



Distribution and Human Health Risk Assessment of Heavy Metals in Tissues of *Callinectes sapidus* from Iko River, Akwa Ibom State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author UUU designed the study and performed the statistical analysis. Authors UUU and IOE wrote the protocol, the first draft of the manuscript and managed the analyses of the study. Author IOE managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigates the accumulation of heavy metals in the tissues of *Callinectes sapidus* (blue crab) in the study area and evaluating the human health risk of their consumption. The tissues of concern were the gills, muscles and hepatopancreas in the male and female crab species, while the heavy metals assessed were Cd, Ni and Pb. Heavy metals levels in the tissues of *Callinectes sapidus* ranged as follow: Ni: hepatopancreas (182.97–313.53 mg/kg), gills (129.93–192.14mg/kg), muscles (93.09–165.84 mg/kg); Pb: hepatopancreas (22.86–39.65 mg/kg), gills (13.46–21.35 mg/kg), muscles (2.73 – 6.32 mg/kg); Cd: hepatopancreas (2.04 –9.03 mg/kg), gills (0.12–4.995 mg/kg), muscles (0.105–2.835 mg/kg), respectively. The highest metal concentrations were observed in the hepatopancreas tissue and in the female of the crab species. Furthermore, the metals' levels were generally higher than the permissible level by FAO/WHO in seafood. To assess the heavy metals health risk to the inhabitants of the study area, the daily intake of metal (DIM) and total hazard Quotient (THQ) were estimated and were found to exceed the recommended values. The results reflect the risk of exposure for the period of life expectancy considered, and revealed that the inhabitants of the study area are highly exposed to the health risks associated with these metals.

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1. INTRODUCTION

Heavy metals pollution is a critical environmental issue of concern across the globe. As the human population increases, the intensities of the anthropogenic threats it exerts on the environment increases as a result of industrialization and aqua-cultural activities [1]. Heavy metal pollution of aquatic environment is the cause of concern because of their toxicity; high presence, being non-biodegradable and tendency to bio accumulate in organisms. These metals when ingested by humans, could be circulated in the blood stream and accumulate in the target organ. Subsequently it appears in toxic form in both aquatic organism and human who consume sea food [2]. The increase of these metals concentration in the environment can be transferred to organism through the food chain with the resultant effect of damaging flora and fauna and ultimately causing death [3-5].

Ubong et al. [6], has stated that the increased use of metal-based fertilizer in agricultural revolution of government could result in continued rise in concentration of metal pollution in fresh water reservoir due to water run-off. As human population increases, the intensity of anthropogenic threat exerted on the environment increases as a result of industrialization and agricultural activities [7]. Furthermore, atmospheric inorganic contaminants of natural or anthropogenic sources with heavy metals and/or trace elements at high concentrations could lead to serious ecological consequences and pose human health risks [8]. Heavy metals are potentially hazardous to humans and various ecological receptors because of their toxicity, persistence, bioaccumulative and non-biodegradable nature. Therefore, monitoring and evaluation of heavy metal concentrations in soils, groundwater and atmospheric environment is imperative in order identify hazards to human health, to prevent bioaccumulation in the food chain and further degradation of the ecosystem [8].

Accumulation of heavy metals in an organism depends on various bioorganic and environmental factors such as size, age, feeding habit, temperature and dissolved oxygen [9]. Feeding habit plays significant roles in the accumulation of metals in organism tissues because metals have the tendency to be bio-magnified through food chain. Most aquatic food

chain begins from the invertebrates, which can accumulate high levels of metals, selenium and arsenic. For this reason, it is important to determine the chemical composition of the marine organism such as crab particularly the heavy metal content, in order to evaluate the possible health risk of this aquatic product to human.

Human health risk assessment is considered as the characterization of the potential adverse health effect of human as a result of exposure to environmental hazard [10]. This process employs the statistical identification to measure the hazard and determine possible routes of exposures and finally use the information obtained to determine the potential risk [11]. This study assesses the human health risk posed by the the consumption of *Callinectes sapidus* obtained from Iko River, Eastern Obolo LGA in Akwa Ibom State, Nigeria. The influence of *C. sapidus* sex on heavy metal bioaccumulation and human health risk is also investigated in this study.

2. MATERIALS AND METHODS

2.1 Sampling Site and Study Area

Iko River is in Eastern Obolo L.G.A, Akwa Ibom State bounded in South-West by Andoni, Rivers State and empties into the Atlantic Ocean in the south. Iko River has many adjoining tributaries, part of it drains into Andoni River estuary, which open to the Bright of Bonny. The study station sites 1 and 2 lie along latitude 4°30" N and 4°45" N; longitude 7°35" E and 7°40"E respectively.

2.2 Sample Collection, Preparation and Instrumental Analysis

Blue crab (*Callinectes sapidus*) were caught in the day, stored in an insulated box, transported to the laboratory and stored at 4°C. Prior to the analysis, the crabs were clean with running tap water and thawed at room temperature. The gills, hepatopancreas and the muscles of the crab were dissected using a pair of sterile stainless steel scissors. All the parts were replicated and transferred to Petri dishes. The tissues were dried in an oven with foil plate for three days. The dry-weight of the crab tissues crushed inside mortar with pestle 1g of the sample was weighed and 10ml of HNO₃ and 2 ml of HClO₄ added. It was digested on a hot plate until it dissolved and

evaporated to near dryness. 10 ml of deionized water was added to the completely digested sample, allowed to cool to room temperature. The resulting solution was filtered through whatman filter paper and washed with deionized water into 100ml volumetric flask, which was then made up to the mark by adding deionized water. The metal concentration in the crab tissues were analyzed using Atomic Absorption Spectrophotometer (Perkin-Elmer Model 2380).

2.3 Human Health Risk Assessment

The potential health risk of heavy metal consumption through *Callinectes sapidus* were assessed based on the daily intake of metal (DIM) [10], health risk Index (HRI) and the target hazard quotient (H/Q) [2,11].

These parameters were estimated thus;

$$DIM = \frac{(Metal \times C \text{ factor} \times C_{food \text{ intake}})}{WAB} \quad (2.1)$$

- DIM (Daily Intake of Metal)
- Metal Concentration in mg/kg
- C Factor: Conversion factor for *C. sapidus* is 20.5 [12]
- C food intake: The daily intake of crab was estimated as 60g/day [12]
- WAB (Average body weight): 60kg

$$HRI = \frac{DIM}{RfD} \quad (2.2)$$

- RfD = Oral reference dose (Pb:0.0035mg/kg/day, Cd:0.001mg/kg/day, Ni:0.020mg/kg/day [13])

$$THQ = \frac{EF \times ED \times FIR \times C}{RfD \times WAB \times TA} \times 10^{-3} \quad (2.3)$$

- EF = Exposure frequency (350 days) years
- ED = Exposure duration 54 years, equivalent to the average lifetime of the Nigeria population.
- FIR = Food Ingestion Rate = 60g/person/day
- C = Concentration of the metal
- WAB = Average body weight = 60kg
- TA = Average exposure time for non-carcinogens ED x 365 days/year) (54 x 1 years)

If THQ is greater than 1, the exposure is likely to cause adverse health effect.

3. RESULTS AND DISCUSSION

The present study indicated that there were differences in heavy metals concentration in two

sample areas. This difference in heavy metals concentration depends on the availability of metal and the source of pollution in the different habitats.

3.1 Physico-chemical Parameters

The physico-chemical analyses of water obtained from the study site reveal a high salt concentration; especially in S2 (Table 1). This can be attributed to the closeness of the river to the Atlantic Ocean, whereby S2 has the closest proximity to the ocean. For S2, salinity was 0.08%, TDS (762 mg/l), Conductivity (1529 μ S/cm) while S1: salinity (0.03%), TDS (324 mg/l), Conductivity (649 μ S/cm) as shown on Table 1. The mean metal concentration in water obtained from site 1 were Pb (15.413 mg/l), Cd (11.927 mg/l), Ni (1.642 mg/l) while for S2: Pb (12.107 mg/l), Cd (8.649 mg/l), Ni (1.126 mg/l). Generally, the results of S1 showed that lead was the highest metal concentration, followed by cadmium while the least metal concentration was nickel. Lead is transportation-related arising from fuel combustion and rainfall washes particles out of the air and stops re-entrainment of particles. The origin of the high lead and cadmium levels in the sample area can be linked to the numerous oil spillages and bunkering activities in the region; this should be a major cause for concern, since these metals are very harmful to exposed humans.

3.2 Heavy Metals in Tissues of *Callinectes sapidus*

Heavy metals levels in the tissues of *Callinectes sapidus* ranged as follow: Ni: hepatopancreas (182.97 – 313.53 mg/kg), gills (129.93 – 192.14 mg/kg), muscles (93.09 – 165.84 mg/kg); Pb: hepatopancreas (22.86 – 39.65 mg/kg), gills (13.46 – 21.35 mg/kg), muscles (2.73 – 6.32 mg/kg); Cd: hepatopancreas (2.04 – 9.03 mg/kg), gills (0.12 – 4.995 mg/kg), muscles (0.105 – 2.835 mg/kg) respectively, with site 1 showing higher values than site 2. These results showed that metal concentration in the different organs followed the hierarchical pattern Hepatopancrease > Gills > Muscle. Nickel gave the highest concentration while cadmium was least in concentration. Several studies have indicated that the high levels of metals in the hepatopancreas in comparison to other tissues may be attributed to the synthesis of low-molecular-weight metal-binding methallothionein-like protein, which have been widely recorded in different crustacean species [14-16]. The

metallothionein protein contains a high percentage of amino groups, nitrogen, sulphur that sequester metal in stable complexes. It has been reported [17] that the accumulation of metals in the tissues of crab species depends on the abundance of metallothionein protein in the tissue. Accordingly, this study has demonstrated that the hepatopancreas is the main site of storage and detoxification of metals, as indicated in the highest metal concentration recorded in this tissue. Gills usually reflect the concentration of metals in surrounding water, since they are directly in contact with water and can absorb suspended materials from the aquatic environment. They also serve a variety of physiological functions such as osmo-regulation and gas exchange. Due to these functions, gills have a remarkable influence on the exchange of toxic metals between a crab and its environment. Furthermore, result of this study has shown that the crab muscles tend to accumulate less metal in comparison to other tissue, suggesting a relatively low concentration of metallothionein in the muscles.

This investigation has considered the influence of sexuality of the organism on metal accumulation and distribution among its tissues; revealing that metals accumulation were higher in tissues of female species than the male, which disagrees with a previous study [17] on the new Calabar river in the same region as the present study. Metal concentration in male *C. saipidus* ranged as follows: Ni (93.09 – 231.17 mg/kg), Pb (2.73 – 29.76 mg/kg), Cd (0.05 – 4.10 mg/kg) while the range for female crab species were: Ni (165.63 – 313.53 mg/kg), Pb (4.77 – 37.08 mg/kg), Cd (0.26 – 4.10 mg/kg). According to a study [18], male crab feed more on fish and bivalvia while the female consume mostly shrimp plant and detritus. Since plants are rooted in the sediment, they receive more sediment associated metals. These metals are subsequently accumulated in the female crabs, which feed mainly on the plants. Furthermore, the elevated levels of metals in female crab may be attributed to their relatively larger sizes, higher metabolic activities and better feeding habit.

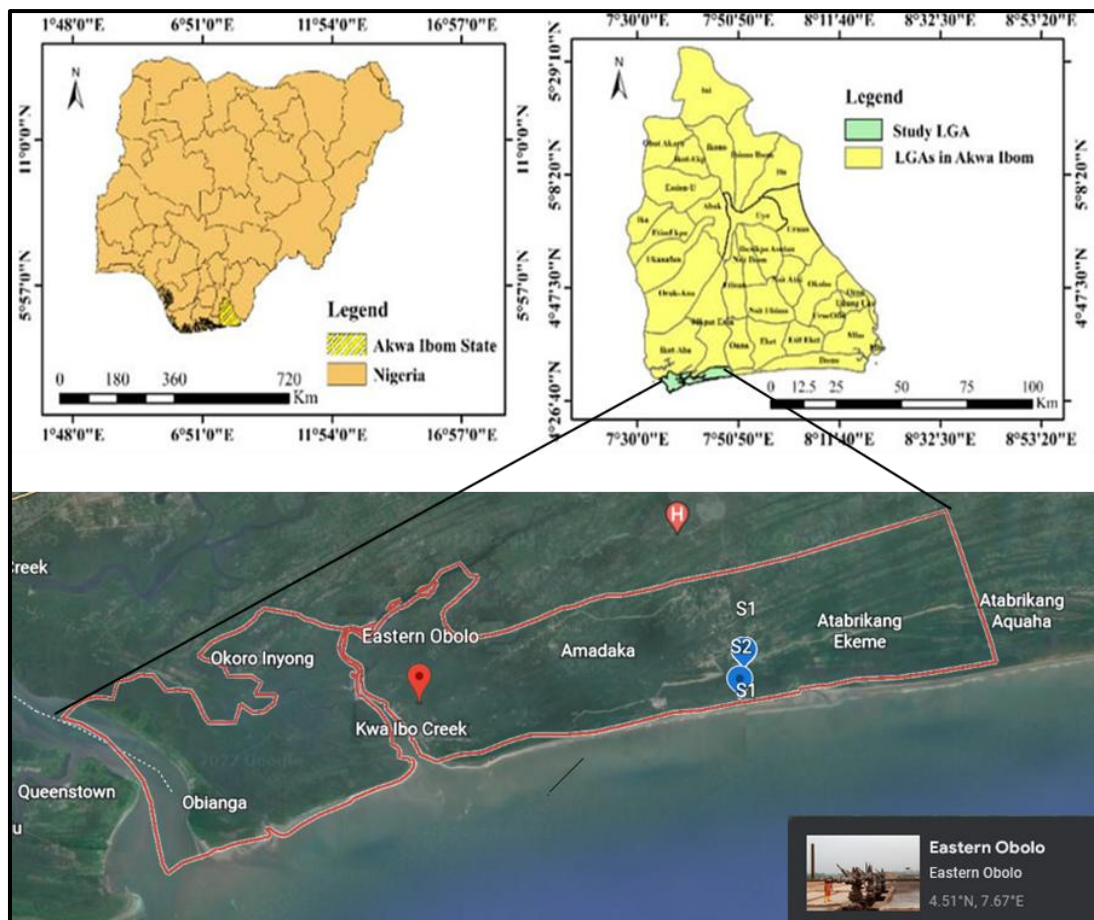


Fig. 1. Map of the study area along Iko river in Eastern Obolo LGA, Akwa Ibom state Nigeria

Table 1. Physico-chemical parameters of water obtained from Iko River

Parameters	S1	S2
Salinity (%)	0.03	0.08
TDS (mg/l)	324	762
Temperature (°C)	27.1	27.1
Conductivity (us/cm)	645	1529
Current I (nA)	1295	1343
pH	6.9	6.5
DO (Dissolved oxygen)	46.7	52.1

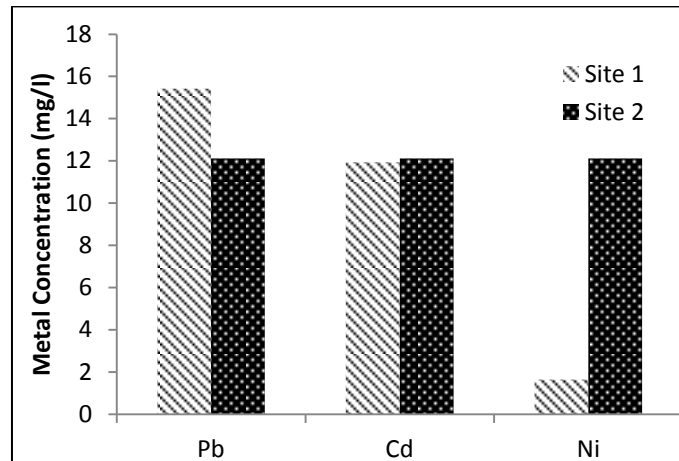


Fig. 2. Mean levels of trace metals in water

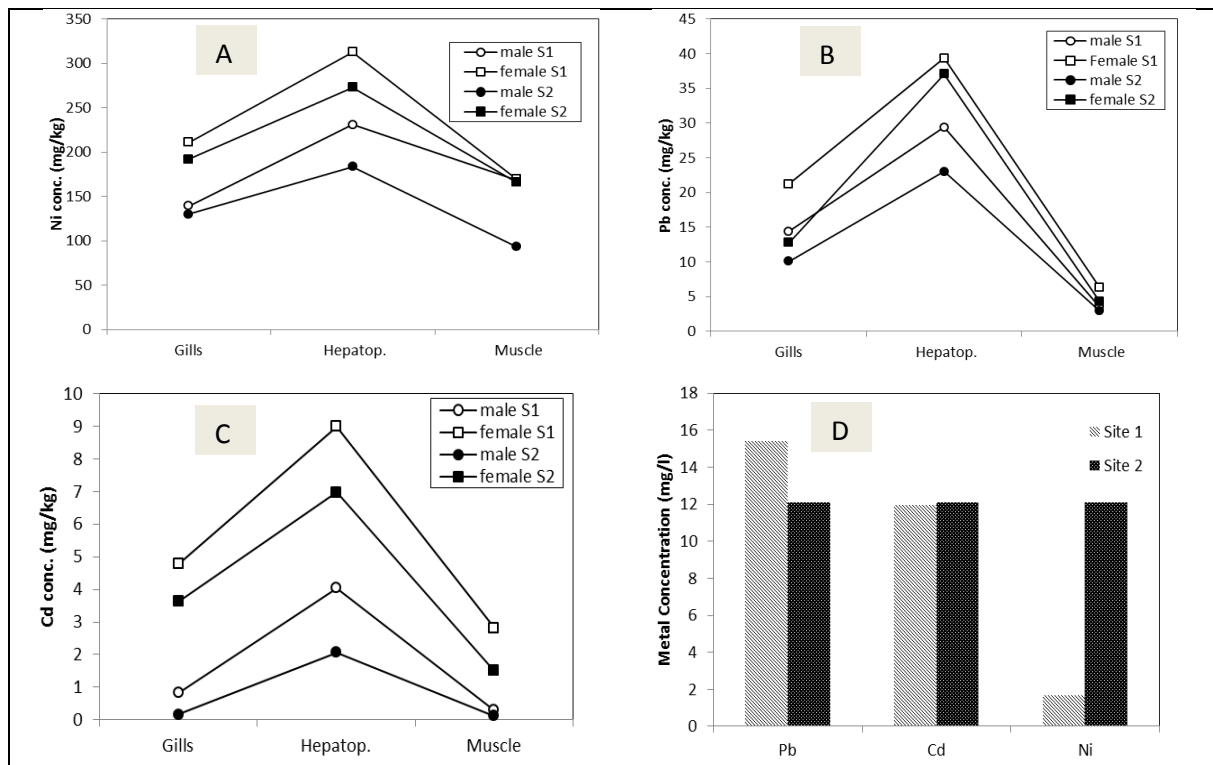


Fig. 3. Metals in tissue of *Callinectes sapidus* and water obtained from Iko River (A) Ni in *C. sapidus* (B) Pb in *C. sapidus* (C) Cd in *C. sapidus* (D) Metals in water of Iko river

Table 2. Daily intake of metal (DIM) and total hazard quotient (THQ) of sample

Heavy metal	<i>C. Sapidus</i> tissue	DIM (mg/kg)	THQ
Ni (S1)	Male Gills	2.85	6.67
	Male Hepatopancreas	4.73	11.11
	Male Muscles	2.14	5.00
	Female gills	4.33	10.19
	Female Hepatopancreas	6.41	15.0
	Female muscles	3.47	8.15
Pb (S1)	Male gills	0.29	3.89
	Male Hepatopancreas	0.61	8.15
	Male Muscles	0.07	0.98
	Female gills	0.44	5.83
	Female Hepatopancreas	0.81	10.74
	Female muscles	0.13	1.67
Cd (S1)	Male gills	0.02	0.74
	Male Hepatopancreas	0.083	3.89
	Male Muscles	0.01	0.37
	Female gills	0.12	4.26
	Female Hepatopancreas	0.184	8.70
	Female muscles	0.15	3.70
Ni (S2)	Male gills	2.67	6.30
	Male Hepatopancreas	3.76	8.89
	Male Muscles	1.91	4.44
	Female gills	3.94	9.26
	Female Hepatopancreas	5.59	14.44
	Female muscles	3.40	8.33
Pb (S2)	Male gills	0.21	2.78
	Male Hepatopancreas	0.47	6.30
	Male Muscles	0.06	0.56
	Female gills	0.26	3.50
	Female Hepatopancreas	0.76	10.15
	Female muscles	0.1	1.34
Cd (S2)	Male gills	0.0034	0.16
	Male Hepatopancreas	0.04	1.99
	Male Muscles	0.0025	0.12
	Female gills	0.007	3.48
	Female Hepatopancreas	0.18	6.69
	Female muscles	0.031	1.44

3.3 Human Health Risk Assessment

To assess the health risk of associated with the consumption of the study crab, the daily intake of metal (DIM) and target hazard quotient (THQ) is presented in Table 2. The DIM were compared with the recommended daily intake of metals and the upper tolerable daily intake level (UL) [19,20]. The THQ is a ratio between the measured concentrations and the oral reference dose, weighted by the length and frequency of exposure, amount ingested and body weight [21]. THQ value greater than 1 denotes health risk to the exposed population for the period of life expectancy considered and exposure duration. Generally, Pb and Cd are not important nutrients for human and are considered to be of much higher risk to human [22], compared to nickel.

Table 2 indicates that THQ values for all metals considered were far greater than 1 in *C. sapidus* tissues, except Cd in the muscle of the male crab species. Thus, *C. sapidus* consumption in the study area poses serious health risk concern and the inhabitants are highly exposed to health risk associated with these metals in the order of Ni > Pb > Cd. For special populations like those with a weak constitution (eg. pregnant women and children), the potential health risk of heavy metal consumption through consumption will likely to be higher than for the normal population.

4. CONCLUSION

This research has presented data on the concentration of heavy metal in water and *C. sapidus* at the two sites (S1 and S2) from the Iko

River, indicating that S1 was more polluted. This site is close to the Utapete operational zone, an oil exploration facility, which received sewage discharge. Thus, the high bioaccumulation of metals in the crab tissues is believed to be due to the rigorous anthropogenic input of bioaccumulative contaminants into the aquatic environment. It was evident from the study that heavy metal accumulations in *C. sapidus* were higher than almost all the international standard level. Research findings showed that the water body is contaminated with heavy metals such as Pb, Cd, and Ni and hence can contaminate seafood and humans as a whole. The heavy metals in water were above the permissible limits of national and international standards and pose a serious environmental and human health risk. Most of the physicochemical parameters were slightly above the permissible limits of regulatory agencies, implying that Iko River is heavily contaminated and potentially hazardous to the exposed populace.

Results indicate a high metal bioaccumulation in the crab species studied, with higher accumulation in female species than the male species. Human health risk assessment showed that THQ in all tissues of *Callinectes sapidus* (except Cd in some male gills and muscle) is far greater than 1. Therefore, benthic crab obtained from the study sites in Iko River is considered unsafe for consumption.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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