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Investigation of the Concentration of some Metals in Blood Cockle (*Senilia senilis*) and Oyster (*Crassostrea gasar*) from Bonny Estuary and Assessment of the Human Health Risk Associated with their Consumption

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ABSTRACT: There is a growing concerns over the health risk of consuming seafood contaminated with poisonous metals from human activities. This study examined the concentration and human health risk of Pb, Cd, Cr, Ni, Co, Zn, V and Hg associated with the consumption of blood cockle (*Senilia* senilis) and oyster (*Crassostrea gasar*) from Bonny Estuary, Rivers state Nigeria by analyzing the levels of the selected metals using Atomic Absorption Spectrometer Model A 200 AA. Data were used to calculate the non-carcinogenic health risk using Daily Intake Rate (DIR) Target Hazard Quotient (THQ) and Hazard Index (HI). The results showed variations in the concentration of the different heavy metals in the two shellfish. The trend of the metal concentrations in decreasing order was: *Crassostrea gasar* > *Senilia senilis*. Levels of metals in *Senilia senilis*, were all below the WHO and FAO permissible limit for fish. Also, all metals except Pb were below the WHO maximum permissible limit in *Crassostrea gasar*. The DIR (9.4684E-06 - 0.00281); THQ (8.286E-07 – 1.842E-04) and HI (2.519E-05 -2.595E-04) for all metals were below one indicating no risk for non-carcinogenic effects individually and collectively. The shell fish analyzed from the Bonny Estuary are free from potential human health hazards from heavy metals. However, continuous monitoring of levels of metals in fish from the estuary is recommended.

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Heavy metals are persistent environmental pollutants, non-biodegradable, ubiquitous in nature and pose threat to public health because of their toxicity. Heavy metals occur in the environment through natural and anthropogenic sources. Humans are easily exposed to them via food, water, air and contact in excess, deficiency or through imbalance in the body (Jaishankar *et al.*, 2014). Increase in population, industrialization, advanced technology, urbanization, agriculture and ineffective pollution control and mitigation measures have elevated the levels of heavy metals in the environment (Anyanwu *et al.*, 2018). Heavy metals have been reported in almost all kinds of foods, water, air, and soil. The toxicity of heavy metals like Pb, Cd, Hg and As have been reported as a serious threat to life. (Adakole and Abolude, 2012; Babatunde *et al.*, 2015). The human population and aquatic ecosystems are at risk because of heavy metal pollution (Rai, 2008). Aquatic organisms take up nutrients and heavy metals from the environment they live in and the food they consumed. These metals are absorbed into their muscles and gill surfaces, and bioaccumulated over a period of time. In the aquatic environment heavy metals are assimilated by fish via ingestion, ion exchange across gills or membrane surface and adsorption by tissues of the fish (Ahmed *et al.*, 2014). Fish also have diverse bioaccumulation characteristics and accumulation can occur in the head, liver, bones, kidney, stomach, heart, muscle, operculum, vertebrae and flesh of fish species (Murtala *et al.*, 2012; Zhao *et al.*, 2012). It happens in different parts of different fish species depending on the degree of solubility of the metal in water, bioavailability, nature of the habitat, life cycles, feeding pattern, ecology and physiological nature of the fish (Perugini *et al.*, 2014; Anandkumar *et al.*,

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2017). Food safety on the other hand is an important issue of concern worldwide. The increasing demands for food and food safety have drawn attention to the risks linked with consumption of foodstuffs contaminated with heavy metals, other pollutants and toxins (DMello, 2003). Fish constitute a portion of daily meal for human population worldwide. Food of aquatic origin are the major source of toxic trace metal via ingestion especially in the Niger Delta region except for those who are exposed to heavy metals via their job. Heavy metals contaminated foods have become an unavoidable challenge (Bhat & Gómez-López, 2014). Long-term low-level body buildup of heavy metals causes damaging effects which becomes obvious after several exposures.

Metals such as Cu, V, Mg and Zn are essential nutrients required in minute quantities for proper physiological and biological functions of the human body, but when they are higher than the amount needed by the body, they cause adverse effects. Inadequate amounts of essential metals like Zn, Cu, Fe, V and Mn can cause adverse effects and increase the vulnerability of humans to other stressors including those connected with other metals and the environment (USEPA, 2006b). However, metals such as Pb, Hg and Cd have been established as potential toxic metals which could affect the proper functioning of the human body. Furthermore, the non-essential heavy-metals in food can directly and indirectly affect the essential metals in the body and can cause retard immunological defenses, impaired psycho-social behaviors, malnutrition, reduced intrauterine growth and cancerous growth (Arora *et al.*, 2008). These metals displace the original metals from their natural binding sites thereby lowering energy levels, damaging the brain, liver, kidney and lungs and causing cell malfunction (Alkesh, 2017). Children and aged people do not regulate metal ingestion and distribution effectively and may be at risk of amassing toxic levels (USEPA, 2006b). Metal toxicity is dependent on the absorbed dose, exposure route and exposure duration. Prolonged exposure to heavy metals causes progressive physical, muscular and degenerative processes that initiate diseases such as multiple sclerosis, Parkinson's, Alzheimer's and muscular dystrophy (Alkesh, 2017). According to Ferner, (2001) heavy metal toxicity is a clinically significant condition when it occurs. Although many scientific studies suggest that benefits of fish consumption outweigh the risks (Park and Mozarrafian 2010; FAO/WHO 2011), there are studies raising concerns both about contaminants in fish and their human health risks (Virtanen *et al.*, 2007). The objectives of this study is to investigate the concentration of lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Cobalt (Co), Zinc (Zn), Vanadium (V) and Mercury (Hg) in blood cockle (*Senilia senilis*) and Oyster (*Crassostrea gasar*) from Bonny Estuary and assess the human health risk associated with consumption of these shell fishes.

MATERIALS AND METHODS

Study Area: The Bonny estuary empties into the Atlantic Ocean and is the largest of the Niger Delta network of estuaries, rivers and creeks. It is environmentally stressed due to various human activities such as intense oil and gas exploitation activities (Babatunde *et al., 2015;* Jamabo and Chinda, 2010). It lies on the eastern flank of the Niger Delta and has 127 km long tidal estuary which falls between latitudes 4.25° and 4.50° N and longitudes 7.00° and 7.15°E. (Fig 1) it is a broad belt of mangrove swamps, which harbors diverse fish with an influx of seawater for the major part of the year as the characteristic features of the estuary. Its lithology is typical of the Niger Delta, (Babatunde *et al*., 2015). It has a brackish water type and is a central point for commercial activity, fishing, and jetty for local transportation but have the reputation of being the most polluted. (Onojake *et al*., 2015).

Sample Collection: Samples of *Senilia senilis* and *Crassostrea gasar* were obtained monthly from artisanal fishermen using casting nets in the Estuary at Bonny area from February 2018 to July 2018. On capture, the fish samples were labelled, stored in a box filled with ice and transported to the laboratory and stored at 4^{o}C in the refrigerator for subsequent analysis for each month.

Preparation of shell fish specimens: A preliminary shell clean-up of the specimens was done with distilled water. The tissues of the shellfish were separated from the shell using a stainless-steel knife and rinsed with distilled water severally to make sure dirt were removed. Samples were dried in the oven at 60-65^{0}C for 48 hours. The dried samples were ground to obtain a homogenous powder and sieved with a 600µm-mesh. Each specimen was labelled and stored in a bag and kept in a desiccator until digestion.

Sample Preparation: Digestion of Samples: Three (3) grams of each of the samples collected were measured in a beaker using analytical weighing balance with 0.0001g calibration. Each of the samples were digested 10 ml of HCl was poured into 250 ml beakers containing the samples and 30 ml of HNO_3 was added to the sample mixture using a measuring cylinder. The mixture was placed on a hot plate to boil until the fumes disappears with clear solution. The digested solutions were allowed to cool at room temperature, and filtered with 11mm filter paper; transferred to a conical flask, the filtrate was made up to 100 ml with distilled water, labelled in a plastic vial for metal analysis. The metal concentrations were determined by Atomic Emission Spectrometer Model A 200 AA. Deionized water was used for all dilution.

Heavy Metal Analysis: The metal concentration were determined using Atomic emission Spectrometer (Spectra AA-100). Standard solution of the metals Zn, Pb, Cd, Hg, Cr, Cu, Ni and V were prepared from their 1000 ppm stock solutions. The equipment was calibrated to ensure precise and accurate analysis. Three replicates of each experiment were carried out to obtain a standard deviation.

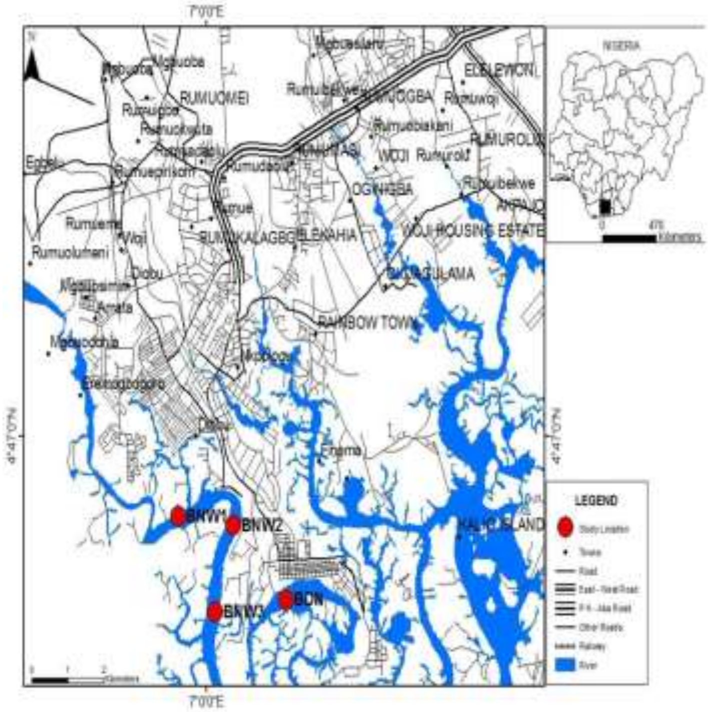

Fig 1 Map of the sampling locations in the Bonny Estuary

Quality Assurance and Quality Control: American Public Health Association (2012) process of sampling and analyzing samples were carried out. Analytical grade of chemicals were used and every set of samples analyzed in triplicates. Standards and blanks were run and no interference was noted. All equipment were calibrated to accurate analysis. Proper care was taken to avoid contamination and cross contamination.

Estimation of Health Risk Assessment: A human health risk assessment is the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future.

Daily Intake Rate of Metals (DIR): The daily consumption rate were calculated according to USEPA (2009) equation.

$$DIR = \frac{Cmetal\ X\ Cfood}{Baverage\ weight}$$

Where C_{metal} is Heavy metal concentration in shellfish (mg/kg), D_{food} intake is daily intake of shellfish (kg/person) and $B_{average}$ is average body weight. The average adult body weights were considered to be 65kg, while average daily of intakes of *Crassostrea gasar* and *Senilia senilis* for adults is considered to be 0.058 kg/person/day (Vanguard, 2014)

The Target Hazard Quotient equation by USEPA, 2011.

$$THQ = \frac{EFxEDxFIRxC}{RfDo\ x\ BWxATn(EFxED)} X\ 10^{-3}$$

Where; EF = The exposure frequency 365 days/year; ED = The exposure duration, average life span of a Nigerian (55 years) (World bank, 2019); FIR = Nigerian shellfish ingestion rate (kg/person/day) 0.058kg/person/day. (Vanguard, 2014); Cm = Metal concentration in shell fish mg/kg d-w); Bw = Nigeria's average body weight (bw) (65g); TA = Average exposure for non-carcinogens (It is equal to EF×ED);

Rfd = Oral reference dose (mg/kg bw/day) values used are in Table 1.

Table 1 Reference dose for metals in food

Metals	**Reference dose (mg/kg/day)**
Pb	0.0035
Cd	0.001
Zn	0.3
Ni	0.02
Cr	0.0015
Hg	0.001
V	0.001
Cu	0.04

Source: USEPA, 2011, 2012

Hazard Index (HI): Hazard index was used to calculate the summation of risk of individual heavy metals using (Guerra *et al*., 2010) equation. The hazard index is the summation of the hazard quotients for all the individual heavy metals which is calculated as

$$HI = \sum HQs = \sum (HQ\ Cr + HQ\ Cd + HQ\ Pb + HQ\ Zn + HQ\ Ni + HQ\ Cu + HQ\ V + HQ\ Hg)$$

***Statistical Analysis*:** Microsoft Excel Software package was used. The results were presented as Mean ± Standard Deviation (SEM). Differences between means was assessed using Analysis of Variance (ANOVA) and used to determine significant difference ($P<0.05$) between groups.

RESULTS AND DISCUSSION

Heavy metal concentration: The concentration trends of heavy metals in *Crasstrostrea gasar* for six months is shown in Table 2. Concentrations of heavy metals followed a decreasing trend of Zn > Cu >Ni >Pb> Cd>Cr>V=Hg. Zn had the highest Concentrations with the peak in in February (7.616 ±0.018mg/kg) and the least in May (0.870 ±0.030mg/kg) followed by Cu which had the highest in April with a value of 1.156 ±0.527mg/kg and the least in July with a value of 0.335 ±0.008mg/kg. Pb and Ni had the highest levels in March with values of 0.243 ±0.002mg/kg and 0.191 ±0.001mg/kg respectively while their least levels were found in May with values of 0.017 ±0.008 mg/kg for Pb and 0.008 ±0.000 mg/kg in April for Ni. Cd and Cr had the highest levels in June with values of 0.040 ±0.025 mg/kg and 0.021±0.002 mg/kg and the least levels in July with a mean concentration of 0.005 ±0.000 mg/kg for Cd and 0.009 ±0.001 mg/kg for Cr. Hg and V were below detectable limit. In *Senilia senilis*, the heavy metal concentration The trend for heavy metal concentrations in *Senilia senilis* for six months is shown in Table 3 and it followed a decreasing trend of Zn >Cu >Ni>Pb>Cd>Cr>V = Hg. Zn had the highest level in March with a value of 1.602 ±0.020mg/kg and the least in July with a value of 0.841 ±0.064mg/kg. While Cu had the highest level in April with a value of 0.450 ±0.397mg/kg and the least in March with a value of 0.002 ±0.000mg/kg. Pb, Ni and Cd had the highest levels in February with values of 0.136 ±0.002mg/kg, 0.164 ±0.097mg/kg and 0.022 ±0.000mg/kg respectively while their least levels were found in May with value of 0.034 ±0.008mg/kg for Pb and 0.008±0.000 for Ni in April, May and June and 0.013 ±0.006mg/kg for Cd in July. Cr was highest in June with a value of 0.030 ±0.005mg/kg and the least in February and March with a value of 0.001±0.000mg/kg. V and Hg were beyond detectable limit.

Table 2. Mean concentrations (mg/kg) of metals in *Crassostrea gasar between* February to July, 2018

	February	**March**	**April**	**May**	**June**	**July**
Zn	7.616±0.018^{e}	6.462±0.073^{d}	1.898±0.908^{c}	0.870±0.030^{a}	1.146±0.275^{b}	0.872±0.019^{a}
Pb	0.217±0.026^{d}	0.243±0.002^{d}	0.044±0.027^{c}	0.017±0.008^{a}	0.047±0.024^{c}	0.038±0.004^{b}
Cu	0.708±0.084^{c}	0.752±0.005^{c}	1.156±0.527^{e}	0.457±0.032^{b}	0.926±0.280^{d}	0.335±0.008^{a}
Cd	0.015±0.001^{a}	0.018±0.001^{a}	0.010±0.004^{a}	0.015±0.002^{a}	0.040±0.025^{b}	0.005±0.000^{a}
Ni	0.112±0.002^{c}	0.191±0.001^{c}	0.008±0.000^{a}	0.018±0.006^{b}	0.145±0.123^{c}	0.030±0.001^{b}
Cr	ND	ND	0.017±0.003^{b}	0.014±0.003^{b}	0.021±0.002^{b}	0.009±0.001^{a}
V	ND	ND	ND	ND	ND	ND
Hg	ND	ND	ND	ND	ND	ND

**ND= Not detected; same superscript indicate there is no significant difference.*

Table 3. Metal mean concentrations (mg/kg) of *Senilia senilis* from February to July

	February	March	April	May	June	July
Zn	1.556±0.003^{e}	1.602±0.020^{f}	1.309±0.425^{d}	1.080±0.212^{c}	0.943±0.189^{b}	0.841±0.064^{a}
Pb	0.136±0.002^{d}	0.125±0.002^{c}	0.048±0.013^{b}	0.034±0.008^{a}	0.050±0.020^{b}	0.035±0.003^{a}
Cu	0.003±0.001^{a}	0.002±0.000^{a}	0.450±0.397^{d}	0.192±0.125^{b}	0.214±0.171^{c}	0.164±0.097^{b}
Cd	0.022±0.000^{a}	0.018±0.000^{a}	0.020±0.014^{a}	0.017±0.004^{a}	0.019±0.001^{a}	0.013±0.006^{a}
Ni	0.164±0.002^{d}	0.112±0.001^{c}	0.008±0.000^{a}	0.008±0.000^{a}	0.008±0.000^{a}	0.013±0.006^{b}
Cr	ND	ND	0.015±0.001^{a}	0.022±0.007^{b}	0.030±0.005^{c}	0.020±0.006^{b}
V	ND	ND	ND	ND	ND	ND
Hg	ND	ND	ND	ND	ND	ND

****ND= Not detected;** Same superscript indicate there is no significant difference.*

Daily Intake Rate of Metals (DIR): The Daily Intake Rate (DIR) of Zn, Pb, Cu, Cd, Ni and Cr in *Senilia senilis* was 0.00109, 6.37009E-05, 0.00015, 1.61607E-05, 4.65983E-05 and 1.32855E-05 respectively while the daily intake rate of Zn, Pb, Cu, Cd, Ni and Cr in *Crassostrea gasar* 0.00281, 9.0024E-05, 0.00064449, 1.5387E-05, 7.4954E-05 and 9.4684E-06 respectively, Hg and V were not detected as represented in Figure 2.

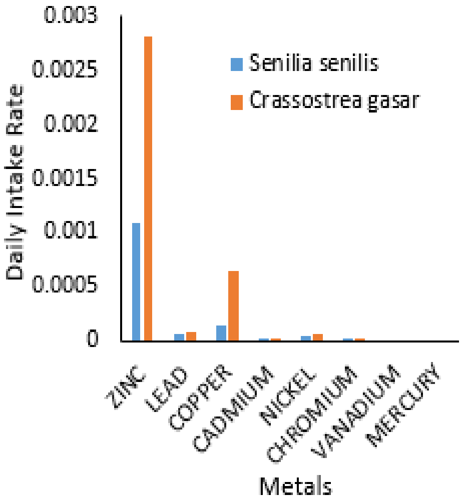

Fig. 2 Daily Intake of metals in *Crasstrostrea gasar* and *Senilis senilia*

THQ: Crasstrostrea gasar showed Pb >Cd>Cu>Zn>Ni >Cr>V and Hg in decreasing order (Figure 3). The THQ values of Zn in *Crassostrea gasar* were estimated at 2.103E-05, 1.785E-05, 5.242E-06, 2.404E-06, 3.166E-06 and 2.408E-06 from February to July respectively. The respective values of Pb were 1.795E-04, 2.013E-04, 3.618E-05, 1.409E-05, 3.894E-05 and 3.149E-05. THQ values of Cu were 1.466E-05, 1.558E-0, 52.395E-05, 9.460E-06, 1.918E-05 and 6.939E-06 respectively during the period. THQ values of Cd for the months of February to July was 1.466E-05, 1.491E-05, 8.672E-06, 1.243E-05, 3.287E-05 and 4.143E-06 respectively. THQ values of Ni for the month of February to July was 4.654E-06, 7.913E-06, 3.314E-07, 7.457E-07, 5.993E-06 and 1.243E-06 respectively. Cr values were 2.762E-07, 2.762E-07, 4.603E-06, 3.959E-06, 5.892E-06 and 2.578E-06 for the month of February to July

Metal levels in Senilia senilis in decreasing order were as follows: Pb>Cd >Cr>Ni>Zn>V and Hg. *Senilia senilis* showed Pb >Cd>Cu>Zn>Ni >Cr>V and Hg in decreasing order. The THQ values of Zn in *Senilia senilis* was estimated at 4.30E-06, 4.43E-06, 3.61E-06, 2.98E-06, 2.60E-06 and 2.32E-06 from February to July respectively, THQ values of Pb was 1.13E-04, 1.04E-04, 4.01E-05, 2.79E-05, 4.14E-05 and 2.87E-05 for February to July. THQ values of Cu was 6.21E-08, 3.45E-08, 9.33E-06, 3.97E-06, 4.44E-06 and 3.40E-06 for the month of February to July. THQ values of Cd for the months of February to July was 1.82E-05, 1.46E-05, 1.63E-05, 1.38E-05, 1.60E-05 and 1.11E-05 respectively. THQ values of Ni for the month of February to July was 6.81E-06, 4.63E-06, 3.31E-07, 3.31E-07, 3.31E-07 and 5.52E-07 respectively. Cr values was 2.76E-07, 2.76E-07, 4.14E-06, 6.08E-06, 8.29E-06 and 5.62E-06 for the month of February to July as seen in Figure 4.

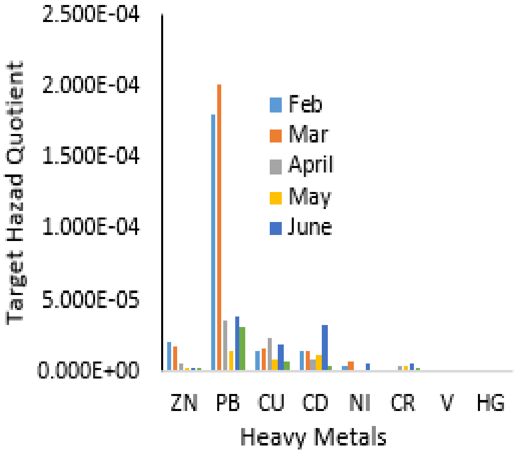

Fig. 3. THQ values of different metals in THQ values in *Crassostrea gasar* from February to July

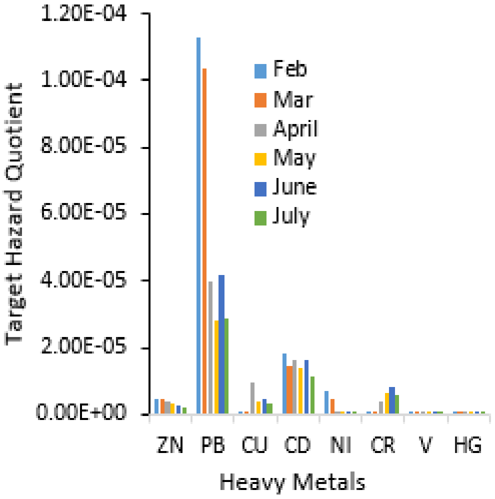

Fig. 4. THQ values of different metals in THQ values in *Senilia senilis* from February to July

Hazard Index: The hazard index values of *Crassostrea gasar* for the months of February-July were 0.000236, 0.000259, 8.06E-05, 4.47E-05, 0.000108 and 5.05E-05 respectively while those for *Senilia senilis* were .000144, 0.000129, 7.54E-05, 5.67E-05, 7.48E-05 and 5.33E-05 respectively as shown in Figure 5.

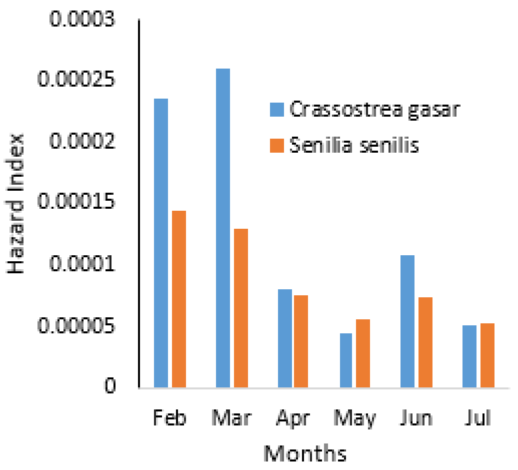

Fig 5 Hazard Index of *Cassostrea gasar* and *Senilis senilia* from Feb-July

Heavy Metal Bioaccumulation: Zn, Pb, Cu, Cd, Ni and Cr were observed among all samples with considerable variations in the concentrations. This may be likely owing to differences in their feeding and bioaccumulation patterns. Bioaccumulation in shell fish is dependent on both their uptake and excretion rates (Sivaperumal *et al.*, 2007). Heavy metals are taken up through various organs and concentrated at different levels in each organism (Abdallah, 2013). The concentration of Zn in *Senilia senilis* fell below the WHO and FAO limits. Zn in this study was lower than its value of 60µ/g in the Bonny Estuary (Claude and Azokwu, 1999) and 2.50- 17.64 mg/kg in Blood cockle from Thailand (Sudsandee *et al.*, 2017). Pb values were also below the permissible limits of WHO and FAO of 0.3 mg/kg and 0.5 mg/kg respectively, although lower concentrations of 0.0012 -0.0573 mg/kg and 0.17 µg/g were reported by Sharma *et al.*, (2014) and Claude and Maureen, (1999) respectively. Hossen *et al*., (2014) reported a high concentrations of 2.65 - 4.36 µg/g of Pb. Cu values were also below the WHO and FAO permissible limits. Sudsandee *et al.*, (2017) reported 0.31- 1.74 mg/kg in their study, while values of 3.4- 5.2 µg/g were reported in *Senilia senilis* from the Bonny Estuary (Claude & Azokwu, 1999). Cd valves were reported to be below WHO and FAO limits in Blood cockle from Thailand and Malaysia: 0.07- 0.74 mg/kg and 1.35 - 2.22 µg/g respectively (Sudsandee *et al.*, 2017 and Hossen *et al.*, 2014). Claude and Maureen, (1999) reported a higher levels of 0.17 – 0.18 µg/g in *Senilia senilis* from the Bonny Estuary which differed from the results of this study. Ni and Cr were lower than the FAO permissible limit while V and Hg were below detectable limits. In *Crassostrea gasar* the concentration of Zn ranged from 1.293 to 11.315mg/kg which were lower than WHO/FAO permissible limits. The vital role Zn plays in the biosynthesis of nucleic acids, nucleic polymerase makes it an important factor in the tissue healing (WHO, 2003) Pb ranged from 0.025 to 0.361 mg/kg which is below the FAO permissible limit but above the WHO permissible limit in February and March. Pb toxicity, which include tiredness, sleeplessness, irritability, headaches, joint pains and gastrointestinal symptoms, may appear in adults at blood Pb level of 50-80 µg/ml. (Jaishankar *et al.*, 2014) Cu ranged from 0.498 to 1.717 mg/kg and were lower than WHO and FAO permissible limit. Cd ranged from 0.007 to 0.059 mg/kg and were lower than WHO regulatory limit but above the FAO limit in June. Bradl, (2005) reported that Cd could cause general pain, bone loss, skeletal deformities and kidney damage. Ni ranged from 0.012- 0.284 mg/kg and were below WHO and FAO permissible limit. The toxicity of Ni is observed only when it's above recommended level. Cr ranged from 0.001 to 0.032mg/kg and were below WHO and FAO permissible limit. Cr does not undergo biomagnification, it does undergo bioconcentration, low concentrations of hexavalent Cr causes sub lethal toxic effects in aquatic plants and animals. (Wright and Welbourn, 2002). V and Hg were beyond detectable limit in all the samples for six months were below the WHO/FAO permissible limit of and 40 mg/kg/30mg/kg respectively.

Daily Intake Rate: DIR is the amount of metal taken by an adult per day. DIR values from this study were many folds lower than their respective reference doses indicating no potential health hazard to the public. Estimating heavy metal exposure level is indispensable in determining organism health risk (Singh *et al.*, 2010). Various routes of exposure to humans do exist, yet the most significant is the food chain. Zn is an important metal in human body as it plays a vital role in normal growth and development. Higher intake of Zn can result in suppression of Cu and Fe absorption, gastrointestinal irritation, and interference of physiological processes (Sahu and Kacholi, 2016). Cr can cause a variety of diseases through bioaccumulation in human body. This ranges from dermal, renal and neurological to the development of several cancers including lungs, larynx, bladder, kidneys, testicles, bone, and thyroid (Fang *et al.*, 2014). Chronic Cd-induced renal damage mainly involves the damage of glomerulus, especially the proximal tubules. Accumulation of Cd in the epithelial cells of the proximal tubules results in impaired reabsorption function, leading to polyuria, glucosuria, and low molecular weight proteinuria. Cd-induced nephrotoxicity is mediated via oxidative stress, apoptosis, and necrosis (Balali-Mood *et al.*, 2021). Cu is a vital element in the human body as it is responsible for upholding central nervous system health, proper working of the metabolic processes, pigmentation, and prevention of anaemia (Alam *et al*., 2003). Continuing low Cu levels have damaging effects to humans due to the nature of the role Cu plays (Demirezen and Aksoy, 2006) People suffering from Wilson's disease are at great risk for health effects when overexposed to Cu (Jarup, 2003). When Cu exceeds safe limits in the human body, it possesses health hazards like hypertension, sporadic fever, coma, anaemia, and liver and kidney damage as well as stomach and intestine irritation (Bermudez *et al*., 2011).

Target Hazard Quotient: Naughton and Petróczi (2008) noted that THQ marker is a complex parameter used in evaluating potential health risk and also connects the concentrations of metals in food with their toxicity, quantity and quality of food consumption and average weight of consumers. THQ values for the shell fish were far below one (1) which indicate no apparent non-carcinogenic risk for each individual metal ingested and of less concern for health challenges. However, a population will experience health risk when the dose is above or equal to the Reference Dose. (US EPA, 2000). The frequency of heavy metals exposure and body level of assimilation determines the degree of toxicity (Orisakwe *et al*., 2012). However, ingested dose is not equal to absorbed pollutant dose because some are excreted with only a smaller proportion left to accumulate in tissues (Ihedioha *et al*., 2014). Therefore, it is possible that the ingested and absorbed amount among consumers may even be lower. The THQ for Zn in this study was far below 0.089 reported for fish in Taiwan and 0.48–0.60 reported for rice in south China. (Zhuang *et al*., 2009). Sudsandee *et al*., (2017) reported low THQ in blood Cockle from the upper Gulf of Thailand; low enough not to pose a risk. This is similar to values reported by Muangdech (2002) and Auiyawong (2008) in which THQ calculated for various heavy metals were less than one (1). Giandomenico *et al*., (2016) suggested that when THQ values are below one it should not be ignored because it could have cumulative effect when other routes of exposure are involved. Onuoha *et al*., (2016) observed THQ values for Pb which were above one (1) in snails consumed in Rivers State Nigeria.

Hazard Index (HI): Hazard Index is the summation of all Target Hazard Quotient values for metals analyzed. The THQ and HI values proposed by EPA are integrated risk indexes, and are widely used for risk assessment of various contaminants in foods (Storelli, 2008; Mok *et al*., 2014). Both THQ and HI are useful parameters for calculating the risk of the ingestion of food contaminated with heavy metals (Abdallah, 2013; Mok *et al*., 2014). A Hazard Index above one (1) indicates that the contaminant is toxic and represents a hazard to human health.

HI assumes that the degree of the adverse effect will be proportional to the sum of multiple metal exposures. It also assumes similar working mechanisms that could linearly affect the target organ. (Amirah *et al*., 2013).

In this study, the HI values calculated were far below one (1) indicating that all heavy metals were not likely to cause risk to the local population. The HI values for all the shellfish harvested from the Bonny estuary are therefore safe for consumption.

Conclusion: **T**he complex DIR, THQ and HI parameters used in the assessment of heavy metal risk in the present study provided a reliable picture than using only a simple parameter like metal concentrations in the specimens. The low Target Hazard Quotient for all metals evaluated indicates no risk of non-carcinogenic effects of consuming these two shellfish species harvested from the Bonny Estuary. It could therefore be concluded that shell fish harvested from the Bonny Estuary do not pose any potential non-carcinogenic human health risk.

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