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Evaluation of Concentrations and Human Health Risk of Cu, Zn, Fe in Two Periwinkles Species from Three Local Government Areas, Bayelsa State, Nigeria.

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ABSTRACT: This study has investigated the concentrations of Cu, Zn and Fe in *P. aurita* and *T. fuscatus* obtained from Bayelsa State, Nigeria and assessed the health risks associated with the consumption of these shellfishes. The concentrations of the metals in mg/kg dry wt basis mean ± SD were Cu (57.0 ± 4.0) Zn (63.8 ± 9.6) and Fe (1260 ± 159.2) in *P. aurita*, while *T. fuscatus* were, Cu (33.3 ± 1.43), Zn (77.7 ± 9.19) and Fe (1985 ± 4.89). These values were higher than the guideline of WHO, and FEPA. The estimated daily intake of the metals in the periwinkles was all higher than their provisional tolerable daily and weekly intakes set by regulatory bodies. The non-carcinogenic risks (THQ and HI) of the individual and combined risk of the metals were within the limit of 1 set by USEPA, indicating no health risk at the moment. However, considering the bioaccumulative nature of heavy metals moderate intake of these periwinkles are recommended to avoid human health risk to consumers in future.
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Key words: Heavy metal, Pollution, Risk Assessment, Perewinkles.

Heavy metal pollution in the aquatic ecosystem over the years has been recognized as a serious environmental concern (Balkase *et al.,* 1982). These pollutants are regularly introduced into the environment through industrial activities especially metal production (mining process), oil and gas activities, transportation processes, agricultural applications, domestic applications etc (Nrigu, 1988; Adoki, 2007). Once released, these pollutants persistent in the environment and its biota due to their non-biodegradable, bioaccumulative and biomagnification properties. (Otitoloju *et al*, 2002). This pose a serious health risks to plants, animals and human directly or indirectly (Chary *et al,* 2008). However, heavy metals such as Cu, Zn and Fe play important biochemical roles in the life processes of many organisms including human and their presence in trace amount are nutritional essential for healthy life (IOSHIC, 1999). Nevertheless, at high concentrations toxic effects are observed. Example, Fe is a key constituent of the haemoglobin required for the production of red blood cells but at high concentration, Fe causes pathological effects such as iron oxide deposition in Parkinson diseases (Altamura and Muckenlthaler, 2009). Cu which is a key constituent of blood pigment, haemoglobin in mollusce and crustacean had been known to be associated with liver damage in excess and Zn may produce adverse nutrient interaction with Cu (Johnson and Lamy, 2009). Also, high level of Zn reduces immune function and the levels of high density lipo-proteins (Spear, 2000).

Risk assessment is one of the method used to evaluate the impact of hazards on human health and also to determine the level of treatment which are tend to solved environmental problems that occur in daily life (Kim and Wolt, 2011). Several methods have been proposed for estimation of the potential risks to human health and this may be divided into carcinogenic and non-carcinogenic effects (USEPA, 1989, 2011; Naughton and Petroczi, 2008). The non-carcinogenic risk assessment is typically based on the target hazard quotient (THQ). The THQ provide an indication of the risk level associated with pollutant exposure. This method of risk estimation has been recently used by many researchers (Chien *et al.,* 2002, Wang *et al.,* 2005) and has been proven valid and useful. The health protection standard of lifetime risk for non-carcinogens is 1 (USEPA, 2011). Ratio less than 1 signifies non-obvious risk, relatively an exposed population of concern will experience risk if the dose is equal to or greater than 1 or the oral reference dose (ORD). Hence, the non-carcinogenic risk assessment was also employed in this study.

In the Niger Delta Area of Nigeria, the entire coastline is dotted by several communities which rely

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closely on environmental resources particularly fisheries for survival. These aquatic organisms are typically processed for consumption and commercial purposes. Among the fish and shellfish species *P. auria* and *T. fuscatus* (periwinkles) is the commonest aquatic organism easily harvested by local resident. These marine organisms live in the mangrove swamps in the intertidal zone and heavy metal present in high concentrations in the aquatic habitats are bioaccumulated within the tissues of intertidal organisms (Odu *et al.,* 2011). Generally, these shellfish species are considered very healthy for human consumption. They contain high quality protein and other essential minerals and vitamins, low lipid content, low saturated fatty acids and omega-3 fatty acids which help to promotes healthy cardiovascular systems and prevent heart related diseases (Ajayi, 1978; Funmilayo,2008). Therefore, we thought it necessary to investigate the levels of Cu, Zn and Fe in *P. aurita* and *T. Fuscatus* (commonly known as periwinkles) from Bayelsa State, Niger-Delta Area, Nigeria and assessed the health risk associated with consumption of these organisms by human.

MATERIALS AND METHODS

Sample collection: The marine periwinkles (*P. aurita and T. fuscatus*) were collected from three local government areas in Bayelsa State, Niger Delta Area, Nigeria. These include Nembe in Okoroba community, Southern Ijaw in Koluama community and Ekeremor in Peretorugbene community. Three (3) samples each of *P. aurita* and *T. fuscatus* were collected.

Sample preparation: The soft tissues (the edible parts) of the periwinkles were obtained by cracking the shells, thoroughly washed and kept in the freezer. Samples were transported to the post-graduate laboratory in the Department of Pure and Industrial Chemistry, University of Port Harcourt Choba, Nigeria, oven dried at 105^0C to a constant weight, ground and sieved to uniform particle size.

Sample digestion: 0.5g of each sample was digested with 10ml aqua regia (3:1 v/v HCL / ($HN0_3$) and 1ml $HClO_4$ (all chemical are analytical grade). The mixtures were place on hot plate, heated and boiled off to near dryness. After digestion samples were cooled and diluted with 25ml distilled water and filtered with Whatman filter paper (N541).

Instrumentation: Heavy metal concentrations (Cu, Zn and Fe) were measured with flame atomic absorption spectrophotometer, FAAS (GBC Avanta 2.02 model). For all the metals analyzed, acetylene gas was used, while the oxidant was compress air. For quality assurance, the samples were digested and analyzed in triphicate along with blanks to minimize error. The instrument was calibrated with series of standard solution supplied by manufacturer.

Health risk evaluation: Since there was no agreed limit for acceptable maximum levels for non-carcinogenic risk, therefore, the United State Environmental Protection Agency (USEPA) models provided in Region III risk based concentration table were employed (USEPA, 2011; 2001, 1989). These include: Estimated Dietary Intakes (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI).

Estimation of dietary intake (EDI): The estimated dietary intake of the heavy metals in the periwinkles (*P. aurita* and *T. fuscatus)* were evaluated using the formula;

$$\text{EDI (mg/kg-bw/day)} = \frac{MLs\ X\ MCs}{BW}$$

Where; MI is the mass of the periwinkles ingested, the per capital consumption of fish and shellfish in Nigeria for human food is averaged 9.0 kg (WHO, 2011) which is equivalent to 24.7 g per day, MC is the metal concentration and BW is the body weight of the consumers (60 kg for adult).

Target hazard quotient (THQ): The THQ is an estimate of the risk level for non-carcinogenic due to pollutant exposure. The equation used for estimating THQ is as follows;

$$\text{THQ} = \frac{EF\ X\ ED\ X\ MI\ X\ MC}{ORD\ X\ B\omega\ X\ AT_n}\ x\ 10^{-3}$$

Where; EF is the exposure frequency (365 days/year), ED is the exposure duration (51.86years) which corresponds to average life span of a Nigerian (World Bank, 2011), ORD is Oral Reference Dose (i.e a daily dose of the metals that is likely to pose no appreciable risk of deleterious effects during a life time). This includes Cu (0.04), Zn (0.3), Fe (0.7), AT_n is the average exposure time for non-carcigens (EF X ED, 365 days/year x 51.86 years) and 10^{-3} is the unit conversion factor.

Hazard Index: Hazard Index is the combine effects of the individual metal in the periwinkles and was calculated as follows;

$$HI = \Sigma THQi$$

Where; ΣTHQi is the sum total of the individual metal in the periwinkles and I is the distinct heavy metal tested.

RESULTS AND DISCUSSION

Heavy metal concentrations in *P. aurita* and *T. fuscatus* were investigated using Atomic Absorption Spectrophotometry and the results are present in table 1

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Table 1: Heavy metal concentrations (mg/kg dry wt, range, mean ±SD) in periwinkle (*P. aurita* and *T. fuscatus*) from Bayelsa State.

Periwinkle sample	**Statistics**	**Trace Heavy Metals**		
		Cu	**Zn**	**Fe**
P. aurita	Range	54.0 - 62.5	50.5 - 73.0	1035 - 1380
	Mean ±	57 ± 4.0	63.8 ± 9.6	1260 ± 159.2
T. fuscatus	Range	31.5 - 35.0	66.5 - 89.0	735 - 2330
	Mean ± SD	33.3 ± 1.43	77.7 ± 9.19	1985 ± 4.89
Guidelines				
WHO, 1985		3.0	10 - 75.0	0.5
FEPA 2003		1 - 3.0	75.0	2.0
MAFF 1995		20.0	50.0	-

The concentration of Cu in the samples ranged from 54.0 - 62.5 mg/kg with mean ± SD of 57 ± 4.0 mg/kg in *P. aurita* and 31.5-35.0 mg/kg with mean ± SD of 33.3 ± 1.43 mg/kg in *T. fuscatus*. The concentrations of Cu in *P. aurita* is also higher than *T. fuscatus*. The concentrations of Cu in these organisms are higher than the recommended limits of WHO (1985), FEPA (2003) and MAFF (1995) respectively. Although Cu is an essential element in living system, it is a key constituent of the respiratory enzymes and protein which help in oxygen transportation and electron transfer. However excessive intake of Cu is known to cause adverse health effects ranging from reduced immune body function to shortage of blood (anemia), liver and kidney damage, stomach and intestinal irritation (John and Larry, 2008).

Zinc: Zn concentrations in the periwinkles ranged from 50.0-73.0 mg/kg with mean ± SD of 63.8 ± 9.6 mg/kg in *P. aurita* and from 66.5-89.0 mg/kg with mean ± SD of 77.7 ± 9.19 mg/kg in *T. fuscatus*. The concentration of Zn in *T. fuscatus* is higher than *P. aurita, but these values are* within the limits recommended by WHO and FEPA. Zn is an essential trace metal needed by plants, animals and micro-organisms. In human Zn plays ubiquitous biological roles. It interacts with a wide range of organic ligands and has a role in the metabolism of RNA and DNA, signal transduction and gene expression. It also modulate the brain excitability (Hambridge and Krebs, 2007). Hence *P. aurita* and *T. fuscatus* are good sources of Zn in the diet of the consumers.

Iron: Fe concentrations ranged from 1035-1380 mg/kg with mean ± SD of 1260+ 159.2 mg/kg in *P. aurita* and 735-2330 mg/kg with mean ± SD of 1985 ± 4.89 mg/kg in *T. fuscatus.* Fe concentrations in these periwinkles are extremely high as compared to other heavy metals in this study. Fe is an essential mineral for life. It is present in every living cell and necessary for the production of haemoglobin, myoglobin and certain enzymes. Fe deficiency can cause weakness, inability to concentrate and susceptibility to infection. According to the World Health Organization, Fe deficiency (anemia) is one of the most common nutrient deficiencies in the world (Spear, 2000). Therefore, the high concentrations of Fe in these periwinkles make them good sources of Fe in the diet of the consumers.

Generally, it was observed that the concentration of the heavy metals in the periwinkle species are in the decreasing order Fe > Zn > Cu. The concentration of the trace metals in this study are higher than the values obtained by Osakwe et al., (2014), Akoto et al., (2014), Ijeomah et al., (2015) and Amirah et al., (2013) in some fish and shellfish species. The high levels of heavy metals observed in this study can be attributed to the massive oil exploration and exploitation activities as well as other industrial activities in Bayelsa State. According to Howard et al., (2006), elevated chloride contents tend to enhance chloride complex formation which decreases adsorption of heavy metals on water and sediment and result in greater solubility and mobility of the metals. Also, Horfall and Spiff, (2005), reported that acidic and oxidizing condition tends to release large amount of heavy metals into solution due to decrease sorption capacity of metal species. Howard et al., (2008), in another study observed that, in the Niger Delta region of Nigeria, an acidic and oxidizing conditions have been created due to industrial activities, and there is high chloride content due to sea water intrusion. The combination of these two factors may enhance the release of heavy metal ions from soil and sediment to overlying water system for ingestion by filter feeding organisms such as periwinkles.

Health risk evaluation: the human health risk evaluation of the trace heavy metals, Cu, Zn and Fe in the *P. aurita* and *T. fuscatus* were evaluated using the estimated dietary intakes (EDI), target hazard quotient (THQ) and the hazard index (HI).

Estimated Dietary Intake (EDI): An important aspect in assessing risk to human health from potentially toxic chemical in food is the knowledge of the dietary intake of such substance in comparison with safe margins or guidelines. The estimated dietary intake (EDI) of the metals in the periwinkles is presented in table 2. Intake estimates were expressed as per unit body weight (mg/kg-bw/day or weekly).

Table 2: Estimated dietary intake (mg/kg-bw/day or week) of Cu, Zn and Fe in periwinkle species.

Periwinkle samples	**Trace Heavy Metals**					
	Copper		**Zinc**		**Iron**	
	EDI	EWI	EDI	EWI	EDI	EWI
P. aurita (periwinkle with sharp spine)	23.47	164.29	26.26	183.84	518.70	3630.9
T. fuscatus (periwinkle without spine)	13.71	97.93	31.99	223.93	817.16	5720
Guideline	PTDI	PTWI	PTDI	PTWI	PTDI	PTWI
EU, (2003)	0.083	0.581	-	-	-	-
NRC (1989)	-	-	.2	1.4	0.21	1.47

EDI: Estimated Daily Intake
EWI: Estimate Weekly Intake
PTDI: Provisional Tolerable Daily Intake
PTWI: Provisional Tolerable Weekly Intake

As indicated in table 2, the estimated EDI and EWI for Cu were 23.47 mg/kg-bw/day and 164.29 mg/kg-bw/week in *P. aurita*, while *T. fuscatus* values were 13.71 mg/kg-bw/day and 97.93 mg/kg-bw/week respectively. These values are higher than the PTDI of 5000 µg/day for intake of Cu recommended by European commission (2003), which is equivalent to 0.083 mg/kg-bw/day and 0.581 mg/kg-bw/week. For Zn the EDI and EWI were; 26.26 mg/kg-bw/day an 183.84 mg/kg-bw/day in *P. aurita*, while *T. fuscatus* values were 31.99 mg/kg-bw/day and 223.93 mg/kg bw/week. Also these values are higher than the PTDI of 1200 µg/day of Zn, recommended by National Research Council, (1989), which is equivalent 0.2 mg/kg-bw/day and 1.4 mg/kg-bw/week.

Finally, the EDI and EWI for Fe in the periwinkles were; 518.70 mg/kg-bw/day and 3630.9 mg/kg-bw/week in *P. aurita*, while *T. fuscatus* were; 817.16 mg/kg-bw/day and 5720 mg/kg-bw/week. Again these values are higher than the recommended daily intakes of NRC of Fe which is 12500 µg/day and this is equivalent to 0.21 mg/kg-bw/day.

Target hazard quotient (THQ) and hazard index (HI). The results of non-carcinogenic health risks due to trace metal exposure in marine snails (periwinkles) from Bayelsa State are presented in table 3. The THQ of the metals in *P. aurita* (periwinkle with sharp spine) are in the decreasing order of Fe > Cu > Zn with risk values of 0.74, 0.59 and 0.09 respectively. The THQ of the individual metals were below the maximum acceptable limits of 1, however, the combine effects of the trace metals which is express in form of Hazard Index (HI) is within the acceptable limit of I. while in *T. fuscatus* the THQ of the individual trace metals are also in the decreasing order of Fe > Cu > Zn with risk values of 1.17, 0.35 and 0.11 respectively. The HI value of *T. fuscatus* is 1.62 which is a little above the maximum acceptable limits of 1 (i.e approximately 2.0).

Among the elements studied, Fe posed the greatest risk in the two marine snails studied. This is a reflection of the high concentration of Fe in these organisms which is often associated with the endogenous nature of Fe as an important biological element in living cells as well as the organisms physiology. In relation to the marine snails, *T.*

fuscatus presented risk above the acceptable level and also above the level reported by Markmanuel and Horsfall, (2015) in land snail (*L. flammea*) and Osakwe et al, (2015) in (*learias garipinus*). However, this value is similar to the value obtained by Markmanuel and Horsfall (2015) in land snail (*A. achitina*).

Table 3: Target hazard quotient (THQ) and Hazard Index (HI) for intake of Cu, Zn and Fe from the consumption of 24.7g of the periwinkles per day

Periwinkle sample	Target hazard quotient (THQ)			Hazard index
	Cu	Zn	Fe	(HI)
p. aurita	0.59	0.09	0.74	1.42
(%) HI	41.26	6.29	51.75	
T. fuscatus	0.34	0.11	1.17	1.62
(%) HI	20.99	6.79	72.22	

% HI = Percent (%) contribution of each metal to the hazard index.

*Conclusion***:** The concentrations of Cu, Zn and Fe in the marine snails investigated in this study were higher than the guideline values set by different regulatory bodies, except Zn that the concentrations were within the limits of WHO and FEPA. The assessment of non-carcinogenic risk evaluated in this study showed that the target hazard quotient of the individual metals were below the acceptable limits of 1, but the combine effects of these metals in the snail (i.e HI) were within the limits of 1, indicating no health concern at the moment. However considering the bio accumulative nature of heavy metals these snails should be consumed moderately. Also, in view of the high levels of heavy metals in these periwinkles more intensive study is needed to determine other heavy metals and persistent organic pollutants (e.g DDT, PCB etc) of health concern as well as other fishes and shell fish species from Bayelsa State.

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