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Bio-accumulation of Pb and Cd in the Soft tissues of *Tympanotonous fuscatus,* Sediment quality of the Upper Bonny Estuary Niger Delta and their Human Health Implications

By

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Abstract

Environmental Pollution is a global problem especially as it affects the human health. Appreciable levels of heavy metals in the ecosystem exert deleterious effects on plants, animals and humans due to their high level of toxicity, persistence and bioconcentration and transfer along the food chain. So, studies to bio-monitor the levels of heavy metals in the environment and determine potentially hazardous levels for humans are necessary. In this study the concentration of Pb and Cd in the soft tissues of *Typanotonous fuscatus* and sediment quality of the upper Bonny Estuary, Niger Delta was carried out. Samples of *Tympanotonous fuscatus* were caught in 5 selected stations namely; Okochiri Creek (S1), Ekerekana Creek (S2), Okari-Ama Creek (S3), Ogoloma River (S4) and Bonny Estuary (Control). Concentrations of Pb and Cd in the soft tissues of *Tympanotonous fuscatus* and Sediment were analyzed following standard procedures using atomic absorption spectrophotometer (AAS) by GBC Avanta. The concentration of Pb and Cd in the soft tissues of *Tympanotonous fuscatus* ranged from 11.49 to 15.42mg/kg and 0.06 to 0.13mg/kg respectively, these values were not significantly different using turkeys multiple comparison at $P<0.05$ amongst the sampled stations. The concentrations of Pb were higher than the FAO permissible limit of 0.5mg/kg while Cd was below permissible limit. However, the Estimated Daily Intake (EDI) showed that Pb concentration ranged from 6.57mg/kg to 8.81mg/kg which is above FAO/WHO,(2010) Permissible Tolerable Daily Intake of 0.25mg/kg while estimated daily intake for Cd showed the range of 0.01 to 0.07mg/kg, which were within FAO / WHO, (2010) Permissible Tolerable Daily Intake of 0.07mg/kg. Sediment concentration range of 6.13 to 11.66 mg/kg and 0.01mg/kg to 0.07mg/kg were recorded for Pb and Cd respectively. These levels were below sediment quality guideline of 40mg/kg and 1mg/kg respectively (USEPA, 1999). The results above indicated that consumption of *Tympanotonous fuscatus* from the sampled areas of Upper Bonny Estuary would likely pose a potential health risk because of the high concentration of Pb which could bio-accumulate in the soft tissues and bones of the consumer, further more the sediment quality also revealed that Pb and Cd in sediment of the sampled stations were below permissible limit as specified by USEPA, SQG (1999) indicating them not to be up to pollution levels. This study emphasized that due to continuous exposure of the study area to industrial effluent discharges into the water bodies, over time, the accumulation of Pb and Cd in sediment and soft tissues of *Tympanotous fuscatus* could increase and become very detrimental for the coastal area. This will then constitute a health risk to the individuals in the area that consume these organisms and definitely become an issue of serious environmental concern.

Key words: Lead, Cadmium, .sediment quality, *Tympanotonus fuscatus,* health risks

1.0 Introduction

Environmental pollution is a global problem expecially as it affects human health (Sadatipour *et al.,* 2004). Heavy metal accumulation in the environment is increasingly attracting attention of policy makers and researchers due to their high enrichment factor, slow removal rate, toxicity, persistence and bio-concentration in the food chain (Monday and Nsikak, 2007). Studies on heavy metals in rivers, lakes, fauna and sediments have been a major environmental focus especially during the last decade. *Typanotonous fuscatus* (Gastropoda: Potamidae) is a univalve gastropods of the Phylum mollusca. The genus is commonly referred to as ‘‘Periwinkle’’ in Nigeria. Periwinkles are shell fish found in the littoral region of the sea, brackish or estuarine waters which are seasonally in submerged regions like the mangroove swamps (Bobmanuel, 2012).

They inhabit the quiet waters where the substratum is rich in decaying organic matter and muddy sediments (Jamabo and Chindah, 2010). They are euryhaline that is, they have the ability to tolerate a wide range of salinities between 0.1mg/l to 25mg/l and are deposit feeders, feeding on the mud and other decaying organic matter, and they have high economic importance in the Niger Delta area of Nigeria. They are noted also to have high binding affinity for heavy metals and other pollutants in any contaminated aquatic ecosystem (Jamabo and Chindah, 2010).

Studies have revealed that periwinkles have the tendency to bio-accumulate heavy metals from about 2 - 729 times than that in sediment (Otitoloju and Don-Pedro, 2002). Heavy metals such as Pb bio-accumulates in most organisms, but biomagnifications from one trophic level in the food web to the next is not a characteristic feature of this metal. Lead bioaccumulation is primarily dependent on the amount of active lead compounds (predominantly aqueous species) in the environment and the capacity of animal species to store it (Prosi, 1989). Lead is toxic at very low exposure levels and has acute and chronic effects on human health. It is a multi-organ system toxicant that can cause neurological, cardiovascular, renal, gastrointestinal, haematological and reproductive effects. The type and severity of effects depend on the level, duration and timing of exposure. Lead is a neurotoxin that causes behavioural deficits in fish, birds, and mammals within days of exposure to sublethal concentrations and these effects can persist after removal from contaminant (Burger and Gochfed, 1997). Lead also causes deficits or decreases in survival, growth rates, development, behaviour, learning and metabolism as well as increased mucus formation in fish (Eisler, 1988).

Cadmium (Cd) on the other hand is a metal pollutant and causes a range of pathological alterations (Zheng *et al.,* 2009). They are bio persistent and once absorbed by an organism, remains resident for many years before it is eventually excreted. Exposed humans will never get rid of all of the Cd in their bodies because it will take 20-30 years to get rid of 50% of it, 40-60 years to get rid of 75%, and 60-90 years to get rid of 87.5% (Ugbomeh and Bako, 2015). As shown in Itai-Itai Disease, Cd effects on human health include skeletal deformities and bone damage including osteoporosis and osteomalacia, kidney damage, and generalized pain. Chronic Cd exposure causes kidney damage by inhibiting enzymes responsible for resorption processes, ingestion, by a Japanese population, of water and food contaminated with Cd was associated with a crippling condition, ital- itai (ouch-ouch) disease. The affliction is characterized by pain in the back and joints, osteomalacia (adult rickets), bone fractures, occasional renal failure especially in women with multiple risk factors such as multiparity and poor nutrition.

Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota (Camusso *et al.,* 1995), which generally exists in low levels in water and attain considerable concentration in sediment and biota. So, studies to bio-monitor the levels of heavy metals in the environment and determine potentially hazardous levels for humans are necessary. In this study the concentration of Pb and Cd in the soft tissues of *Typanotonous fuscatus* and sediment quality from the upper Bonny Estuary, Niger Delta was carried out.

2.0 Materials and Methods

2.1 Description of the study area

The sampling stations were established along the stretch of Upper Bonny Estuary in Rivers State, Niger Delta. The creek is a brackish inter-tidal mangrove swamp. The vegetation consist of *Rhizophora racemosa, Leguncularia racemosa, R. Mangle, Nypa frutcan, Avicennia nitida* which lines the shores of the creek. Anthropogenic activities along the creek include discharge of industrial effluents, sand mining or dredging, fishing, navigation, washing, bathing and recreational activities. A major industrial outfit which is situated in station 2 (Ekerekana) is the Port Harcourt Refinery Company (PHRC) [a subsidiary of the

Nigerian National Petroleum Corporation (NNPC)], which generates several volumes of effluents that is channelled into the creek via a drainage system.

Five (5) sampling stations were selected and established for this study as indicated in Fig. 1 and are described below:

- Okochiri River as station 1 (S.1):Representing the upstream of the river N04º44'36.4' and E007º06'31.7''
- Ekerekana Creek as station 2 (S.2) which serves as the Point Of Discharge into the river N04º44'46.9' and E007º06'06.0''
- Okari-ama River as station 3 (S.3):Representing the Downstream of the river N04º44'23.2' and E007º05'51.8''
- Ogoloma River as station 4 (S.4): Representing the Downstream of the river N04º43'59.0' and E007º05'05.7''
- Bonny River (Control station) N04º43'16.0' and E007º04'40.8''

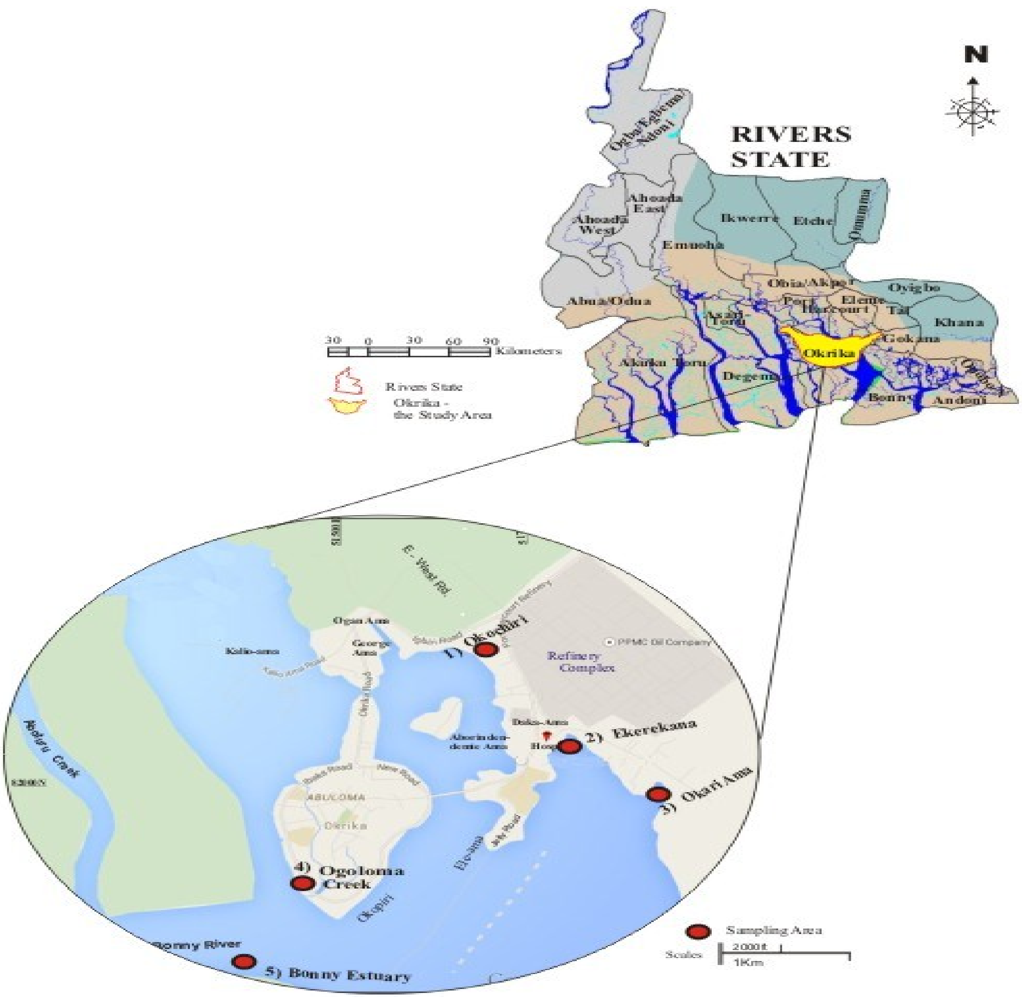

Fig. 1: Map Showing Nigeria, Niger Delta, Rivers State and Upper Bonny Estuary the Study Area.

2.2 Sample Collection and Method

2.2.1 Sediment Sample Analysis

Sediment samples were collected within the intertidal at low tide with the use of a stainless Eckman's grab. Collected samples were placed in a pre-cleaned well labelled polythene bags and placed in an ice chest before transporting to the laboratory for further chemical analysis. In the laboratory the sediment samples were air dried for two to three weeks under room temperature. After drying, the dried samples were disaggregated; visible remains of organisms and debris were removed. The dried samples were then crushed in a mortar, sieved through a 200µm sieve to normalize particle size and then homogenized in a porcelain mortar, sieved (using a 2-mm mesh size) and stored in plastic container. Then 2g of crushed sample was then weighed out and dissolved in 5ml of ultra pure nitric acid 10% in HCL solution for acid digestion before heavy metal analysis. The solution was filtered into a cleaned and dried 20ml standard volumetric flask. The solution was then aspirated on to the Atomic Absorption spectrophotometer (AAS) model GBC Avanta Ver 2.02 and the metal concentrations were recorded. The AAS reading was in ppm.

2.2.2 *Typanotonous fuscatus* sample collection and Analysis

Thirty six (36) samples of *Tympanotonous fuscatus* used in this study were collected from the stretch of upper Bonny Estuary Rivers State at the five (5) selected stations. The collection was carried out bimonthly for six months (6) from September 2016 to February 2017 by handpicking. The samples were preserved in ice and taken to the laboratory for subsequent analysis. In the laboratory, the whole biota samples were properly cleaned (by copiously rinsing all exposed and partially enclosed parts) with distilled water to remove debris and all external adherents before processing for analysis. The samples were weighed out to the nearest 0.01g on a top pan electronic balance and measured in cm to the nearest millimeter using a ruler.

2.2.1 Sample preparation

Samples were prepared according to APHA (1995) and Ademoroti (1996) methods. The samples were thawed on a cleaned plastic sheet, and the whole soft tissue of *Tympanotonous fuscatus* were extracted and separated from the shell, placed in cleaned silica dishes, dried and ignited at 500^{o}C for 30 minutes until it turned to ash. Then the samples were allowed to cool in a desiccator and crushed into powdery form. Then 2g of the sample was weighed out for acid digestion.

2.2.2 Preparation of solution of the ash

The ash was dissolved in 0.5ml of ultra pure nitric acid 10% in HCL solution. The solution was filtered into a cleaned and dried 20ml standard volumetric flask. The solution was then aspirated on to the Atomic Absorption spectrophotometer (AAS) model GBC Avanta Ver 2.02) and the metal concentrations were recorded. The AAS reading was in ppm.

2.2.3 Quality control

Precautions were followed during the trace metal determinations to prevent contamination. Periwinkle samples were washed in deionised water before extracting the edible tissues from their shell. All aqueous solutions were prepared with deionised water; glassware and plastic containers used were rinsed and soaked overnight in 10% (v/v) HNO_3 and subsequently rinsed and dried before use. Quality control standards were run at selected intervals during the analysis to ensure consistent instrument performance over the period of the analysis. The effect of interfering elements on the determination of Pb, and Cd was assessed by measuring the response of standards with and without an interfering ion. Each set of samples was accompanied by a system blank, a spiked blank and reference material. Accuracy and precision were verified using certified reference materials (IAEA 2003). Analytical results of the quality control samples indicated a satisfactory performance of trace metal determinations within the range of certified values of 95–111% recovery for the metals studied. Each sample was analysed in triplicate for repeatability with a relative SD <5% for all metals analysed.

2.2.4 *Statistical analysis*

Metal data for soft tissues of *Tympanotonous fuscatus* were subjected to analysis of variance (ANOVA), Turkeys multiple comparison was used to compare the significant difference of the mean values amongst the sampled station. Probabilities less than 0.05 was considered statistically significant. All statistical analyses were carried out with the SPSS 20 software program.

3.0 RESULTS

3.1 Mean concentration of Pb and Cd in sediment

The mean concentration of sediment samples in the selected stations are shown in Table 1 below:

Table 1: Mean concentration of Pb and Cd in sediment and Sediment Quality Guideline USEPA, SQG (1999)

Parameters	Okochiri Creek	Ekerekana River	Okari-Ama River	Ogoloma River	Bonny Estuary	USEPA, SQG (1999)
Pb (mg/kg)	6.90±7.41	6.13±8.46	11.66±14.41	11.10±14.41	10.77±14.56	40
Cd (mg/kg)	0.07±0.16	0.07±0.16	0.05±0.06	0.01±0.02	0.01±0.02	1

The mean Pb concentration in sediment ranged from 6.13±8.46 to 11.66±14.41. The highest concentration was recorded in Okari-Ama River (11.66±14.41) followed by Ogoloma River, Bonny Estuary (control), Okochiri River, and Ekerekana Creek with mean concentrations of 11.10±12.12, 10.77±14.56, 6.90±7.41 and 6.13±8.46 respectively. While the concentrations of Cd in the sediment ranged from 0.01±0.02 to 0.07±0.16. The highest concentration was recorded in Okochiri river (0.07±0.16) followed by Ekerekana Creek, Okari-Ama River, Ogoloma Creek and Bonny Estuary (control) with mean values of 0.07±0.11, 0.05±0.06, 0.01±0.02 and 0.01±0.02 (Fig. 2). Statistically the values at $P<0.05$ were not significantly different amongst the sampled stations. Comparison using international limit following USEPA Sediment Quality Guideline. USEPA(SQG) of 40mg/kg showed that the values were below permissible limits.

3.2 Bio-Sediment Accumulation Factors (BSAF)

Bio-Sediment accumulation factor was estimated as the ratio of the concentration of heavy metal in animal divided by heavy metal concentration in the sediment (BSAF, that is, bio-sediment accumulation factor) as shown in Table 2.

$$BSAF = \frac{\text{Heavy metal concentration in animal tissue}}{\text{Heavy metal concentration in sediment}}$$

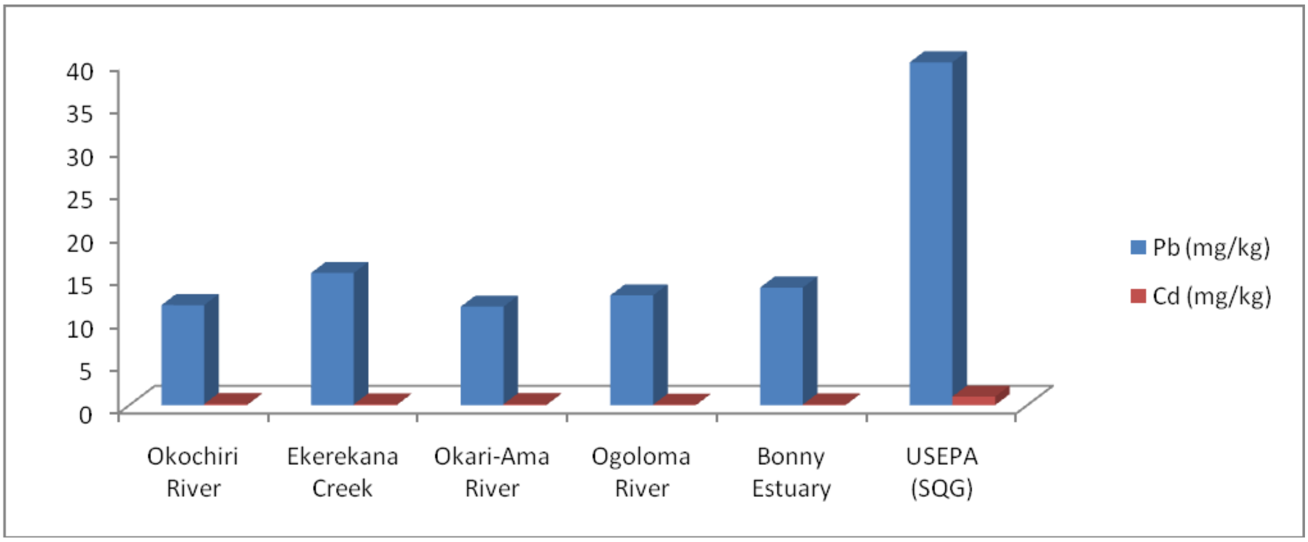

Fig. 2: The concentration of Pb and Cd in sediments from the sampled stations

Table 2:Bio-sediment Accumulation Factor amongst the Sampled Stations

Stations	**Pb (mg/kg)**	**Cd (mg/kg)**
Okochiri River	1.69	1.85
Ekerekana Creek	2.51	1.42
Okari-ama River	0.98	2.20
Ogoloma Creek	1.15	6.0
Bonny Estuary(control)	1.27	7.0

3.2 Mean bioaccumulation of Pb and Cd in the soft tissues of *Tympanotonous fuscatus*

The mean concentration of *Tympanotonous fuscatus* in the selected station is shown in Table 3 below.

Table 3: Mean concentration of Pb and Cd in the soft tissues of *Tympanotonous fuscatus*

Parameters	**Okochiri River**	**Ekerekana Creek**	**Okari-Ama River**	**Ogoloma River**	**Bonny Estuary**	**FAO / WHO (2010)**
Pb (mg/kg)	11.67±16.50	15.42±21.81	11.49±16.25	12.81±18.12	13.71±19.38	0.5
Cd (mg/kg)	0.13±0.19	0.10±0.14	0.11±0.15	0.06±0.08	0.07±0.09	0.5

The mean concentration of Pb in the Soft tissues of macrobenthic invertebrate (*Tympanotonous fuscatus*) ranged from15.42±21.81 to 11.49±16.25. The highest concentration of Pb in the soft tissue of *Tympanotonous fuscatus* was recorded in Ekerekana Creek 15.42±21.81 followed by Bonny Estuary (Control), Ogoloma Creek, Okochiri River and Okari-Ama River with mean values of 13.71±19.38, 12.81±18.12, and 11.67±16.50 . Statistically using Turkey's multiple comparison at $P<0.05$, showed that the values were not significantly different. However comparing the values with international limit FAO / WHO, (2010) of 0.5mg/kg, the values were shown to be above permissible limit while the concentration of Cd in *Tympanotonous fuscatus* ranged from 0.06± 0.08 to 0.13±0.19. the highest concentration was recorded in Okochiri River (0.13±0.19) followed by Okar-Ama River, Ekerekana Creek, Bonny Estuary (control), Ogoloma River with mean values of 0.11±0.15, 0.10±0.14, 0.07±0.09, and 0.06±0.08. statistically using Turkey's multiple comparison at $P<0.05$, showed that the values were not significantly different amongst the sampled station, also comparing with international safe limit with FAO/WHO (2010) for the concentration of heavy metals in biota showed that the levels are within permissible limit (Fig. 3).

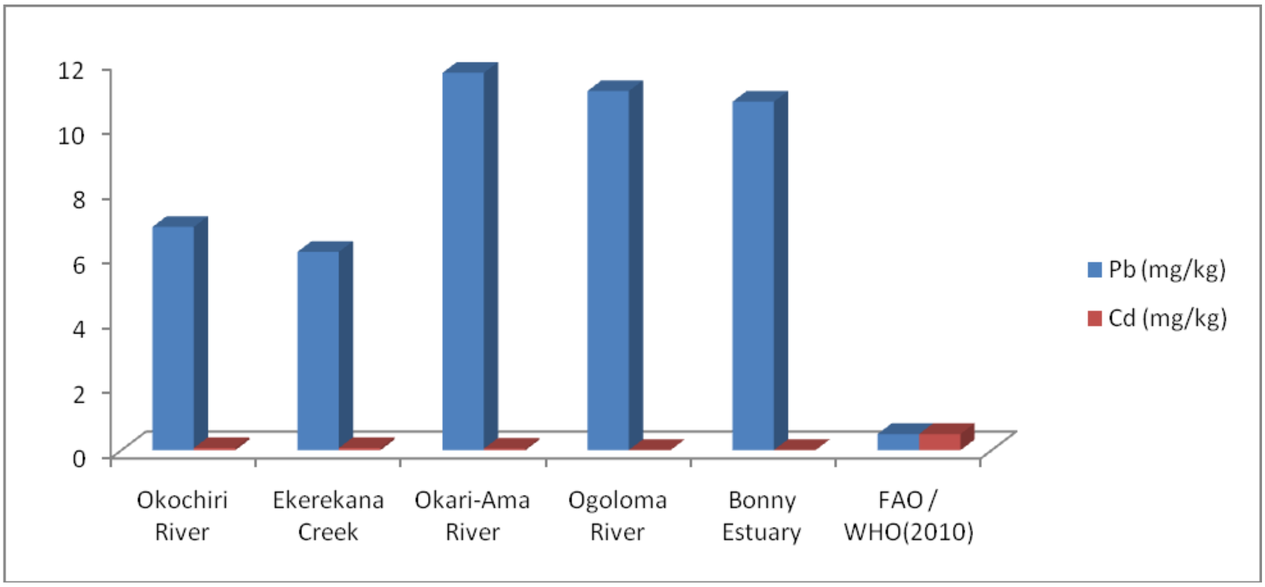

Fig 3. Mean bioaccumulation of metals in *Tympanotonous fuscatus* from the different sampled stations and the safe permissible limit

3.3 Heavy Metal: Human Exposure Risk Assessment

Risks associated with human consumptions of the potential contaminated periwinkle with heavy metals measured in the study was derived by comparing the levels quantified in the soft tissues and International safe limits (Maximilian *et al.,* 2015). Exposure doses from ingestion of contaminated biota among population can be derived following the equation of Agency for Toxic Substances and Disease Registry on calculation of exposure doses assuming that all biota consumed are caught from one contaminated water body (Maximilian *et al.,* 2015)

ADD = (C×IR ×EF ×ED) /(BW × AT)

- C = the mean total trace metal concentration (mg/kg)
- IR = Intake rate of contaminated medium (0.04kg/day = 40g))
- EF = Exposure frequency (365days per year)
- ED = Exposure duration over a life time (53years)
- BW=The body weight of adult person is 70 kg.
- AT= Average life time (53×365days per year)

Table 4: Calculated Average Daily Dose and Provisional Tolerable Daily intake for metal ingestion

Stations	Pb (ADD)(mg/kg/day)	Cd ADD)(mg/kg/day)
Okochiri river	6.61	0.07
Ekerekana creek	8.81	0.06
Okari-ama river	6.67	0.03
Ogoloma creek	7.32	0.01
Bonny Estuary(control)	7.83	0.04
PTDI	0.25	0.07

*Provisional Tolerable Daily Intake (FAO/WHO, 2010)

4.0 Discussion

4.1 Concentration of Pb and Cd in the sediment

The mean levels of Pb and Cd in the sediment were below permissible limit USEPA, SQG(1999) but the values recorded reviewed sediment as a sink for heavy metals in the study area and can be attributed to continuous anthropogenic discharge of heavy metals contaminated wastes / effluents into the water bodies over the past decades. The result further revealed a high bio-accumulating capacity (BSAF) of Pb and Cd in the sampled stations which confirms the study area as a major source for bioaccumulation of Pb and Cd into the food chain. These heavy metal concentrations in the sediment may also play a significant role in the remobilization of the metals into the aquatic system under favorable conditions and in the interactions between sediments and biotas which feed on mud flat (decayed detritus) like the periwinkles

(Rasheed, 2001). The river been tidal in nature constantly distributes heavy metals front and back thereby localizing pollution around the study area and could indeed be a major contributing cause of heavy metal distribution along the stretch of the upper Bonny Estuary.

4.2 Bio-accumulation in the soft tissues of *Tympanotonous fuscatus*

Lead accumulation in the soft tissues of *Tympanotonous fuscatus* amongst the sampled stations was compared with international safe limit FAO/WHO, (2010) which showed that the levels were higher than the permissible limit and so portrays it as unfit and unhealthy for human consumption. Further calculations on the risk associated with consumption of *Tympanotonous fuscatus* (Table 4) clearly revealed that the level of accumulation in the soft tissues of the shell fish were above the Permissible Tolerable Daily Intake (PTDI) thereby affirming a possible long term adverse health effects associated with constant consumption of these biota from the sampled water bodies. Cd concentration showed that the values were within permissible limit and below Provisional Tolerable Daily Intake (PTDI) but the levels in BSAF revealed that over time there could be possible increase of this metal in the soft tissues of *Tympanotonous fuscatus* if the continuous discharge of contaminated wastes / effluents into the water ways is not discontinued or controlled.

5.0 Conclusion

This study emphasized that due to continuous exposure of the study area to industrial effluent discharges and other anthropogenic activities into the water bodies, over time, the accumulation of Pb and Cd in sediment and soft tissues of *Tympanotous fuscatus* could increase and become very detrimental for the coastal area. Thus, it will constitute a health risk problem to the individuals in the area that consume these organisms and definitely become an issue of serious environmental concern.

Concentrations of pollutants in periwinkles are useful bioindicators due to their high binding affinity for heavy metals and other pollutants and are also a reflection of how stressed the estuarine environment could be, so it can be assumed that the environment from where these organisms are caught to be contaminated.

Hence further studies on bioaccumulation of other chemicals or pollutants may help elucidate the impact of contaminants on the human body. The study also emphasized the need for constant environmental monitoring of the estuarine environment which should be encouraged by policy / decision makers in order to further safeguard the health of the citizenry.

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