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Rupert C Akpaniteaku
Department of Biological Science, Evangel University Akaeze, P.M.B. 129 Abakaliki, Ebonyi State Nigeria

Precious A Udeozor
Unit of Biochemistry, Department of Chemical Science, Evangel University Akaeze, P.M.B. 129 Abakaliki, Ebonyi State Nigeri*a*

Correspondence
Rupert C Akpaniteaku
Department of Biological Science, Evangel University Akaeze, P.M.B. 129 Abakaliki, Ebonyi State Nigeria

Bioavailability assessment of essential metals in freshwater crab (*Potamon ebonyicum*) at Ebonyi River Basin Nigeria

Rupert C Akpaniteaku and Precious A Udeozor

Abstract
Bioaccumulation of selective essential metals: Magnesium (Mg), iron (Fe), zinc (Zn), selenium (Se) and copper (Cu) in the cephalothorax, cheliped and leg of *Potamon ebonyicum* was analysed using Atomic Absorption Spectrophotometer (AAS). The metals were found at various concentration levels in the body parts of male and female. The concentrations decreased in the following order: Male: Cu< Se< Fe < Zn < Mg and in the female: Cu < Se < Zn < Fe < Mg. The amount of Fe in the cephalothorax of the female was higher than in the other body parts of both sexes. All the metals analysed were found at levels within the NRC recommended dietary allowances. Based on the bioaccumulation in the different body parts, it is concluded that the crab species is relatively wholesome for consumption and cultivation

Keywords: Essential elements, concentration, edible crab, investigation, south east, Nigeria

1. Introduction
Population of fresh water crab at Ebonyi River basin has been depleted by crab hunters (Akpaniteaku 2013) [1]. Habitats of the shellfish have been polluted due to emissions and discharge from stone crushing and mining industries in the area (Akpaniteaku and Emmanuel 2017; Akpaniteaku and Okoye 2018) [2, 3]. Conservation estimate of demand and exploitation in recent time were put at 9.0% and 5.0% respectively. Fishing effort and distribution route increased noticeably due to demand by residents and tourists (Pers. Obs.). Growth in knowledge of nutrition and economic values of the crustacean has also influenced the incremental rates. The bioaccumulation reflects the nutritional quality of crab meat. The difference in accumulation pattern and levels of concentration are associated with contribution from surrounding industries (Arockia Vasanthi *et al*. 2014) [4]. Among the metals known to be essential for human life are Mg, Cu, Fe, Se and Zn (Averil and Eldredge 2011) [5]. Copper (Cu) and iron (Fe) are as essential to life as to play irreplaceable roles in the functioning of critical enzyme system (Lehninger 1975) [6]. However all the metals have three possible levels of dietary intake concentration namely deficient, optimum and toxic. Essential metals such as chromium (Cr), Se and their compounds could be toxic and even cancerous (Averil and Eldredge 2011) [5]. In intact male and female *P. ebonyicum*, Cr was yet to be detected (Akpaniteaku and Okoye 2018) [3]. Studies on bioaccumulation of essential elements in the crustacean would serve as strategic guide to sustainable conservation, and also assist to ascertain nutritional quality and biochemical characteristic of the body parts. The aim of the study is to determine and compare concentrations of selective essential metals (Mg, Fe, Zn, Se and Cu) in the body parts of male and female *P. ebonyicum*.

2. Materials and Methods
Samples of the crab used for investigation were collected in the month of June using the methods of Akpaniteaku (2014) [7]. Male and female *Potamon ebonyicum* numbering 41 were captured at Ishieke community of Ebonyi State. The community is located 12.9 km west from Abakaliki the capital of the state on latitude 6º 15' 18'' N, longitude 8º 05' 55'' E with total area of 5,533 Km^2. The samples were washed with clean borehole water to remove soil particles and transported within 3 hours in plastic container to analytical laboratory. At the laboratory, samples used for analysis were randomly selected (Table 1). They were washed in distilled water, dismembered to cephalothorax, cheliped and leg and stored in refrigerator at approximate temperature of 4 – 5ºC.

Table 1. Morphometric of the sample of *Potamon ebonyicum* used for the essential metal analysis

Measurement	Male	Female
Weight (g)	47.00	47.00
Carapace width (cm)	5.50	5.49
Carapace Length (cm)	3.71	3.72

Wet digestion of sample was done with 20ml of acid mixture (650 ml conc. HN03, 80 ml perchloric acid (HCLO4); 20ml conc. H_2SO_4) in the digestion flask. The flask was heated until a clear digest was obtained. It was allowed to cool and the digest diluted with distilled water to the 50 ml mark. The digest was filtered and stored in bottle for analysis. Series of standard reference metal solutions in the optimum concentration range was prepared by diluting single stock element solutions with water containing 1.5 ml concentrated HNO_3L^{-1}). Calibration bank was prepared by using all the reagents except for the metal stock solution.

Metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer (AAS) according to methods of APHA (1995) [8]. One way analysis of variance was used to determine whether bioaccumulation of essential metals in the body parts reflected male or female at 0.05 level of probability. Correlation coefficient analysis was conducted with the concentration values, to establish relationships between levels of accumulation in the body parts of the male and female.

3. Results and Discussion

Bioaccumulation of essential metals in the body parts of the male and female *P. ebonyicum* is presented in Figs. 1–5. The range of concentration values were significantly ($P< 0.05$) lower than those recorded in the river crabs such as *Paratelphusa lamellifrons.* In the body parts of the crab, concentrations were found in the range of 6.63 – 7729.15 mg kg^{-1} (Islam *et al*. 2016) [9]. Magnesium (Mg) concentration was higher in the cephalothorax of the male than in other body parts of both sexes (Fig 1). There was no significant difference ($P> 0.05$) in the bioaccumulation levels of the element.

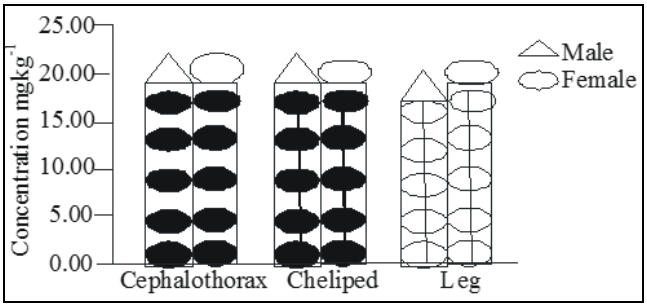

Fig 1: Composition of magnesium in the body parts of *Potamon ebonyicum* (freshwater crab) at Ebonyi River basin Nigeria

The cephalothorax of the female had the highest concentration of Fe, which was also significant compared to other body parts especially those of the male (Fig 2). According to Abdel-Salam (2014) [10], differences in bioaccumulation of metal in the crustacean was due to variation of species, sex and species location. Perhaps the reproductive behaviour and morphological differences in their organs are responsible for variation. Bioaccumulation of Fe in the body parts of the female may seem to indicate that the essential element is more abundant in their meat than those of the males. Akpaniteaku (2014) [7] reported that population of the males in the river basin (Ebonyi) could be higher than that of the females

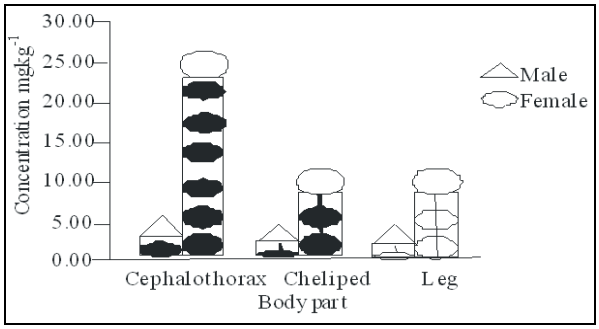

Fig 2: Composition of Iron in the body parts of *Potamon ebonyicum* (Freshwater Crab) at Ebonyi River basin Nigeria

According to Islam et al. (2016) [9], Fe and Cu are respectively found in the range of 423.22 – 487.03 mg kg^{-1} and 6.63 – 64.78 mg kg^{-1} in the body parts of river crab irrespective of sex. The concentration range of Fe in different body parts of the fresh water crab was lower in the present investigation. Result of the regression analysis (Table 2) revealed that the relationship ($r = 0.93$) between concentration levels in the cheliped was more significant ($P < 0.05$) than in other body parts. Functional equilibrium of the appendage may seem to be the reason for relatedness of the composition level in both sexes. This is contrary to coefficient of relationship ($r = 0.70$) for accumulation levels in their cephalothorax, with different reproductive organs (abdominal flap).

Table 2. Regression equation and coefficient of correlation (R) for the concentration relationship between the male and female crab *(Potamon ebonyicum)* at Ebonyi River Basin.

BP	Variable	RE	CC
Cephalothorax	Male and Female	y = 3.75 + 0.90x	0.70
Cheliped	Male and Female	y = 1.98+0.93x	0.93
Leg	Male and Female	y = 1.63+0.96x	0.81

BP = Body part; RE = Regression equation; CC = Correlation coefficient

Consumption of the cheliped may have been justified by the healthy practice of selecting crab legs, which are packed with essential vitamins and minerals (HRF, 2016) [11]. According to seafood World (2009) [12] the legs of king crab (*Paralithodes camtschaticus)* are excellent food source of Mg, P, Se, Zn and Cu. In the present investigation, information on P is lacking, but other essential elements were detected in the male and female. However, result of the regression analysis may seem to highlight similarity in the concentration relationship between male and female. Zinc (Zn) concentration in the body parts of the male was higher than in those of the female (Fig. 3). Dange and Manoj (2015) [13] reported that body concentration of Zn can be regulated against fluctuations in intake by crustaceans. The ways in which it is achieved may vary among species. Short fall in the female *P. ebonyicum* in the present investigation may seem to be species specific.

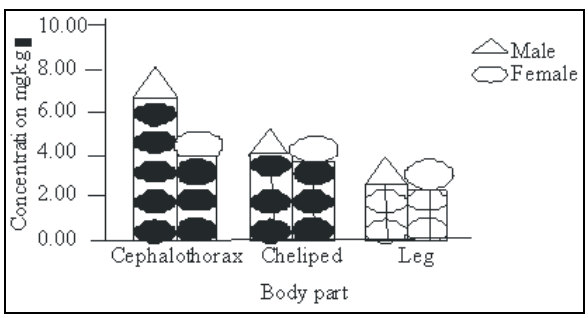

Fig 3: Composition of Zinc in the body parts of *Potamon ebonyicum* (Freshwater Crab) at Ebonyi River basin Nigeria

According to Dange and Manoj (2015) [13] the rate at which Zn is regulated may increase with molting frequency, temperature, salinity, chelators in the medium and bioavailability of uncomplexed free metal ions. With the exception of cephalothorax, more Se was detected in the body parts of the male than in the female (Fig. 4). Islam *et al* (2016) [9] reported that in the river crabs, minerals in the cephalothorax were found to be higher than in the legs and cheliped.

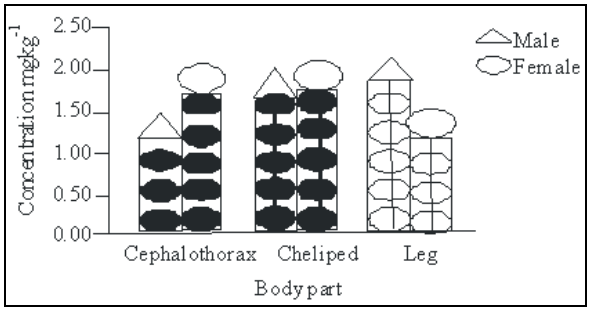

Fig 4: Composition of Selenium in the body parts of *Potamon ebonyicum* (Freshwater crab) at Ebonyi River basin Nigeria

Despite negative correlation in the concentration of Zn and Se between male and female (r = 0.56 and r = 0.58 respectively), the coefficients were higher than in those of other metals. There was more Cu in the male than in the female (Fig. 5). The male accumulated more Cu in the cheliped and leg than the female. Despite relatively high concentration of the metal in the cephalothorax of the male, none was detected in that of the female.

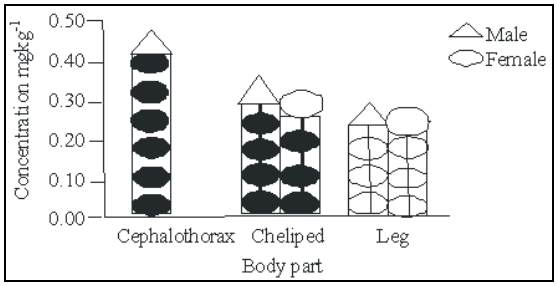

Fig 5: Composition of copper in the body parts of *Potamon ebonyicum* (Freshwater crab) at Ebonyi River basin Nigeria

The concentration gradient (Fig. 6) revealed that the amount of Mg in the male was significantly ($P < 0.05$) higher than other metals. Bio accumulation of Mg, Zn and Fe in the male was higher than other metals, but could be found in the cephalothorax and cheliped. The metals also accumulated more in the cephalothorax and cheliped of the female (Fig 7) than in other body parts. Perhaps those body parts are the rich sources of the elements in the freshwater crab. Arockia Vasanthi *et al*. (2014) [4] reported that concentration of Zn and Fe were highest in the gills and significantly lower in the muscles of crab

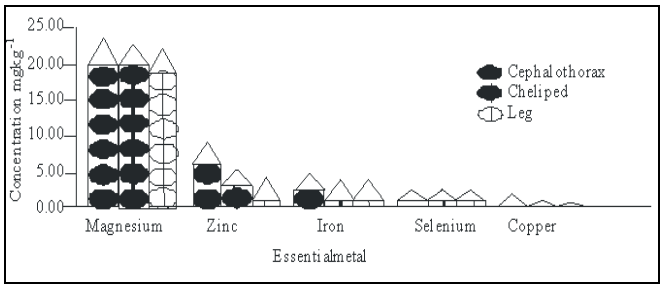

Fig 6 Comparative composition of essential metals in the male crab (*Potamon ebonyicum)* at Ebonyi River basin Nigeria

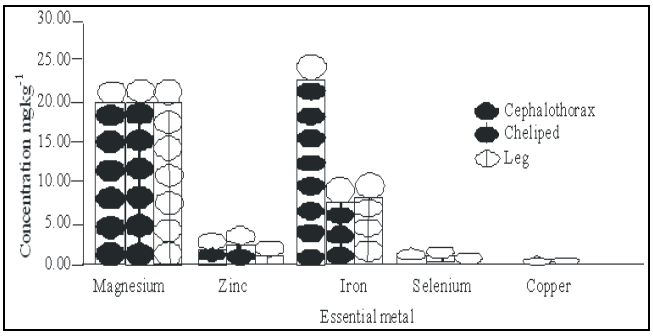

Fig 7: Comparative composition of essential metals in the female crab (*Potamon ebonyicum)* at Ebonyi River basin Nigeria

Despite the undulating levels of concentration, the Mg content was lower than the Fe. The levels of metals found in both sexes did not exceed recommended NRC dietary allowances (Table 3) for the essential metals.

Table 3: Comparison values of essential metal composition in the muscles of *Potamon ebonyicum* with dietary recommended allowances of National Research Council (1989) [13].

Essential metal	Concentration (mg kg^{-1})		NRC Recommended dietary allowance
	Male	Female	
Mg	23.13 (0.26)	23.01 (0.00)	–
Fe	4.86 (1.21)	16.32 (6.46)	15 mg day^{-1}
Zn	5.60 (1.64)	4.14 (0.66)	12 – 15mg day^{-1}
Se	1.93 (0.32)	1.98 (0.31)	55-70µg day^{-1}
Cu	0.38 (0.07)	0.20 (0.14)	1.5-30mg day^{-1}

(Standard deviation in paranthesis)

4. Conclusion

The assessment of essential-metal concentration in the crab species at Ebonyi River basin revealed that *Potamon ebonyicum* contained the selected elements: Mg, Fe, Zn, Se and Cu. The accumulation levels of the elements in the cephalothorax, cheliped and leg were below the dietary allowances recommended by NRC, which indicated that the wild stock was wholesome. The female could be a rich source of Fe due to its cephalic content. Consumption preference might adversely affect population status and reproduction activities. Relevant authority could ensure that appropriate exploitation and cultivation plans for the freshwater crab should be incorporated in rural and aquaculture extension programme.

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