

## Bioaccumulation of Heavy Metal Concentrations on the Tissues of Crab (*Cancer pagurus*) Found in Oferekpe River, Ikwo, Ebonyi State, Nigeria

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**Abstract:** Crabs are important and cheap source of animal protein to human beings and a large number of people depend in crabs for their livelihood. Increasing human influence through heavy metal pollution have however led to the depletion of the aquatic resources and substantial reduction in the nutritive value. Six fresh (live) samples of crab used for analysis were randomly collected directly, by hand picking. The soil sediment samples were sun dried for one week and the dried samples were ground into fine powders using laboratory mortar and pestle. Chemical analysis of the crab tissues and the soil samples were performed. Heavy metals concentration in the samples were determined using Atomic Absorption Spectrophotometer (SOLAAR 32 AA) and bioconcentration factors (BCF) were obtained. The data were subjected to analysis of variance (one-way ANOVA) at the 5% level using SPSS 10.01 version. Heavy metals in the body of the crabs were measured. Comparing concentration of heavy metals per crab collected, it was observed that in crab A the concentration of the metals were significantly different ( $p < 0.05$ ). Lead (Pb) had the highest concentration and Zinc (Zn) had the lowest concentration. The order of the concentrations were  $Pb > Cu > Fe > Zn$ . When concentration of each heavy metal was compared between the crabs (A, B, C, D, E and F), Pb concentration was significantly different ( $P < 0.05$ ) in some crabs. The highest concentration was found in Crab F. The bioconcentration factor (BCF) of Pb was found to be greater than all other metals. The concentration of the heavy metals; Lead (Pb), Zinc (Zn), Copper (Cu) and Iron (Fe), in the soil and crab samples in this study showed that there were variations in the concentration of these heavy metals in the crab samples and soil samples. The results obtained from these samples were below the international standards for heavy metals in food like the World Health Organization, European Commission and Food and Agricultural Organization permissible limits for consumption,

**Key words:** Bioaccumulation • Crabs • Soil

### INTRODUCTION

The aquatic environment with its water quality is considered the main factor controlling the state of health and disease of habitats in that environment. Pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing serious threat to the survival of aquatic organisms including crabs. The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals [1]. The most anthropogenic sources of metals are industrial, petroleum contamination and sewage disposal [2]. Metal ions can be incorporated into food

chains and concentrated in aquatic organisms to a level that affects their physiological state. Of the effective pollutants are the heavy metals which have drastic environmental impact on all organisms. Trace metals such as Zn, Cu and Fe play a biochemical role in the life processes of all aquatic plants and animals; therefore, they are essential in the aquatic environment in trace amounts [2].

Crabs are important and cheap source of animal protein to human beings and a large number of people depend in crabs for their livelihood. Increasing human influence through heavy metal pollution have however led to the depletion of the aquatic resources and substantial reduction in the nutritive value [3]. The danger of these heavy metals is their persistent nature as they remain in

the biota for long period of time when they are released into the environment [3]. As a result of these heavy metals pollution several aquatic species have become threatened [3]. Realizing this, concern for assessment of heavy metals in the tissues of aquatic species in most water bodies have increasingly been gaining ground throughout the world. Studies have also indicated that aquatic species are able to accumulate and retain heavy metals from their environment and that accumulation of metals in their tissues is dependent upon exposure concentration and duration as well as other factors such as salinity, temperature, hardness and metabolism of the animals [4].

Considering Crabs as important food item and source of protein to man, since there is no formal control of effluent discharge from industries and homes into the river, it is important to monitor the levels of metals in the reservoir in comparison. The concentration of Arsenic and Chromium were measured in the gills, muscle, hepatopancrease and whole body of crabs in order to assess the food consumption safety. It could also establish a baseline for future studies of heavy metal pollution. Pollutants enter through numerous factories established in this area as well as farmers using pesticides and fertilizers in the farm. The factory effluents (treated and untreated), pesticides and fertilizers as well as waste from the nearby markets and workshops may finally get discharged into the water bodies.

Ikwo is a tourist hotspot and an academic environment, where washing, bathing and fishing takes place. Diverse amount of wastes from these activities and numerous quarry companies engaged in mining and crushing stones are also constantly discharged to these fresh water ecosystem and there is the possibility of buildup of heavy metals in aquatic organisms especially crabs. This study is thus to assess the level of Fe, Zn, Cu, Mn, Pb and Cr in the tissues of crab of the fresh water ecosystem at Ikwo; with a view to understand whether the concentration level of these metals constitute health hazards to consumers.

## MATERIALS AND METHODS

**Sample Collection:** Six fresh (live) samples of crab used for analysis were randomly collected directly, by hand picking, from their holes at the bank of Ikwo River, a shallow, narrow, but all seasons river, located at Ebonyi state, Nigeria. Sediment samples were also randomly collected for analysis from the river bank at the same spots as the crab samples. The crabs were kept in a

bucket containing the river water and transported to the laboratory until dissection took place.

**Sample Preparation:** The soil sediment samples were sun dried for one week and the dried samples were ground into fine powders using laboratory mortar and pestle. The ground samples were stored in polythene bags and clearly labeled. Representative sample was finally made for the sediment samples and labeled.

The crabs were removed from the river water; washed with tap water; rinsed thoroughly with distilled water and were dissected using surgical knife. Tissues from the chest region and the appendages were separately extracted. The tissue from the chest was labeled A1, while those from the appendages were labeled A2. The tissues were placed in clean watch glasses and were oven dried at 105°C for 1 hour and later cooled in the desiccators.

**Chemical Analysis of Crab Samples:** 2.0g of each dried crab tissue was weighed into empty clean beaker with cover. The sample was digested in 5.0ml of distilled water and 3.0ml of concentrated sulphuric acid on a hot plate until the digest neared 5.0ml when the beaker was removed from the hot plate and allowed to cool slightly.

Another 3.0ml of concentrated nitric acid was then added and the sample was gently heated on a hot plate for additional 15 minutes. The beaker was removed, allowed to cool and another 2.0ml of distilled water and 3.0ml of 30% hydrogen peroxide ( $H_2O_2$ ) was added. The beaker was heated until the effervescence from the  $H_2O_2$  ceased. Another 3.0ml of  $H_2O_2$  was added and the beaker was heated until only about 2.5ml remained. The beaker was removed from the hot plate and the digest was diluted with distilled water and filtered through Whatman No1 filter paper. The residue was washed with distilled water thoroughly and the combined wash and filtrate was made up to mark in a 100ml volumetric flask with distilled water. The solution was stored in clean labeled plastic containers until analysis.

**Chemical Analysis of Sediment Sample:** 2.0g of the dried sample of soil sediments was weighed into a clean beaker and a mixture of concentrated nitric and hydrochloric acids (3:1) were added. The contents were digested on a hot plate for 1.5 hours. The digest was cooled and filtered through Whatman No1 filter paper. The residue was thoroughly washed with distilled water and the combined filtrate and wash was made up to mark in a 100ml volumetric flask with distilled water. The solution was stored in clean labeled plastic container until analysis.

Heavy metals concentration in the samples were determined using Atomic Absorption Spectrophotometer (SOLAAR 32 AA).

The bioconcentration factors (BCF) of the heavy metals in the crab samples were obtained using.

$$BCF = \frac{C_{org}}{C_{sed}} \quad \text{Equation 1}$$

where BCF = bioconcentration factor;

C<sub>org</sub> = concentration of metal in the organism;

C<sub>sed</sub> = Concentration of the same metal in the ambient environment, soil sediment in this case.

**Data Analysis:** The coefficient of variation was calculated to determine whether or not the BCF obtained for the various heavy metals in the two tissues of the tropical crab were significantly different from one another. Statistical analysis of data was carried out with SPSS statistical program. The data were subjected to analysis of variance (one-way ANOVA) at the 5% level using SPSS 10.01 version and Duncan's multiple range tests were performed to separate difference among means.

## RESULTS

Table 1 shows the heavy metals concentration of various crab samples analyzed. In the analysis Pb, Zn, Fe and Cu were determined in the different crab samples. Table 2 shows the concentration of metals in the soil samples taken from the holes in which various crabs were collected, Pb, Zn, Fe and Cu were also determined in the soil samples. Table 3 calculated the Bio-Concentration Factor (BCF) for each crab sample.

**Heavy Metals in Body of Crabs:** Comparing concentration of heavy metals per crab collected (Table 1), it was observed that in crab A the concentration of the metals were significantly different ( $p < 0.05$ ). Lead (Pb) had the highest concentration and Zinc (Zn) had the lowest concentration. The order of the concentrations were  $Pb > Cu > Fe > Zn$ . In crab B, Pb differed from all of them significantly and had the highest concentration while Cu had the least. There was no significant difference ( $p > 0.05$ ) in the concentration of Zn and Fe. In crab C, there was no significant difference ( $P > 0.05$ ) between the concentrations of Pb and Cu while Fe had the least concentration. In crab D, there was a significant difference ( $P < 0.05$ ) in the concentration of the metals and their order was  $Pb > Cu > Zn > Fe$ . In crab E, the concentration of the metals was significantly different ( $P < 0.05$ ) and the order was

$Pb > Fe > Zn > Cu$ . In crab F, the metals were also significantly different ( $P < 0.05$ ) and the order was  $Pb > Zn > Cu > Fe$ .

When concentration of each heavy metal was compared between the crabs (A, B, C, D, E and F), Pb concentration was significantly different ( $P < 0.05$ ) in some crabs. The highest concentration was found in Crab F followed by Crab E while the least concentration was found in Crab B followed by Crab A and C. There was no significant difference ( $P > 0.05$ ) in Pb concentration in Crab A and Crab C. In Zn concentration analysis, there was significant difference ( $P < 0.05$ ) in the amount of zinc present in the crab samples. For Fe concentration, Crabs A and D, C and F had no significant difference ( $P > 0.05$ ). However, crab E had the highest concentration while Crab A and Crab D had the lowest concentration. For Cu concentration comparison, there was no significant difference ( $P > 0.05$ ) between crabs A, B and E (Table 1).

**Heavy Metals in Soil Samples of River Oferekpe:** As shown in Table 2, in soil A the concentration of the metals were significantly different ( $P < 0.05$ ) from each other in the following order  $Fe > Pb > Cu > Zn$ . Similarly, soil B also differed significantly ( $P < 0.05$ ) in this order  $Fe > Zn > Pb > Cu$ . In soil C, there was no significant difference ( $p > 0.05$ ) in the concentration of Pb and Fe and, Zn and Cu. In soil D, the concentration of the metals were significantly different ( $P < 0.05$ ) from each other in the following order  $Pb > Cu > Fe > Zn$ . Also in soil E, the concentration of the metals were significantly different ( $P < 0.05$ ) from each other in the following order  $Pb > Fe > Cu > Zn$ . In soil F, there was also significant difference ( $P < 0.05$ ) in the concentration of the metals ( $Cu > Pb > Zn > Fe$ ).

As presented in Table 2, Pb concentration was significantly different ( $P < 0.05$ ) in this order soil  $D > F > E > C > A > B$  while for the Zn concentration, the highest concentration was found in soil F, followed by soil D. There was no significant difference ( $P > 0.05$ ) in the concentration of Zn found in soil B and soil C. The Fe concentration in the soil was significantly different ( $P < 0.05$ ) with soil B having the highest concentration followed by soil A. There was no significant difference ( $P > 0.05$ ) in Fe concentration of soil D and soil E. Soil F had the lowest Fe concentration followed by soil C. For the Cu concentration in the soil samples, soil F had the highest concentration followed by soil D. There was no significant difference ( $P > 0.05$ ) in the Cu concentration of soil C and soil E. There was also no significant difference ( $P > 0.05$ ) in the Cu concentration of soil A and soil B and they had the least concentration of Cu.

Table 1: Concentration of heavy metals in various crab samples

Element	Crab A	Crab B	Crab C	Crab D	Crab E	Crab F
Pb	$0.20 \pm 0.005^{a5}$	$0.29 \pm 0.005^{a4}$	$0.20 \pm 0.005^{a5}$	$1.34 \pm 0.005^{a3}$	$1.90 \pm 0.005^{a2}$	$1.95 \pm 0.005^{a1}$
Zn	$1.01 \pm 0.0005^{d6}$	$0.24 \pm 0.005^{b4}$	$0.12 \pm 0.005^{b5}$	$0.35 \pm 0.005^{c2}$	$0.26 \pm 0.005^{c3}$	$0.81 \pm 0.005^{b1}$
Fe	$0.04 \pm 0.005^{c4}$	$0.22 \pm 0.005^{b2}$	$0.09 \pm 0.000^{c3}$	$0.05 \pm 0.005^{d4}$	$0.49 \pm 0.005^{b1}$	$0.11 \pm 0.005^{d3}$
Cu	$0.10 \pm 0.00^{b4}$	$0.02 \pm 0.005^{c4}$	$0.20 \pm 0.005^{a3}$	$0.56 \pm 0.005^{b2}$	$0.09 \pm 0.005^{d4}$	$1.67 \pm 0.005^{c1}$

Results are expressed as Mean+S.E.

\*Mean values in a column with different alphabets as superscript are significantly different ( $p < 0.05$ )-comparing heavy metals per crab

\*Mean values in a row with different figures as superscript are significantly different ( $P < 0.05$ )-comparing crabs per heavy metal

Table 2: concentration of metals in the various soil samples

Element	Soil A	Soil B	Soil C	Soil D	Soil E	Soil F
Pb	$0.40 \pm 0.005^{b5}$	$0.29 \pm 0.01^{c6}$	$1 \pm 0.005^{a4}$	$1.95 \pm 0.005^{a1}$	$1.78 \pm 0.005^{a3}$	$1.87 \pm 0.005^{b2}$
Zn	$0.08 \pm 0.01^{d5}$	$0.35 \pm 0.005^{b3}$	$0.32 \pm 0.02^{b3}$	$0.45 \pm 0.000^{d2}$	$0.17 \pm 0.01^{d4}$	$1.02 \pm 0.01^{c1}$
Fe	$1.06 \pm 0.005^{a2}$	$1.19 \pm 0.005^{a1}$	$0.97 \pm 0.01^{a4}$	$1.01 \pm 0.000^{c3}$	$1.02 \pm 0.005^{b3}$	$0.78 \pm 0.007^{d5}$
Cu	$0.21 \pm 0.005^{c4}$	$0.20 \pm 0.005^{d4}$	$0.25 \pm 0.01^{b3}$	$1.52 \pm 0.005^{b2}$	$0.36 \pm 0.01^{c3}$	$2.96 \pm 0.005^{a1}$

Results are expressed as Mean+S.E.

\*Mean values in a column with different alphabets as superscript are significantly different ( $p < 0.05$ )-comparing heavy metals per crab

\*Mean values in a row with different figures as superscript are significantly different ( $P < 0.05$ )-comparing crabs per heavy metal

Table 3: Bio-concentration factor of heavy metals in the crab from soil sample

Element	BCF A	BCF B	BCF C	BCF D	BCF E	BCF F
Pb	$0.49 \pm 0.01$	$1.00 \pm 0.00$	$0.2 \pm 0.005$	$0.69 \pm 0.00$	$1.07 \pm 0.00$	$1.04 \pm 0.00$
Zn	$0.12 \pm 0.01$	$0.69 \pm 0.03$	$0.46 \pm 0.04$	$0.78 \pm 0.02$	$1.50 \pm 0.06$	$0.79 \pm 0.01$
Fe	$0.04 \pm 0.005$	$0.18 \pm 0.00$	$0.09 \pm 0.00$	$0.05 \pm 0.005$	$0.48 \pm 0.00$	$0.14 \pm 0.005$
Cu	$0.49 \pm 0.01$	$0.49 \pm 0.02$	$0.58 \pm 0.02$	$0.38 \pm 0.02$	$0.24 \pm 0.005$	$1.23 \pm 0.005$

\*Values greater than 1.00 are bio-accumulated in the body of the animal

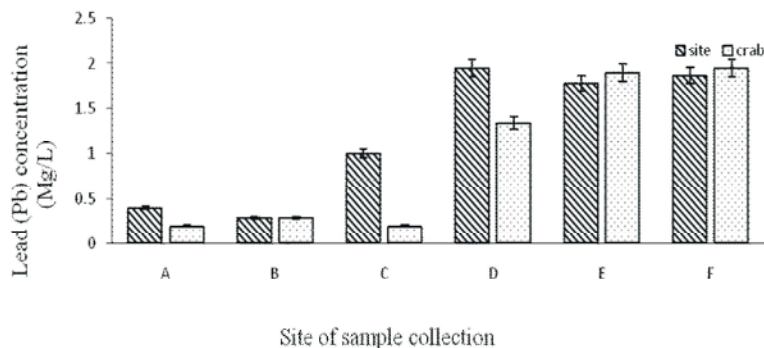


Fig. 1: Lead concentration in the crab and soil samples from the six different sites

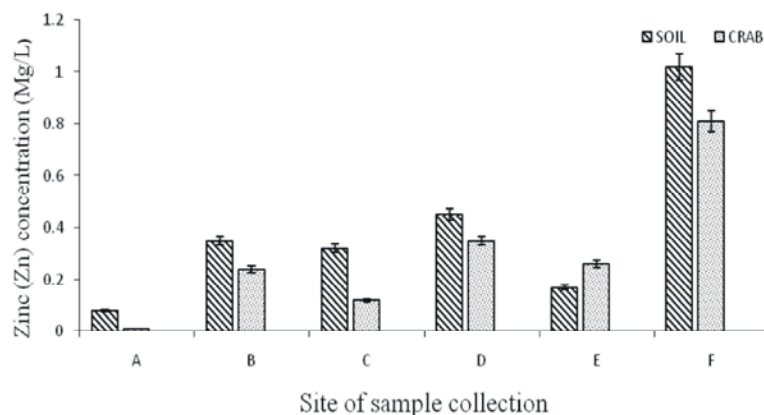


Fig. 2: Zinc concentration in soil and crab samples from the six different sites

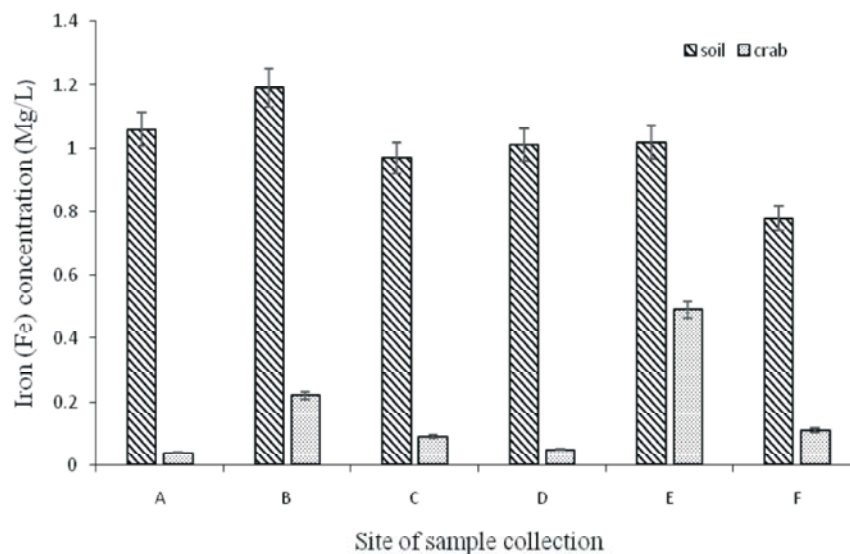


Fig. 3: Iron concentration in the soil and crab samples from the six different sites

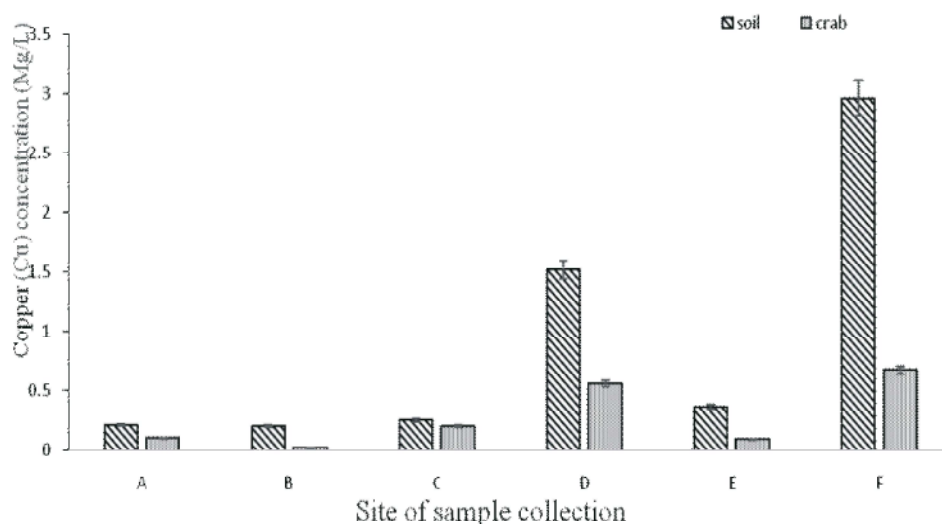


Fig. 4: Copper (Cu) concentration in the soil and crab samples

**Bio-Concentration Factor (BCF) of Heavy Metals in the Crabs from Soil Sample:** The bioconcentration factor (BCF) of Pb was found to be greater than all other metals. The higher BC factor of heavy metal indicates the stronger accumulation of the respective metals by the crabs. The trend of transfer of these metals is found to be in the order of  $Pb > Zn > Cu > Fe$ . The greater the transfer factor value than 0.50, the greater the chances of crab for metal contamination by anthropogenic activities will be and so there is need for environmental monitoring of area is required.

Fig. 1 shows that site A, C and D had more lead (Pb) concentration in the soil than in the crab while in site B,

the concentration of Pb was the same in both. In site E and F, the Pb concentration in the crab was greater than that in the soil.

Comparison of the Zn concentration in soil and crab samples is shown in Fig. 2. It was observed that the soil samples had more Zn concentration than the crabs in site A, B, C, D and F, hence Zn was not bio-accumulated in the crabs. In site E, the crab had more Zn content than the soil, these could be as a result of its feeding habit in the environment.

In all the six sites, the Fe concentration in the soil was more than the concentration in crabs (Fig. 3). This shows that Fe is not bio-accumulated in the crabs.

In Fig. 4 below, it was observed that the soils all had higher copper (Cu) content than the crabs. This shows that Cu is not bio-accumulated in the crab samples.

## DISCUSSION

Generally in this study, the concentration of heavy metals in the crab sample was in the following order:  $Pb > Zn > Cu > Fe$  while the concentration of the heavy metals in the soil samples was in the following order  $Pb > Fe > Cu > Zn$ .

Lead (Pb) is the most abundant heavy metal in the samples and its mean concentration varied from 0.4 -1.87 Mg/L in the soil and 0.2-1.95 Mg/L in the crab. The result obtained in this study was in agreement with that of Wyse *et al.* [5], their result ranged from 1.15-1.85 Mg/L in fishes from Turkey. The obtained result for lead are higher than the standard permissible levels of 0.4Mg/L [6] and 0.5Mg/L [7]. The mean value obtained for the lead (Pb) in the crabs was also above the prescribed standard safe limits of 0.5 Mg/l by WHO[8]. The mean bio-concentration factor value obtained for lead in the body of the studied crabs was 0.49-1.07. Crabs with lead bio-concentration factor greater than 1.00 indicate that these crabs could pose health related hazards to consumers. Lead toxicity can cause palsy and encephalopathy, characterized by muscular fatigue, anoxia and gradual seizure in children [9]. Lead is also known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults [10].

In this study, zinc concentration ranged from 0.01-0.81 Mg/L in crab and 0.08-1.02 Mg/L in the soil samples. According to Bartik and Priscal[11] normal concentration of Zinc in meat samples were 0.35-0.45Mg/L. Five samples out of the six investigated samples were within their normal range of values, i.e., they contained normal level of zinc. However, results obtained from this study for Zn are similar to those recorded by Salisbury and Chain [12]. These authors stated that Zn concentration in meat and special organs such as kidney and liver ranged from 0.23-1.47Mg/L. Zinc was also bio-accumulated in some of the crabs. Though the mean bioconcentration factor was less than 1.00, but regular consumption of these samples, could pose Zinc health related conditions in consumers.

The Fe concentration in the crab and soil sample ranged from 0.04-0.22 Mg/L in the crab and 0.78-1.19 Mg/L in the soil sample in this study. The soil was found to

contain higher Fe concentration than the crab and the mean bio-concentration factor of Fe was 0.17 which showed that the Fe was not bio-accumulated in the tissues of the crab. However, the results are in concordant with that obtained by Canli and Athi[13]. Iron in all the studied crab samples fell within the recommended tolerable level of 0.45 Mg/L [14].

The Cu concentration of the samples in this study ranged between 0.02-0.67 Mg/L in the crabs and 0.2-2.96 Mg/L in the soil. The bio-concentration factor from the samples showed that Cu was not bio-accumulated in the crabs. Although Cu is essential for good health but very high intake can cause health problem such as liver and kidney damage [15]. The maximum copper concentration for meat and meat products has been proposed as 0.90-30 Mg/L. The Cu concentrations obtained from this study were lower than those recorded by Canli and Athi [13].

Heavy metal contamination of these crab samples arise from automobile emissions, agricultural practices, mining activities going on in the area. There is need to educate and advocate for good farming and mining practices among the inhabitants of the area. Routine heavy metal assessment of food and soils should be carried out to monitor the quality of food especially edible crabs sold in our markets and the environment in general. Also relevant agencies in Nigeria such as Consumer Protection Rights, National agency for Food and Drug Administration Control (NAFDAC) and Standard Organization of Nigeria (SON) need to ensure and enforce compliance through public awareness the need for the desired levels of essential elements in the aquatic products they consume.

## CONCLUSION

The concentration of the heavy metals; Lead (Pb), Zinc (Zn), Copper (Cu) and Iron (Fe), in the soil and crab samples in this study showed that there were variations in the concentration of these heavy metals in the crab samples and soil samples. The results obtained from these samples were below the international standards for heavy metals in food like the World Health Organization, European Commission and Food and Agricultural Organization permissible limits for consumption, except for lead (Pb) and Zinc (Zn) that were above the permissible limits in some samples. This could be related to the organism mobility, food preference, or to other characteristics of behavior with respect to the environment.

The mean BCF for these metals never exceeded 1.00. The individual BCF value indicates high level of concentration for lead (Pb) and Zinc (Zn) in some of the crab sample even though within safe limits. Continuous consumption of these crabs may not constitute health hazards as a result of the present level of the heavy metals. However, bioaccumulation of these metals in the human body could be possible following prolonged ingestion due to their reported toxicities.

### REFERENCES

1. ECDG, 2002. European Commission DG ENV. E3 Project ENV. E.3/ETU/0058. Heavy metals in waste. Final report.
2. Santos, I.R., E.V. Silva-Filho, C.E. Schaefer, M.R. Albuquerque-Filho and L.S. Campos, 2005. Heavy metals contamination in coastal sediments and soils near the Brazilian Antarctic Station, King George Island. *Mar. Poll. Bull.*, 50: 85-194.
3. Bolormaa, O., J. Baasansuren, W. Kawasaki and T. Hattori, 2006. Analysis of heavy metals in water samples from a mining area in Monogolia. *Journal of Nuclear Instrument and Methods in Physics Research*, 243: 161-166
4. Yoon, C.A., E.R. Daka, C.A. Ekeh and M.A. Moslen 2008. Cadmium and lead levels in some fish species from Azuabie creek in the Bonny Estuary, Nigeria. *African Journal of Biotechnology*, 7: 63-64.
5. Wyse, E.J., S. Azemard and S.J. Mora, 2003. Report on the World-wide Intercomparison Exercise for the Determination of Trace Elements and Methylmercury in Fish Homogenate IAEA-407, IAEA/AL/144 (IAEA/MEL/72), IAEA, Monaco.
6. EC, 2001. European Commission Regulation No 466/2001 of 8 March 2001, official Journal of European Communities L.77/1
7. FAO, 1983. Compilation of legal limits for Hazardous substance in fish and fishery products (Food and Agricultural Organization). FAO Fishery Circular, 464: 5-100.
8. WHO (World Health Organization), 1993. Guidelines for drinking water quality. Recommendations, vol. 1, 2<sup>nd</sup> ed., Geneva.
9. Özmen, H., F. Kùlahçı, A. Çukurovalı and M. Doğru, 2004. Concentrations of heavy metal and radioactivity in surface water and sediment of Hazarlake (Elazığ, Turkey). *Chemosphere*, 55: 401-408.
10. Luckey, T.D. and B.P.P. Venugopal, 1977. Metal toxicity in mammals. Plenum press, New York.
11. Bartik, M. and A. Piscal, 1981. Veterinary Toxicology, Elsevier, Newyork.
12. Salisbury, D. and W. Chain, 1991. Multielement concentrations in liver and kidney tissues from five species of Canadian slaughtered animals. *Journal of the Association of analytical Chemistry*, 74l: 587-591.
13. Canli, M. and G. Athi, 2003. The relationship between heavy metals (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of fish species. *Environmental Pollution*, 121: 129-136.
14. Institute of Medicine, 2003. Dietary reference intakes, applications in dietary planning. Institute of Medicine of the National Academics, National Academics Press, Washington, DC, pp: 248.
15. Agency for Toxic Substances and Disease Registry, 2004. Agency for Toxic Substance and Disease Registry, Division of Toxicology, Clifton Road, NE, Atlanta, GA. Available from <http://www.atsdr.cdc.gov/toxprofiles>