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HEAVY METALS IN SHELL FISHES OF OJO RIVER, LAGOS STATE, NIGERIA

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ABSTRACT
The research examined the accumulations of six heavy metals including chromium (Cr), lead (Pb), iron (Fe), zinc (Zn), cadmium (Cd), and copper (Cu) in water as well as in three male and female species of crab (Cardisoma armatum, Callinectes danae, Callinectes ornatus) sampled from Ojo River in Lagos State of Nigeria. Physico-chemical parameters of the water samples from the River and heavy metal concentrations in the crab species obtained also from the River were examined using standard methods. The results showed that total dissolved solute (TDS), electrical conductivity (EC), salinity, dissolved oxygen, biological oxygen demand and chemical oxygen demand varied significantly along the River course, with TDS and EC being relatively high. Heavy metals detected in the crabs were Fe, Cu and Zn, with Cu being the highest in the species examined. The species C. ornatus presented the highest content of Cu followed by C. armatum and C. danae. The content of heavy metals (mg kg^{-1}) among the crab species showed that Cu (23.47±0.10) > Zn (19.06±0.01) > Fe (16.85±0.01) in all the species except in C. armatum where Fe (1.26±0.44) was > Zn (1.19±0.02). Furthermore, Pb, Cd and Cr were not found in the crabs and this could be associated with the fact that the area consists mainly of residential houses, farms and a few industries. Notably, the content of the heavy metals was larger in the female than the male crabs. This study reported different levels of heavy metal accumulation in male and female species of crabs. Also, the shell fishes from Ojo River might be considered safe for consumption, but the need for continuous monitoring to prevent bioaccumulation is recommended.

Key words: crabs, Nigeria, pollution, Ojo River, shell fishes, water

INTRODUCTION
Investigation of heavy metals in the aquatic environments including aquatic organisms is not a new area of science; it exists around more than a century. Heavy metals are natural constituents of the earth crust which unsystematic anthropogenical activities have significantly distorted their geochemical cycles and biochemical balance (Singh *et al.,* 2011). They comprise elements that are necessary for living organisms including manganese, cobalt, zinc, copper, molybdenum and others (Duffus, 2002), but at high concentration, are harmful (Popek *et al.,* 2008).

Heavy metals can be classified as potentially toxic elements such as cadmium, lead and chromium and essential elements such as copper, zinc and iron. Toxic metals naturally occur in aquatic environments in very low concentration, but their concentration level has increased due to persistent discharge of anthropogenic, agricultural and industrial pollutants over time (Anim *et al.,* 2011). Industrial activities as well as agriculture and mining create a potential source of metals pollution in the aquatic environment (Hosseini *et al.,* 2014).

Over the past century, heavy metals have been discharged into rivers and estuarines as a result of rapid industrialization (Adeniyi *et al.*, 2011).Urban and industrial activities in coastal areas introduce significant amount of trace metals into the marine environment, causing permanent disturbances in marine ecosystems, leading to environmental and ecological degradation and constitute a potential risk to flora and fauna species, including humans and through food chains (Boran and Altınok, 2010). Crabs are considered as good bio-indicators in environmental monitoring studies (Wei *et al.,* 2017). The heavy metals in penetrate the mucous membrane of the bronchia, move in the body through the circulatory system and accumulate in the liver and kidney, and the effects include reductions of growth and reproductive capacity (Chandra-Sekhar *et al*., 2004; Fleischer *et al.,* 2011).

Researches on heavy metals in tropical crab (*Carcinus* sp.) from River Aponwe, Ado Ekiti, Nigeria (Falusi and Olaonipekun, 2007) and Pulicat Lake, North Chennai, Southeast coast of India (Thomas and Mohaideen, 2014) reported higher concentration of metals in hepato-pancreas of fish, crab and shrimp. Heavy metals are persistent and non-biodegradable and they can be accumulated through biological chains (Singh *et al.*, 2011).

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The toxic nature of some heavy metals in aquatic environment invariably affects the organism that lives in the environment; hence poses a greater danger to man as final consumer of some of these affected aquatic organisms. Therefore, the accumulation of heavy metals concentration in crabs of Ojo River, Lagos Nigeria became necessary and its implication on human safety. Since crabs are edible, study of heavy metals toxicity on them will give baseline information on their safe level for human consumption. Thus, the thrust of the study was to investigate the accumulation of the heavy metals in shell fishes of the Ojo River, Lagos Nigeria.

MATERIALS AND METHODS

Description of the Study Location

Ojo River is in Lagos, Southwest Nigeria (Figure 1). It is located at an elevation of 114 meters above sea level. Its coordinates are 6°55'60"N and 6°24'0"E. Ojo is located on the eastern section of the Trans-West African Coastal Highway, about 37 km West of Lagos. It is a part of the Lagos Metropolitan Area. The area consists mainly of farmland and a few residential houses. There are also few industrial activities around the location.

Sampling Methods

Sampling was carried out between Aug. and Oct., 2019 fortnightly in accordance with the recommendation of APHA (2005) reference method for marine pollution studies. Four samples each of *Callinectes ornatus, Callinectes danae* and *Cardisoma armatum* crabs were collected from the Ojo River for five times totaling 20 for each crap species. They were identified and categorized into male and female using Tobor and Ajayi (1979), Fischer *et al.* (1981) and Powell (1982) text. All the samples were transported to the laboratory in an ice chest cooler box on same day for metal analysis. Water samples were collected in 60 cl sterile plastic bottle for physico-chemical analysis.

Determination of Physico-Chemical Indices and Heavy Metals of the River Water

Water parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) were measured in-situ using portable pen-type pH Meter (PH-009), EC/TDS meter hold and DO meter probes, respectively. The instruments were dipped into water sample at each sampling point at a recommended level (Awoyemi *et al.,* 2014). Salinity, chemical oxygen demand (COD) and biological oxygen demand (BOD) were measured ex-situ using salinometer, titrimetric and volumetric methods using APHA (2005) standard procedures respectively.

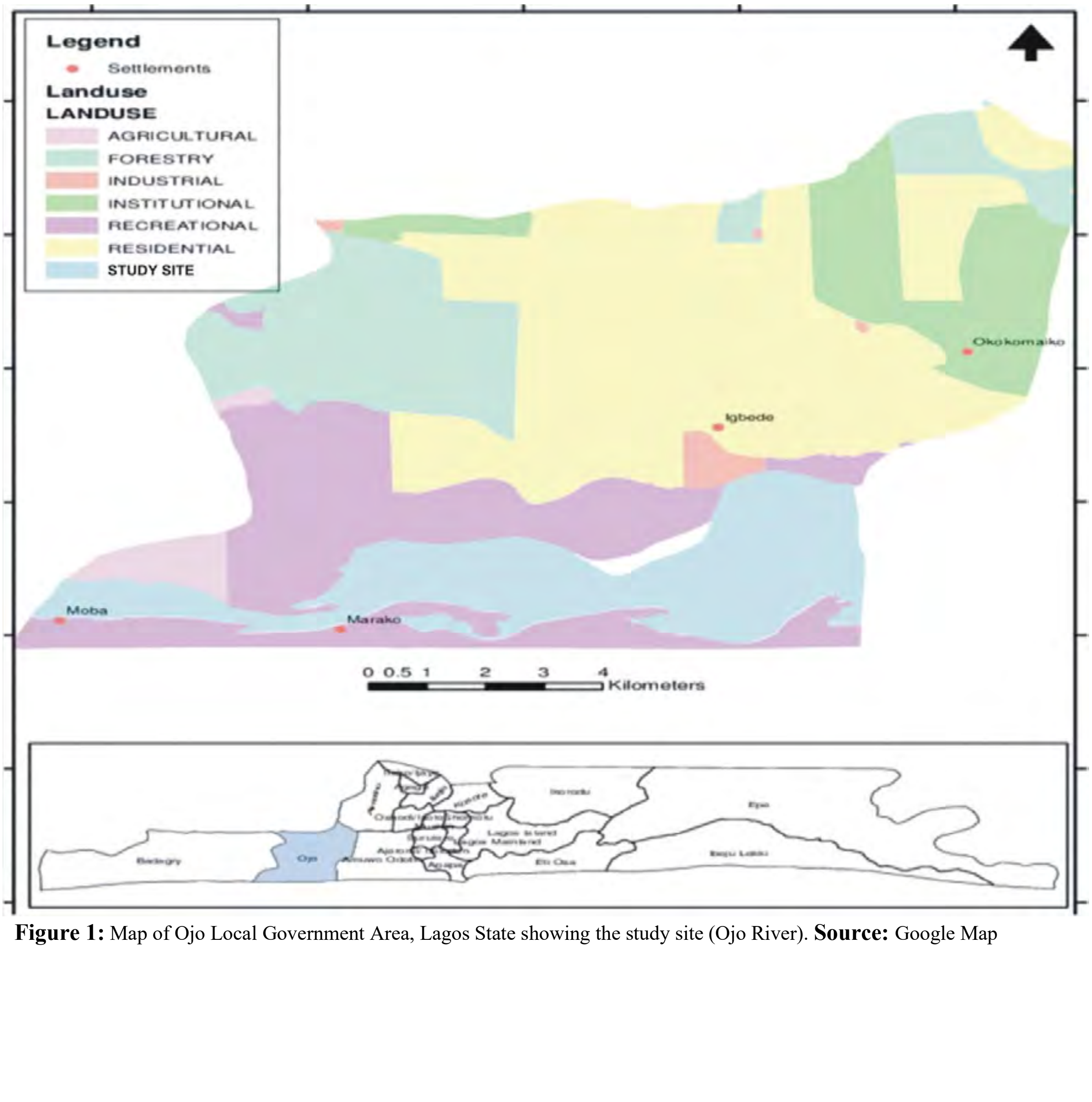

Figure 1: Map of Ojo Local Government Area, Lagos State showing the study site (Ojo River). **Source:** Google Map

The three different species collected from Ojo River were properly cleaned by rinsing with distilled water to remove debris, planktons and other external adherents. The specimens were then drained under folds of filter, wrapped in aluminum foil and then refrigerated at 10°C prior to analysis. After digestion, theresulting solution from the digestion was then aspirated into the flame of the atomic absorption spectrophotometer (Model 210 VGP of Buck Scientific, Germany) using air-acetylene flame for the metal analysis against standard metal solutions. Each metal (cadmium, chromium, copper, lead, zinc and iron) was analyzed using the specific hollow cathode lamp at a specific wavelength according to APHA (2005) standard recommended procedures.

The crab's samples were defrosted for 2 h, and then dried at 80ºC in the Gallenkamp hot box oven. The dried samples of crab were put in a cleaned dried mortar separately and were grounded to fine particles and then sieved using a 0.02-mm sieve. Then 0.50 g of each sample was measured into clean 100-mL dry beaker; 10 mL of aqua regia hydrochloric acid (HCl) and nitric acid (HNO_3) at the ratio of 2:1 was then added to the sample for digestion. The samples were made to be evenly distributed in the acid by stirring with glass rod before placing the beaker on the heater. The digested sample was filtered into a graduating cylinder and the filtrate was made up to 50 mL using distilled water. The atomic absorption spectrophotometer was used to analyze the concentration (µg g^{-1}) of heavy metals in the experimental crabs with air-acetylene gas mixture as oxidant.

Extracts from the above digestion were aspirated and the equipment calibrated for each element. The results were recorded as mg l^{-1} of solution and were calculated to mg kg^{-1} of sample using the weight of sample taken as a denominator of the digest volume (50 ml). The calculations are as follows:

$$Sample = Digest\ concentration\ \times Dilusion\ Factor$$

$$Digest\ Concentration = Analyte\ readings\ on\ AAS$$

$$Dilution\ Factor\ (DF) = \frac{\text{Volume of digest} \times \text{Aliquot}}{\text{Weight of Sample}}\ ;$$

where volume of digest refers to final volume of digested or extracted sample, and aliquot refers to ratio of sample to distilled water.

Statistical Analysis

Data were subjected to statistical analysis using SPSS version 20.0 (IBM Corporation, 2011). Mean values were compared using analysis of variance. Post-hoc test was done using the Student Newman Keuls (SNK). Probability value (p - value) less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Physico-Chemical Parameters of Ojo River

The levels of TDS, EC, salinity, DO, BOD and COD of the Ojo River are shown in Table 1. The values of TDS and EC were relatively high in the water samples.

Levels of Heavy Metals in *Callinectes ornatus,* Male and Female of *Cardisoma armatum* and *Callinectes danae*

Concentration of each heavy metal studied in the Ojo River is described in Figure 2. Lead (Pb), cadmium (Cd) and chromium (Cr) were not detected, while the concentration of copper (Cu) was higher followed by zinc (Zn) and in *C. ornatus*.

The concentration of heavy metals iron (Fe), Pb, Cu, Cd, Cr and Zn in *C. armatum* and *C. danae* from Ojo River, Lagos is shown in Table 2. Concentration of Fe was significantly ($p < 0.05$) higher in the female *C. armatum* than the males. Similarly, concentration of Cu was significantly ($p < 0.05$) higher in the female *C. armatum* than the males. However, there was no significant ($p > 0.05$) difference in the concentration of Zn recorded in both the male and female *C. armatum*. Concentrations of Pb, Cd and Cr were not detected in the male and female *C. armatum* from Ojo River. Similarly, Pb, Cd and Cr were also not detected in the male and female *C. danae* from Ojo River. On the other hand, concentrations of Fe, Cu and Zn were significantly ($p < 0.05$) higher in the female *C. danae* than the males.

Table 1: Physico-chemical properties of water from Ojo River

Parameters (Units)	Mean	Standard error	WHO (2003)
Total dissolved solids, TDS (mg l^{-1})	50318.50	575.50	≤ 500.00
Electrical conductivity, EC (µS cm^{-1})	5070.00	401.00	≤ 1000.00
Salinity (mg l^{-1})	29.87	9.34	≤ 35.00
Dissolved oxygen, DO (mg l^{-1})	2.11	0.12	10.00
Biological oxygen demand, BOD (mg l^{-1})	178.29	53.60	10.00
Chemical oxygen demand, COD (mg l^{-1})	159.44	51.86	≤ 5.00

Table 2: Heavy metals (mg kg^{-1}) in *Cardisoma armatum* and *Callinectes danae* of Ojo River

	Cardisoma armatum					
(mg/kg)	Pb	Fe	Cu	Cd	Cr	Zn
Male	0.00±0.00	0.27±0.01*	4.98±0.06*	0.00±0.00	0.00±0.00	1.15±0.01
Female	0.00±0.00	2.25±0.01	7.96±0.08	0.00±0.00	0.00±0.00	1.23±0.01
	Callinectes danae					
	Pb	Fe	Cu	Cd	Cr	Zn
Male	0.00±0.00	0.21±0.01*	4.19±0.03*	0.00±0.00	0.00±0.00	1.04±0.01*
Female	0.00±0.00	0.32±0.01	5.29±0.04	0.00±0.00	0.00±0.00	1.29±0.01
USEPA (2007)	0.05	0.01	1.00	0.005	0.10	5.00

*represents means (±standard error of mean) that are significantly ($p < 0.05$) different between males and females

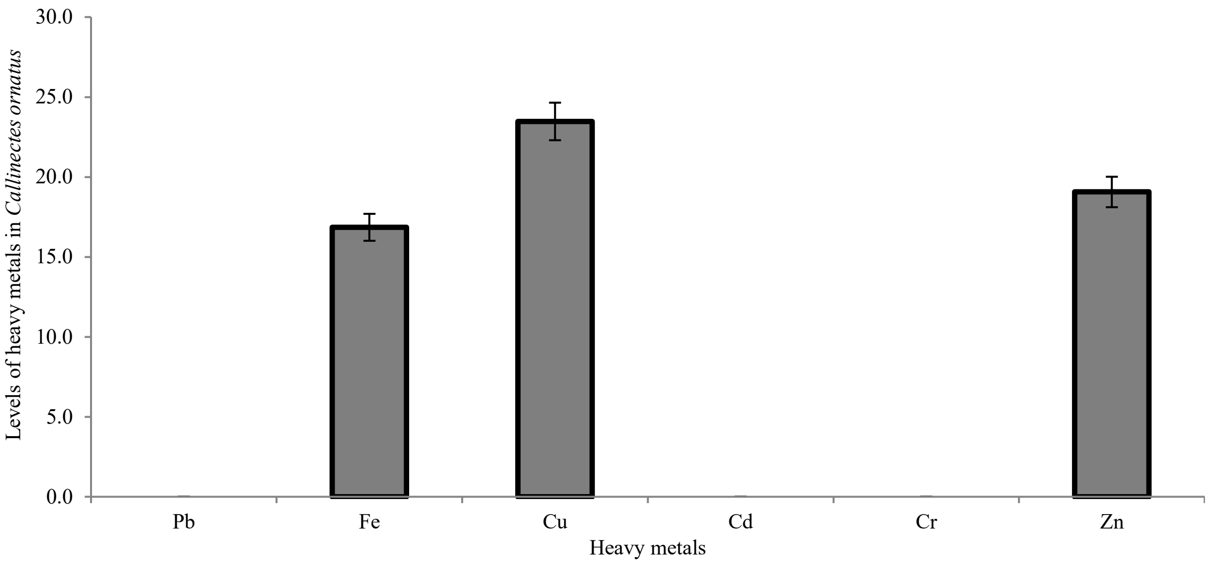

Figure 2: Levels of heavy metals in *Callinectes ornatus* of Ojo River

Overall Level of Heavy Metals in the Crab Species
The overall comparison of the levels of heavy metals in *C. danae, C. armatum* and *C.ornatus* is represented in Table 3. Concentrations of Pb, Cd and Cr were also not detected in the three crab species from Ojo River. However, *C. ornatus* recorded significantly ($p < 0.05$) higher concentrations of Fe, Cu and Zn than the other species, *C. danae* recorded lowest.

Decrease in the value of dissolved oxygen observed implies that the oxygen level in the water has been distorted which might be as a result of predisposition factors of biological living aquatic organisms as opined by Idowu *et al.* (2020) which observed decrease in DO concentration of water used for *Oreochromis niloticus* culture. Among the innumerable contaminants, pollution by heavy metal in aquatic environments has become a global phenomenon due the higher toxicity and persistence for several decades in the aquatic environment (Thomas and Mohaideen, 2014). Total dissolved solids and electrical conductivity was higher than the other parameters which could be as a result of the constant movement on the water and the activities located around the study area. High metal concentration (Fe, Cu and Zn) observed to be higher than the standard regulatory values in shellfish may pose a socio-economic threat to the commercial and domestic consumption of the fisheries resources around the inhabitant of Ojo River, and by extent Nigeria, fishing being one of the predominant activities in the country (Saleh and Ahmed, 2020) may be damaged if her fisheries resources if not properly regulated and monitored.

Several studies showed accumulations of heavy metals in crustacean, yet few studies show effect of sexual changes with respect to their metal accumulation. Differences in accumulation between the genders have been attributed to difference in diet or differences in habitat (Hosseni *et al.,* 2014). They documented that male's crabs feed more on fish and bivalves while the females feed more on shrimps, plant and detritus. Plants have relationship with sediments and receive more sediment associated metals. It is also known that certain forms of metals can readily accumulate within crustacean tissues at much higher levels (Oguzie and Okhagbuzo, 2010). Therefore, female crabs would receive higher levels of metals when they feed on shrimps and plants. Larger organisms generally exhibit higher contaminant level in their bodies and crabs that are higher on the food chain also accumulate more contaminants when compare to crabs that eat a range of different foods or eat smaller organisms. Heavy metals in the female crabs were found higher than the males which may be as a result of being larger and can eat larger food items (Falusi and Olaonipekun, 2007). Some metals (Pb, Cd and Cr) were not found in the crabs and this could be associated with the fact that the area consists mainly of residential houses, farms and a few industries.

Table 3: Heavy metals (mg kg^{-1}) in the crab species from Ojo River

(mg/kg)	Pb	Fe	Cu	Cd	Cr	Zn
C. ornatus	0.00±0.00	16.85±0.01^{a}	23.47±0.10^{a}	0.00±0.00	0.00±0.00	19.06±0.01^{a}
C. armatum	0.00±0.00	1.26±0.44^{b}	6.47±0.67^{b}	0.00±0.00	0.00±0.00	1.19±0.02^{b}
C. danae	0.00±0.00	0.26±0.03^{b}	4.74±0.25^{c}	0.00±0.00	0.00±0.00	1.17±0.06^{b}
USEPA (2007)	0.05	0.01	1.00	0.005	0.10	5.00

a, b, c represent means and standard deviations of duplicate replication.
Mean values within the column with the same superscript were not significantly ($p > 0.05$) different.

CONCLUSION & RECOMMENDATION

In the study, it was found that metals values were larger in female crabs than in the males. *C. ornatus* has the highest accumulation of copper followed by *Cardisoma armatum* and *C. danae*, while Cu>Fe>Zn in all the species collected except *C. armatum* where Fe is greater than Zn. This study has also provided information on the different level of accumulation of heavy metal in male and female species of crab. From the study of crabs sampled from Ojo River, Lagos metropolis may be considered safe for consumption but the need for continuous monitoring to prevent bioaccumulation is necessary. The study provides baseline data that can be used in future to evaluate differences within and across specific geographical areas. Without this database it will be difficult to evaluate and interpret future results from the region, or to identify places with disturbing trends in pollution levels.

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