumulation in the whole tissue of these shell-fishes is attributable to the rural nature of the study area which had minimized lead emitting processes. Other contributing factors to lead accumulation in this ecosystem include water-craft, effluent from industries and runoff from hinterland. Accumulation of Ni, Cr and V are possibly as a result of factors already mentioned.

One of the important assumptions of the BAF model is that it reflects equilibrium conditions between exposure and tissue concentrations (Mc Geer *et al*., 2003). According to Demina *et al.*(2009) BAF values > 1 indicates bioaccumulation of the agent in question. All the heavy metals analysed in the whole tissue of the two shell-fish species were bioaccumulated and BAF values were higher in *M. macrobrachion* than *M. vollenhovenii.* Apart from possible factors responsible for accumulation of these heavy metals already noted above, these accumulations may also have resulted from the fact that many aquatic organisms store metals in detoxified forms, such as in inorganic granules or bound to metallothionein-like proteins (Langston and Zhou 1986; Hylland *et al*.,1994). The use of granules as a storage mechanism is of particular note in the context of BAFs because extremely high tissue concentrations are often associated with this storage mechanism but unrelated to adverse impact. The sequestered and stored heavy metals may not result in direct impacts on the organism itself but there exists the potential for impacts in predators through dietary uptake. There is need for further study in order to ascertain if *M. macrobrachion* and *M. vollenhovenii* exhibits this physiological response.

Conclusion

The observed levels of bioaccumulation of all the heavy metals estimated in this study exceeded international guideline limits of WHO and USEPA. *M. macrobrachion* and *M. vollenhovenii* from Benin River are contaminated with heavy metals. Similar condition is likely applicable to other aquatic fauna therein. Urgent action is required in order to curtail activities that contribute toward the accumulation of this toxic substances into this environment. Regular monitoring of levels of contamination is necessary to further assess the impact of heavy metals in the aquatic system.

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