Assessment of Heavy Metals Concentrations in Selected Crustaceans and Their Potential Health Risk at Qua Iboe Estuary, Ibeno

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Abstract:
This study was conducted to assess the accumulation of some metals (Pb, Cu, Zn, Cd, and Mn) in the tissues of Procambarus clarkii and Callinectes amnicola, in Qua Iboe Estuary, Ibeno. Four locations were selected, three within Qua Iboe Estuary—Mkpenak, Ukpenekang, and Iwo-Okpom, while the fourth, Ifiayong, served as control. Water and Sediment samples were also collected from the same locations. Standard procedures were employed in sample preparation and elemental analysis was carried out using Atomic Absorption Spectrophotometer (AAS). The results obtained showed that the various concentrations of the metals in water was in the order Mn>Cu>Zn>Cd>Pb at all study locations, and that concentrations of Cd (0.058±0.01 mg/l) and Mn (1.086±0.81 mg/l) were found to be higher than WHO permissible range, while others (Pb, Cu, and Zn) were within the range. In sediments, all concentrations of metals were higher than their values in the overlying water and were within the allowable range except for Mn (80.805±6.80 mg/kg). Also, concentrations of Cu and Zn in crustacean tissues were lower than the international permissible levels. However, Pb, Cd and Mn exceeded the allowable limits given by FAO/WHO. From calculations, the estimated weekly intake (EWI) for all metals were considerably below the provisional tolerable weekly intake based on the FAO/WHO standards. The Hazard index (HI) for the studied metals in P. clarkii and C. amnicola were 0.474 and 0.370 respectively, showing the absence of potential significant health risk through the ingestion of the crustaceans. The cancer risk factors for Pb (3.2×10^{-7} and 2.7×10^{-7} in crabs and crayfish respectively) were below the acceptable lifetime carcinogenic risk (10^{-5}). Relative to the permissible limits for metals in seafood, there was an absence of sufficient accumulation of any of the investigated metals in the crustacean samples to indicate a potential significant health hazard from their consumption. The results of this study revealed a safe level of Pb, Cu, Cd, Zn, and Mn contents in the crustaceans consumed by the population.

Keywords: Heavy metals, Bioaccumulation, Qua Iboe Estuary, Procambarus clarkii, Callinectes amnicola

1. Introduction
Coastal environments are usually densely populated and urbanized with various industries operating within its proximity. These industries produce various waste streams with diversified obnoxious constituents, which are usually not managed or treated to recommended limits before disposal. Amongst these components, heavy metals, which are natural constituents of the environment, and generally occur in low concentrations, have attracted global concerns because anthropogenic activities have inadvertently raised the levels of metals in many of the natural water systems, leaving them in polluted conditions.

Mine drainage, offshore oil and gas exploration, industrial effluents (pesticides, paints, leather, textile, fertilizers and pharmaceuticals), agricultural runoff, and acid rain have all contributed to the increased metal burden in these waters being ultimately incorporated into aquatic sediments (Güner, 2010). The uncontrolled discharge of these pollutants into the freshwaters cause many dramatic problems to most of the aquatic fauna. Heavy metals constitute a major problem because they are toxic and tend to accumulate in the body organs (Vilizzi and Takan, 2016). These metals are neither degraded nor metabolised, which leads to their ultimate persistence in the environment.

The ability of heavy metals to accumulate in marine animals is of great scientific interest as far as the knowledge of heavy metal is concerned (Kumar and Hema, 2007). Aquatic animals may absorb dissolved metals from surrounding...
waters and sediments which may accumulate in various tissues in significant amounts and elicit toxicological effects at critical targets. Macro invertebrates are frequently suggested as bioindicators for monitoring changing water conditions in areas of potential contamination (Morse, Bae, and Munkhjargal et al., 2007). Therefore, the determination of harmful and toxic substances in water, sediments and biota gives direct information on the significance of pollution in the aquatic environment.

Qua Iboe Estuary is an outlet of the parent river, Qua Iboe River, which traverses through the rain forests in Abia and Akwa Ibom states. Therefore, pollution by heavy metals is an indicator to the environmental pollution by numerous local economic activities in this terrain. This study aims to determine the bioaccumulation of heavy metals in crabs and crustaceans. This terrain. This study aims to determine the bioaccumulation of heavy metals in crabs and crayfish from the Estuary. The human health risk assessment of the heavy metals by dietary intake through crabs and crayfish is also investigated.

2. Materials and Methods

2.1. The Study Area

The Qua Iboe Estuary is a major and commercially important hydrographic feature in the Niger Delta (Benson and Essien, 2009). The estuary lies within latitude 4°30’–4°45’N and longitude 7°30’–8°45’E on the southeastern coastline of Nigeria and it is in close proximity to the Exxon-Mobil oil terminal and effluent treatment/discharge site. Socio-economic activities thrive along the bank of the estuary, and the population depend mainly on the estuary for most of their domestic, economic and recreational activities. There is a constant exchange of organic and inorganic substances between the estuary and the ocean through backwash infiltration.

In this study, four locations were selected, three within the Estuary– Mkpanak, Ukpenekang, and Iwo-Okpom, while the fourth location, Òrayong, served as control.

![Figure 1: Map of Study Area Showing Sampling Locations](image)

2.2. Sample Collection and Preparation

Water samples from the four study locations were collected in triplicate form into pre-cleaned plastic bottles, and treated with 2ml HNO₃. Before sampling, the bottles were rinsed with water from sampling location, and immersed to about 30-50cm below the water surface to prevent contamination of heavy metals from air. They were stored in aniced box and transported to the laboratory. In the laboratory, triplicate water samples were thoroughly mixed and aliquots of 50ml digested with a mixture of nitric and perchloric acid in a ratio of 4:1. Samples were heated on a hot plate in a fume cupboard at120-150°C for 40 minutes until clear solutions were obtained. Digests were filtered, diluted to 50ml with distilled water and stored in labelled plastic bottles for analysis.

Sediment samples were collected from the same locationsas water. At each point, three sediment samples were taken using Eckman bottom sampler, and stored in pre-cleaned plastic containers and packed separately in polyethylene bags. These samples were air-dried in the laboratory for 72 hours, disaggregated and sieved through a 2mm mesh to remove large debris, gravels and other unwanted materials. Samples were digested with 10ml of concentrated HNO₃ and HClO₄ in the ratio of 4:1 as described by Vowotor et al. (2014).

Samples of P. clarkii and C. amnicola were collected from the study locations using containers and diagonal nets. The collected crustaceans were stored in ice and later transported alive to the laboratory. In the laboratory, the crustaceans were washed with distilled water, oven-dried at 105°C for 24 hours and then transferred to a desiccators until it reached a constant weight. The dried samples were ground to fine particles using a clean mortar and pestle. The mortar and pestle were properly cleaned after grounding each specimen to avoid contamination (Prendez and Carrasco, 2003). 0.5g each of powdered samples was weighed into a clean dried beaker and digested with 5ml of a mixture of HNO₃ and HClO₄ in the ratio of 4:1. The mixture was heated up to 140°C on a hot plate in a fume cupboard to obtain a clear solution. The solution was filtered, diluted to 50ml and stored in bottles for analysis. Metals concentrations were
measured using Atomic Absorption Spectrophotometer (Model A-Varian Spectra 100, Australia), according to IAEA, (1980). The accuracy and precision of the analytical procedure were determined. Metals measured in water, sediment and crayfish tissues were Lead (Pb), Copper (Cu), Manganese (Mn), Zinc (Zn), and Cadmium (Cd).

2.3. Estimated Daily Intake

The Estimated Daily Intake (EDI) was calculated by the following equation:

\[ EDI = E_D \times E_F \times F_{IR} \times C_F \times C_m \times 10^{-3} \]

Where \( E_F \) and \( E_D \) are the exposure frequency (365 days/year) and the exposure duration (60 years) (Saha et al., 2016), respectively; \( F_{IR} \) is the ingestion rate of seafood (25 g/day); \( C_F \) is the conversion factor. A \( C_F \) of 0.208 is to convert fresh weight crustaceans to dry weight (Saha et al., 2016); \( C_m \) is the metal concentration in the crustaceans tissues (mg/kg); \( W_{AB} \) is the average body weight for adults (65 kg); and \( T_A \) is the average exposure time for non-carcinogens (equal to \( E_F \times E_D \)) (Saha et al., 2016).

2.4. Daily Consumption Limit

The daily consumption rate limit (\( CR_{lim} \)) of crab and crayfish, based on the carcinogenic effect of the contaminants, was calculated by the following equation:

\[ CR_{lim} = \frac{(ARL \times W_{AB})}{CSF \times C_m} \]

Based on the non-carcinogenic effects of the contaminants, the maximum allowable daily consumption of crab and crayfish was determined using the following equation:

\[ CR_{lim} = \frac{(RfD \times W_{AB})}{C_m} \]

Where \( CR_{lim} \) is the maximum allowable daily consumption of contaminated crustaceans (kg/day), ARL indicates the maximum acceptable individual lifetime risk level (in the present study, 10^-5 was used as set by USEPA (Yu et al., 2014)), \( W_{AB} \) is the mean body weight of consumer population (kg), CSF is the cancer slope factor; RfD refers to the oral reference dose (mg/kg-day), and \( C_m \) is the metal concentration in the edible part of the crustaceans (mg/kg) (Alipour et al., 2015).

2.5. Target Hazard Quotient

Non-carcinogenic risk was investigated using the target hazard quotient (THQ), which is the ratio between the estimated exposure (estimated daily intake (EDI)) and the oral reference dose (RfD). RfD (mg/kg bw/day) represents an estimate of the daily oral exposure of the human population that is likely to be without an appreciable risk of deleterious effects. The following equation was used to calculate the THQ (Saha et al., 2016):

\[ THQ = \frac{EDI}{RfD} \]

A THQ less than 1 reveals that there is no adverse hazard of the exposed population. A THQ equal to 1 indicates that the concerned receptors may experience non-carcinogenic health risk, while the probability should increase as the THQ value increases. In calculating the THQ, the effect of cooking on the concentration of contaminants was not considered and the ingestion dose was assumed to be equal to the absorbed dose of the contaminant (Saha et al., 2016). Based on literature, exposure to two or more pollutants may cause synergistic effects, and hence, the combined target hazard quotient (CTHQ) may be calculated. The CTHQ gives an overview of health risks of the five studied metals (Pb, Cd, Zn, Cu, and Mn) together through crayfish consumption. The CTHQ was calculated according to the following equation:

\[ CTHQ = \Sigma THQ \]

2.6. Cancer Risk

The cancer risk (CR) over a lifetime of exposure to Pb was estimated using the cancer slope factor according to Equation 6 (Peng et al. 2016; Shaheen et al. 2016):

\[ CR = \frac{E_D \times E_F \times F_{IR} \times C_F \times C_m \times CSF \times 10^{-3}}{W_{AB} \times T_A} \]

Where CSF is the cancer slope factor (mg/kg/day), while the other parameters have been defined previously. The US Environmental Protection Agency set an acceptable lifetime carcinogenic risk of 10^-5 (Saha et al., 2016).

3. Results and Discussion

[Reference to DOI: 10.24940/ijird/2020/v9/i1/JAN20042 Page 66]
3.1. Heavy Metal Contents in Water, Sediment, Crayfish and Crab

Heavy metal concentrations in sample matrices in Qua Iboe Estuary and Ifayong are presented in Tables 1 & 2. Results revealed that average concentrations of heavy metals in all the study samples was in the order Mn > Cu > Zn > Cd > Pb. Generally, metals were lower in water column than in the other samples. These low concentrations of metals in water corroborate studies done by several authors whose findings indicated that metal concentration is usually lower in water, but will show a dramatic increase in the sediment and the biota of the specific aquatic system (Varol and Şen, 2012; Vicente-Martorell et al. 2009).

| Sample       | Pb     | Cu     | Zn    | Cd    | Mn    |
|--------------|--------|--------|-------|-------|-------|
| Water        | 0.008 ± 0.00 | 0.694 ± 0.12 | 0.228 ± 0.06 | 0.064 ± 0.01 | 1.427 ± 1.24 |
| Sediment     | 0.453 ± 0.21 | 19.636 ± 5.78 | 4.774 ± 0.74 | 0.965 ± 0.03 | 80.805 ± 6.80 |
| Crayfish     | 0.472 ± 0.13 | 38.019 ± 1.96 | 5.706 ± 0.47 | 1.186 ± 0.06 | 32.264 ± 4.85 |
| Crabs        | 0.324 ± 0.05 | 42.569 ± 1.67 | 8.458 ± 0.67 | 1.078 ± 0.01 | 54.268 ± 10.40 |

Table 1: Concentration of Heavy Metals Expressed as Mg/L in Water and Mg/Kg in Sediment and Crustaceans in Qua Iboe Estuary

| Sample       | Pb    | Cu     | Zn    | Cd    | Mn    |
|--------------|-------|--------|-------|-------|-------|
| Water        | 0.001 ± 0.00 | 0.099 ± 0.01 | 0.182 ± 0.17 | 0.037 ± 0.03 | 0.063 ± 0.04 |
| Sediment     | 0.020 ± 0.01 | 14.380 ± 0.02 | 3.626 ± 0.30 | 0.410 ± 0.20 | 10.944 ± 0.03 |
| Crayfish     | 0.068±0.04 | 17.610 ± 3.51 | 4.697 ± 1.24 | 1.350 ± 0.07 | 10.868 ± 4.94 |
| Crabs        | 0.025 ± 0.01 | 12.800 ± 1.27 | 3.953 ± 0.50 | 0.000 ± 0.00 | 14.558 ± 2.50 |

Table 2: Concentration of Heavy Metals Expressed As Mg/L in Water and Mg/Kg in Sediment and Crustaceans Ifayong

The concentrations of Pb in all study samples remained considerably low. Pb levels in crabs, crayfish and sediment were relatively higher than the level obtained in water. Also, at the control location, all samples showed lower values of Pb comparative to the estuarine samples.

From Table 1, the mean concentration of Pb in water was lower than 0.01mg/l, the recommended concentration of Pb for drinking water as prescribed by the World Health Organization (WHO, 2004). Pb However, in crayfish and crabs, concentrations were above the WHO recommended level of 0.002mg/kg in seafood, which suggested that the investigated crustaceans had accumulated the metal in considerable amounts.

According to Kouba et al. (2010), Pb is neither essential nor relevant to living organisms and has been known for centuries to be a cumulative metabolic poison. Also, Pb is a mutagen and teratogen, and therefore has carcinogenic properties which could impair reproduction, impede liver and thyroid functions, and interferes with resistance to infectious diseases (Güner, 2010).

The mean concentration of Cu in both the estuarine and control water samples were below the WHO recommended 2mg/l, though they maintained a considerable variation between the two locations (Tables 1 and 2). Copper is moderately soluble in water and binds easily to sediments and organic matter, indicating that there is usually a huge amount of the metal in sediments and benthic organisms than in the overlying water column. The levels of Cu in sediments were markedly elevated relative to those obtained in the water (Table 1), which corroborates the assertion that sediments remain a potential repository for every pollutant that gains entry into the aquatic space. From results, average Cu concentrations were higher in crabs than in crayfish and this could be due to the differences in size, assimilation efficiency or excretory capacity.

As observed in this study, concentrations of Cu in P. clarkii and C. amnicola were above the 3mg/kg recommended by the WHO for seafood. This is an indication that accumulation of Cu may have occurred, as there seemed to be higher levels of the metal in the organism than in the water and sediment samples.

Copper is an essential trace nutrient that is required in trace amounts (5-20μg/g) by humans, other mammals, fish and shellfish for carbohydrate metabolism and the functioning of enzymes. However, copper toxicity may result in gastrointestinal defects, hepatic necrosis, damaged cornea, kidney failure and even death (Ferenci et al., 2015).

The concentrations of Zn in all sample matrices were considerably lower than international permissible limits. From Table 1, Zn in water had a mean value of 0.228±0.06mg/l which is below the recommended 3mg/l for drinking water (WHO, 2004). Sediment, which generally accumulates higher proportion of metals relative to water, had a mean of 4.774±0.74mg/kg which was below the limit of 123mg/kg established by the WHO. Concentrations of Zn in tissues of crustaceans were also below the 30mg/kg respectively, indicating that Zn had low concentrations in the studied environment. Zinc is required for normal physiological function in the human body. Genetic expression, cell division, protein synthesis, immune functions, wound healing, and normal growth are some of the basic functions zinc. Nevertheless, signs of zinc toxicity include vomiting, diarrhea, lack of appetite, abdominal pain, headaches, and lethargy. Other adverse effects include kidney failure, prostatitis, pale gums, menstrual problems, ovarian cysts and muscle spasms (Nriagu, 2007).

Cadmium (Cd) is classified as an environmental pollutant with severe toxicity to humans and animals, and is also recognized globally as an occupational hazard. Industrial applications have caused great abundance of the element in the environment with various routes of exposure. The mean concentration of Cd in water was 0.064±0.01mg/l and was higher than the value obtained at the control location (Table 2). The mean value of Cd in this study exceeded the benchmark of...
According to Güner (2010), cadmium contamination in drinking water sources may originate from untreated industrial sewage, impurities in galvanized zinc pipes and agricultural runoff. The mean concentration of Cd in sediment was 0.96±0.03mg/kg, and was lower than 6mg/kg recommended for unpolluted sediment by the WHO. Hence, concentrations of Cd in sediments might not have reached the nuisance level as a consequence of several economic activities around this ecosystem. Crustaceans in Qua Iboe Estuary accumulated Cd in higher proportions than in water and sediment. Mean concentrations of Cd in crabs and crayfish were slightly above unity and had exceeded the 0.05mg/kg recommended limit (WHO, 2004). Cd is a non-essential metal and has the potential to cause nephro-toxicity, immuno-toxicity, osteo-toxicity, teratogenicity, carcinogenicity, and reproductive toxicity (Samuel et al., 2012).

The mean concentration of Mn in water was 1.427±1.24mg/l and far exceeded the recommended 0.4mg/litre hold prescribed by WHO. However, concentration of Mn at the control location (0.063 ± 0.04) was below the permissible threshold. Manganese is one of the most abundant metals in the Earth’s crust, usually occurring principally in the manufacture of iron and steel alloys, as an oxidant for cleaning, bleaching and disinfection as potassium permanganate and as an ingredient in various products (WHO, 2004).

Manganese revealed very high concentrations in sediments with a mean of 80.805±6.80mg/kg and was the only metal with such elevated level in sediment samples. This mean was higher than 30mg/kg stipulated limit for Mn in unpolluted sediment (WHO, 2004). Mean concentrations in crustaceans were 32.264±4.85mg/kg and 54.268±10.40mg/kg for crayfish and crabs respectively. Results revealed that crabs accumulated more Mn than crayfish in its tissues and this may have been due to differences in size or filtration efficiency. Mn concentrations may have a toxic effect and pose some negative environmental and health problems to the aquatic organisms and humans (Tunca et al., 2013).

### 3.2. Consumption Rate Limits

The Estimated Daily Intake (EDI) of the five heavy metals for both crayfish and crabs is presented in tables 3 and 5. The Estimated Weekly Intake (EWI) were calculated and compared with the Provisional Tolerable Weekly Intake (PTWI) established by FAO/WHO. The EDI of the five heavy metals studied was determined based on the assumption of 65kg body weight per person and exposure duration of 60 years. As presented in tables 3 and 5, the maximum daily intake for both crayfish and crabs was in the order Cu > Mn > Zn > Cd > Pb.

In this study, the average EWI values were significantly below the PTWI values for all the studied metals, indicating that the values are within safe range.

The PTWI is a reference dose set by the WHO/FAO Joint Expert Committee on Food Additive (JECFA). It represents a safe weekly intake of pollutants (Ostos et al., 2015).

| Metal | PTWI (mg/kg bw/week) | EDI (mg/kg bw/day) | EWI (mg/kg bw/week) |
|-------|---------------------|--------------------|---------------------|
| Pb    | 0.025               | 3.81 × 10⁻⁵        | 2.67 × 10⁻⁴         |
| Cu    | 3.500               | 3.07 × 10⁻³        | 2.15 × 10⁻²         |
| Zn    | 7.000               | 4.60 × 10⁻⁴        | 3.22 × 10⁻³         |
| Cd    | 0.007               | 9.56 × 10⁻⁵        | 6.69 × 10⁻⁴         |
| Mn    | 0.980               | 2.60 × 10⁻³        | 1.82 × 10⁻²         |

*Table 3: Estimated EDI and EWI through Consumption of P. Clarkii*  
*Source: Iwegbue (2015)*

| Metal | RfD (mg/kg bw/day) | Non–carcinogenic CR<sub>lim</sub> (kg/day) | Carcinogenic CR<sub>lim</sub> (kg/day) | THQ | CSF | CR |
|-------|-------------------|------------------------------------------|--------------------------------------|-----|-----|----|
| Pb    | 0.004             | 0.551                                    | 0.206                                | 0.010 | 8.5× 10⁻³ | 3.2× 10⁻⁷ |
| Cu    | 0.040             | 0.068                                    | –                                    | 0.076 | –   | –  |
| Zn    | 0.300             | 3.418                                    | –                                    | 0.002 | –   | –  |
| Cd    | 0.001             | 0.055                                    | –                                    | 0.096 | –   | –  |
| Mn    | 0.014             | 0.028                                    | –                                    | 0.186 | –   | –  |

*Table 4: Estimated CR<sub>lim</sub>, THQ, CSF and CR of P. clarkii*  
*Source: Alipour et al. (2015)*
In order to characterize the risk of crustacean consumption by the estuarine population, some health risk parameters were investigated and the results are presented in Tables 4 and 6. From calculations, the combined target hazard quotient for the five studied metals in Crabs and Crayfish were 0.474 and 0.370 respectively, i.e., below 1, showing the absence of potential significant health risk through the ingestion of the crustaceans. The CR factor for Pb over a lifetime of exposure through contaminated crabs and crayfish consumption were $1.7 \times 10^{-7}$ and $2.6 \times 10^{-7}$ respectively. The USEPA set a value of $10^{-6}$ (1 for 100,000) as an acceptable lifetime carcinogenic risk (Saha et al., 2016). In comparison with is value, this implies that the CR of Pb appears to be negligible. The results of this study revealed safe levels of Pb, Cu, Zn, Cd, and Mn contents in investigated crustaceans consumed by this population.

3.3. Conclusion
This study was designed to assess the concentrations of heavy metals in samples of selected crustaceans in Qua Iboe Estuary, Ibeno. The associated health risk assessment of these heavy metals was also investigated to ascertain the degree of safety for consumption. *P. clarkii* and *C. amnicola* harvested from this study location are safe for consumption as there was no sufficient accumulation of any of the investigated metals in crabs and crayfish to indicate a potential significant health hazard from their consumption. However, there is need to conduct continuous monitoring for commercial fish markets to ensure that the concentrations of metals remain within the prescribed safe threshold.

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