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Seasonal Heterogeneity and Health Risk Assessment of Metal Contaminant in *Callinectes amnicola* from Epe Lagoon, Southwest, Nigeria

Rasheed Olatunji Moruf
Department of Fisheries and Aquaculture, Bayero University, Kano, Kano State, Nigeria

Corresponding author, Email address: tunjimoruf@gmail.com

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tunjimoruf@gmail.com
Phone: +2348022429983

Abstract

Seasonal distribution and human health risks of trace metals (zinc, copper, lead, iron, cadmium, chromium and nickel) in water, sediment and crab (*Callinectes amnicola*) from Epe Lagoon, Lagos (Nigeria) were assayed using standard methods. A non-significant variation ($p > 0.05$) in water physicochemical parameters occurred in both dry and wet seasons. Higher metallic contamination was observed in *C. amnicola* compared with water while the sediment acts as a major depository of these metals. In this portunid crab, the ability of metal accumulation from water (Bio-water accumulation) was higher than that from sediment (Bio-sediment accumulation). In addition, the linear regression models revealed positive relationships between tissue and sediment concentrations of all the investigated metals for both seasons. Estimated Daily Intake of the metals for both seasons were lower than the oral reference dose while the target hazard quotient and the total hazard index of individual metal were less than 1, indicating no potential health hazard for the consumption of *C. amnicola* from Epe Lagoon.

1. Introduction

The marine ecosystem is an essential component on earth. It consists of diverse communities such as the estuaries, salt marshes, mangrove forests, coral reefs, the open ocean, and the deep-sea ocean that exist in different regions of the world. This ecosystem acts as habitat for resident and migratory animals and plays a part in carbon sequestration, climate regulation, biological control maintenance and protection against erosion, among other important environmental functions [1]. Food from the sea is produced from wild fisheries and species farmed in the ocean (mariculture), and currently accounts for 17 % of the global production of edible meat [2]. In addition to protein, food from the sea contains bioavailable micronutrients and essential fatty acids that are not easily found in land-based foods, and is thus uniquely poised to contribute to global food and nutrition security [3].

During the last few decades, the marine environment has been witnessing increasing pressure and threats due to demographic growth concentrated essentially on the coastal zones that actually host two thirds of the world's population [4]. The presence of chemical contaminants in the marine environment has been assigned mostly to land-based sources that contribute to about 80% of pollution entering the oceans and seas [5, 6]. Contaminants such as trace metals can enter the marine environment by natural or anthropogenic means. They can be naturally released by geological weathering, erosion, and other phenomena such as forest fires and volcanic activities. Anthropogenic activities include industries, untreated sewage, surface runoff, and dumpsites leachate which release significant amounts of toxic trace metals that are dangerous for marine ecosystems and ultimately to human health [7]. Due to the toxicity, bioaccumulative and biomagnifying characteristics of trace element contamination, public health issues are a matter of serious concern especially in developing countries [8, 9].

Along with the annelids (which are especially important in the sediments), crustaceans are often among the most abundant and influential invertebrates in marine environments. Crustaceans such as portunid crabs have become a global delicacy for seafood lovers because of their vital nutrients that are beneficial to human health [10]. *Callinectes spp*, belonging to the Family Portunidae are decapod crustaceans of high economic importance worldwide and have been subjected to intense aquaculture practices especially in Asia and the Americas [11]. *Callinectes amnicola* (De Rochebrune, 1883) is an economically important macro invertebrate, which supports valuable commercial crab fisheries in Nigeria. Despite the enormous density of nutrients, crustaceans are known to bioaccumulate trace metals in minute amounts over time, and this contamination is then concentrated higher up the food chain [12, 13]. There are two key reasons why metal bioaccumulation in crustacean shellfish needs to be constantly studied. One, shellfish is among the widely and preferred eaten seafood and secondly, shellfish has widespread distribution, easy accessibility, and resistance to toxins while playing a significant role in aquatic ecosystem's food chain [14, 15].

The coastal waters of Lagos are blessed with numerous species of aquatic crabs, which are nutritionally important in the supply of protein, the nine essential amino acids and minerals [16]. However, due to rapid population growth, this area has experienced typical anthropogenic activities and industrialization at the catchment site. Numerous researches have been conducted to assess the trace metal bioaccumulation levels in portunid crabs of Nigeria [17-21]. However, data and information on human health risk assessment of trace metal contamination due to crab consumption are either insufficient or absent in the area. The objectives of this study are to quantify the concentration levels of some trace metals (zinc, copper, lead, iron, cadmium, chromium and nickel) in water, sediment and crab (*C. amnicola*) from Epe Lagoon and to assess the human health risks associated with consumption of the biota.

2. Methodology

2.1 Description of study area

Epe Lagoon lies between Latitudes 6° 23' 27"N - 6° 41' 45"N and Longitudes 2° 42' 20"E - 3° 42' 21"E with a surface area of 243 km^2, and an average depth of about 1.80 m [22, 23]. Epe Lagoon is sandwiched between two other lagoons, the Lekki Lagoon (freshwater) in the east and the Lagos Lagoon (brackish water) in the west. Epe Lagoon supports a major fishery in Lagos State, Nigeria and it is also used as transportation route for people, goods and timber logs from Epe to other places in South-Western Nigeria. Based on increasing anthropogenic effects from heaps of domestic and solid waste dumps, six sampling stations closed to settlements (Epe, Ejirin, Imope, Ikosi, Ijede and Ajeboh) were chosen for this study (Fig. 1). Each site is approximately 1.5km apart.

2.2 Sample collection and preparation

Water, sediment and crab (*C. amnicola*) samples were collected monthly between November 2020 and October 2021 covering dry and wet seasons. Water samples (500 mL per sampling point) were collected at a depth of 1 cm below the water surface in HNO_3 pre-rinsed (1L) containers and 5 mL of concentrated HNO_3 added immediately to minimize chemisorption. Sediment samples were collected with the aid of 0.05 m^2 Eckman Grab during low tide at 10 –15 cm depth. Five (5) water and sediment samples each were randomly collected per sampling station, making 30 samples for the six locations per month. A total of 142 adult crabs (average weight of 145 g) were caught directly from the lagoon during low tide through a basket trap. In each case, samples were properly labelled and kept in clean plastic containers and stored at 20 °C before taking to the laboratory for analyses.

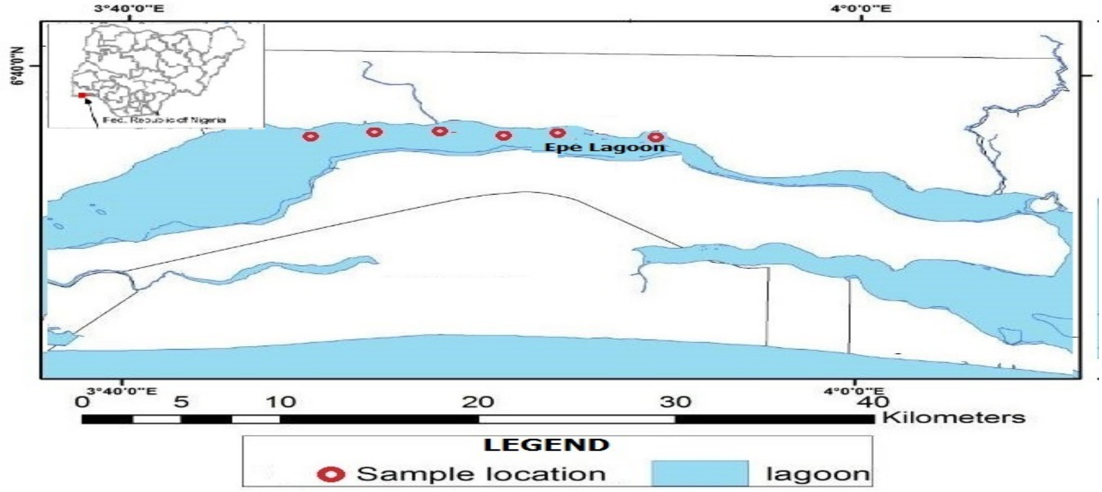

Figure 1. Map showing the study area (Red dots indicating the study site)

2.3 Laboratory analysis

Water temperature and dissolved oxygen were measured in-situ using a mercury-in-glass thermometer and Lutron DO meter (Model: DO 5519) respectively. Separate water samples were collected in 250 ml dissolved oxygen bottles at each station and incubated in the dark for five days for biochemical oxygen demand (BOD) determination as described in APHA [24], while chemical oxygen demand (COD) was determined using Titrimetric method. At the laboratory, the sediments were defrosted by keeping them at room temperature for about 24 h, then dried in an oven at 40 ^{0}C [25], disaggregated and sieved through a 200 µm sieve. The sieved samples were subsequently homogenized in a porcelain mortar and re-sieved. Approximately 5 g of the samples was put in Teflon tubes, and 5 ml aqua regia (HCl: HNO_3 in a ratio of 3:1) added for digestion, following the ISO 11466 digestion method [26].

Sub-samples of approximately 1 g tissue were weighed in a precision scale with decimal resolution (0.001 g) and digested in a mixture of 5 ml of concentrated nitric acid (TMA, Hiperpure, PanReac, Spain) and 3 ml of 30 % w/v hydrogen peroxide (PanReac, Spain) in a microwave-assisted digestion system (Ethos Plus; Milestone, Sorisole, Italy). Digested samples were transferred to polypropylene sample tubes and diluted to 15.0 ml with ultrapure water. As described by Dussubieux and Van Zelst [27], the determination of the trace elements copper, zinc, chromium mercury, lead and cadmium in all the samples was carried out by ICP-MS. ICP-MS-based multi-element determination was performed in an Agilent 7700x ICP-MS system (Agilent Technologies, Tokyo, Japan) equipped with collision/reaction cell interference reduction technology. The continuous sample introduction system consisted of an autosampler, a Scott double-pass spray chamber (Agilent Technologies, Tokyo Japan), a glass concentric MicroMist nebuliser (Glass Expansion, West Melbourne, Australia), a quartz torch and nickel cones (Agilent Technologies, Tokyo Japan). Elemental concentrations were quantified using a Mass Hunter Work Station Software for ICPMS (version A.8.01.01 Agilent Technologies, Inc. 2012, Tokyo, Japan). Analytical quality control was guaranteed through the implementation of laboratory quality assurance and laboratory methods, including the use of standard operating procedures, calibrations with standards and analyses with reagent blanks. Samples were analysed in triplicates, all chemicals and reagents used were of analytical grade. The limits of detection calculated for the investigated metals in all media was

$1.0 \times 10{-}4$ mg/L for water and $1.0 \times 10{-}4$ mg/kg for sediment and biota. The accuracy of determination was evaluated by comparison with the analytical recoveries determined in certified reference materials (fish protein DORM- 3 National Research Council, Ottawa, Ontario, Canada) analysed following exactly the same procedure as for the samples.

2.4 Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20.0. Student's t- test was used to determine the differences between the concentration levels of each of the analysed element in water as well as in sediment samples in the dry and wet seasons. $P < 0.05$ was considered the level of statistical significance.

Bioaccumulation factor (BAF), which is the ratio of the concentration of metal in organism tissue to the concentration of metal in sediment (Bio-sediment accumulation) and water (Bio-water accumulation), was calculated.

To define the potential risk for human health, concentration and toxicity information relative to each pollutant need to be integrated with the exposure assessment [28, 29]. Health risk was estimated based on Environmental Protection Agency guidelines [30]. To assess the potential health risk via the consumption of the *C amnicola*, the estimated daily intake (EDI), target hazard quotient (THQ) and target hazard index (THI) were calculated using equations 1, 2, and 3 respectively with the following assumptions:

- The hypothetical body weight for adult Nigerian was 70 kg [31].
- The maximum absorption rate was 100% while the bioavailability factor was also 100%.

$$Estimated\ Daily\ Intake, EDI(mg/kg/day) = \frac{\mathrm{C\ x\ CR\ x\ AFx\ EF}}{BW} \quad [32] \quad \textbf{Eqn. 1}$$

Where

C = Concentration of the contaminant in the exposure pathway (mg/kg) of C amnicola
CR= Consumption Rate; Nigeria aquatic crab taken/day, 0.0366 Kg/day =13.359 kg/y
AF= Bioavailability factor (100%)
EF = Exposure Factor = 1
Bw = Body weight (70kg)

$$Target\ Hazard\ Quotient, THQ = \frac{\mathrm{EDI}}{\mathrm{RfD}} \quad [33] \quad \textbf{Eqn. 2}$$

Where EDI= Estimated Daily Intake and RfD = the oral reference dose (mg/kg/day),

$$THI = \sum_{i=1}^{n} \mathrm{THQ} \quad [33]\ \textbf{Eqn. 3}$$

Where THQi is the target hazard quotient of an individual trace metal

3. Results and Discussion

3.1 Physico-chemical parameters of water

The seasonal variation of water physico-chemical parameters measured at the Epe Lagoon during the study period is presented in Figure 2. Although temperature was higher in the dry season (30.01± 0.05) than in the wet season (29.03± 0.10), there was no significant difference ($p > 0.05$) in the variation. The range of recorded temperatures (29.39-30.07◦C) is also considered normal with reference to the lagoons found in Lagos State [34]. The climatic conditions, sampling times, sunshine hours, and unique properties of the water environment, such as turbidity, wind force, plant cover, and humidity, all influence the drop or increase in water temperature [35].

The DO, BOD and COD were generally higher during the wet season with the mean values of 6.98 mgL^{-1}, 8.41 mgL^{-1} and 13.33 mgL^{-1} respectively. The lower concentration of DO during the dry season could be owing to large number of bacteria that required a lot of oxygen for chemical oxidation, degradation, or breakdown of organic loads present in the water body. Also, the greater DO measured during the rainy season could be attributed to the cold temperatures and significant run-offs experienced at the study site. According to Abobakr Yahy *et al.* [36], DO is a vital respiratory gas that also serves as a water quality indicator and a measure of a river's health and productivity. The BOD values were lower than the permissible limit of 50 mgL^{-1} for coastal water [37]. The higher COD values recorded may be due to chemical oxidation of some organic substances. The recorded values are comparable to the results of Lawal-Are *et al.* [38] who reported mean DO of 5.3±0.1 mgL^{-1}, BOD of 5.1±0.6 mgL^{-1} and COD of 19.0±3.9 mgL^{-1} for Lagos Lagoon.

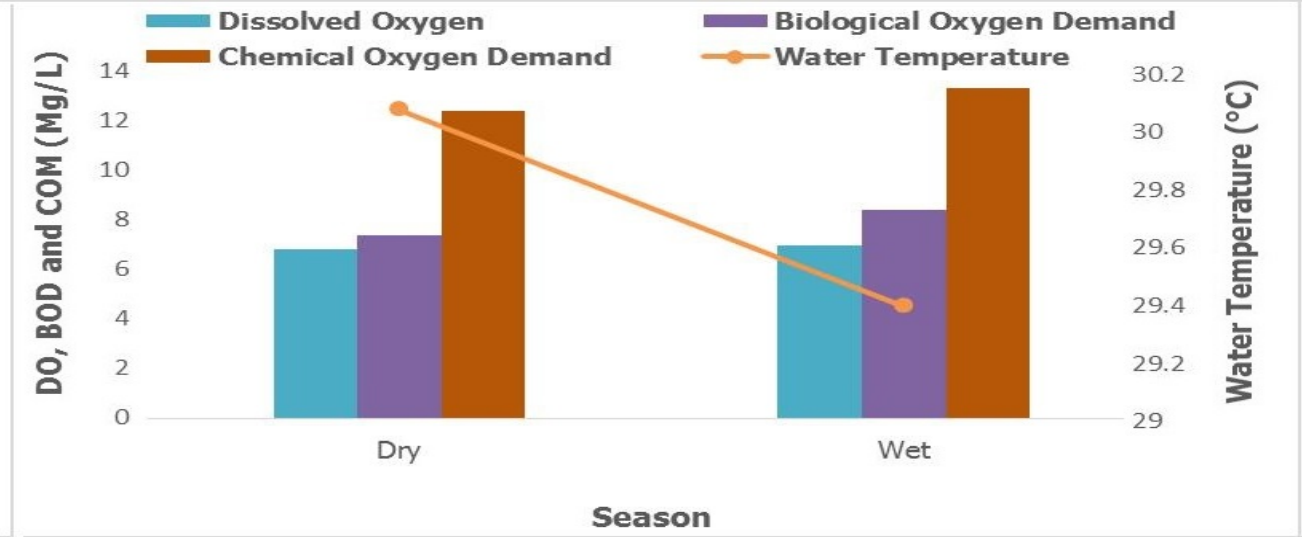

Figure 2. Physicochemical parameters of Epe Lagoon, Nigeria

3.2 Levels of trace metals in samples

Figs. 3–5 depict box and whisker plots showing trace metal concentrations in water, sediment and *C. amnicola* relation to seasonality. In Fig. 3, copper in water showed a widest spread (0.04-0.34 mgL^{-1} in dry season and 0.03 – 0.38 mgL^{-1} in wet season) while the concentrations of other investigated trace metals were moderately spread across seasons. The mean concentration level of lead reported in the lagoon water for both seasons was above the Nigerian Industrial Standard (NIS) limit of 0.01 mgL^{-1} Pb [39] and 0.05 mgL^{-1} Pb in water reported by USEPA [40]. This could be attributed to increased commercial and industrial activities around the coastline and the continuous inflow of domestic wastes into the lagoon. Furthermore, dumping of domestic wastes as well as washing of agrochemical fertilizers from agricultural farmlands into the water bodies by runoffs water when it rains could be the potential sources of trace metals in the studied water body.

In Fig. 4, cadmium in sediment showed a narrow spread for both seasons while the concentrations of other investigated trace metals were moderately spread across seasons. The seasonal trend of trace metal concentrations in sediment during the study period was in the order: zinc < copper < cadmium < lead < nickel < chromium. According to Moruf and Akinjogunla [13], sediment is the most important metal depository, containing up to 99 percent of all metals found in the aquatic environment in some circumstances. When sediment metals are researched, it is possible to gain a better understanding of the aquatic ecosystem's long-term pollution state [41].

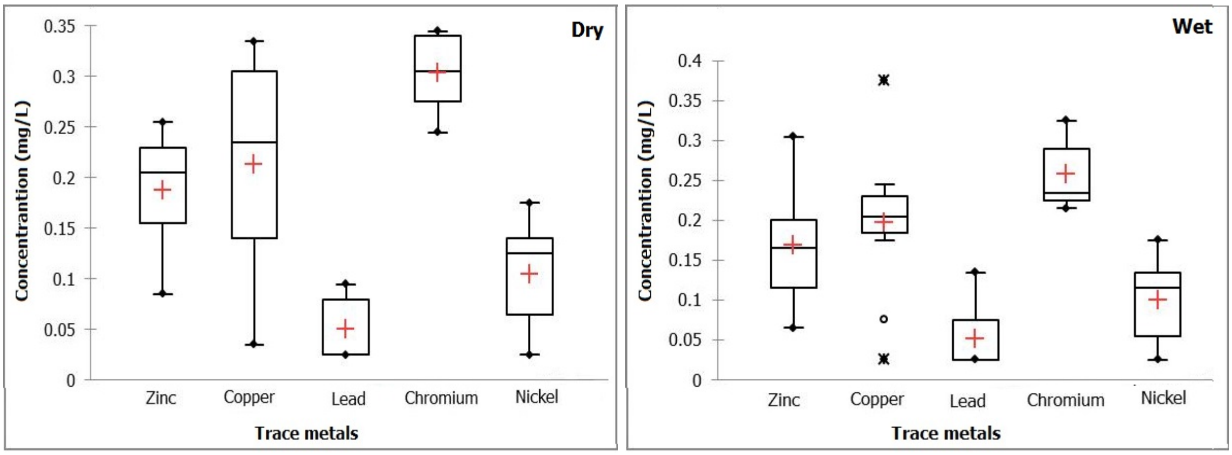

Figure 3. Box plot for seasonal distribution of water trace metals in Epe Lagoon, Nigeria (Lower and upper box boundaries 25th and 75th percentiles, respectively, line inside box median, lower and upper error lines 10th and 90th percentiles, respectively, filled circles data falling outside 10th and 90th percentiles)

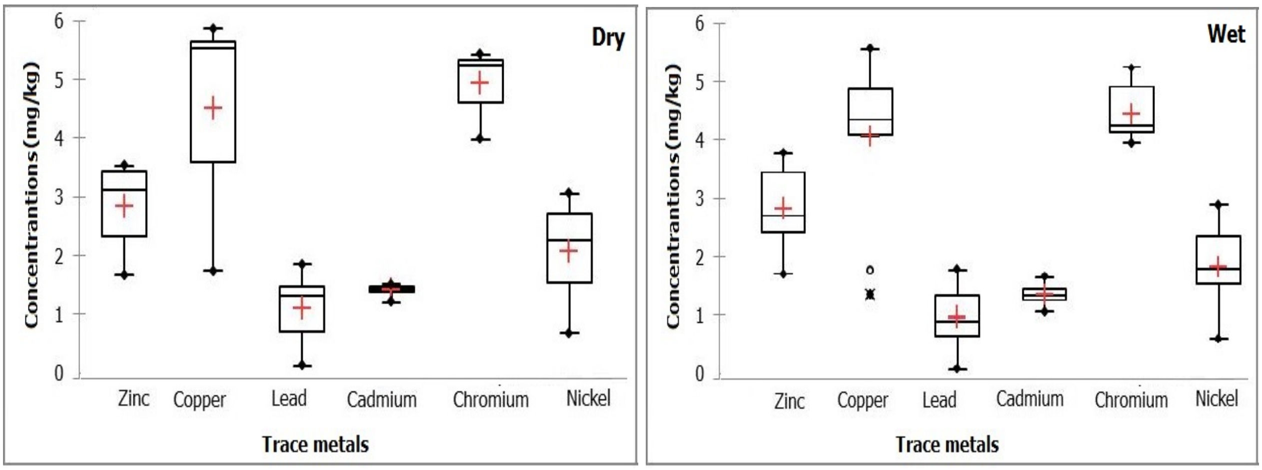

Figure 4. Box plot for seasonal distribution of sediment heavy metals in Epe Lagoon, Nigeria (see Fig. 3 caption for explanation of box-plot)

In Fig. 5, cadmium showed a narrow distribution in *C. amnicola* while zinc, copper and chromium showed a wider distribution across seasons. Chromium (3.22 – 5.61 mgkg^{-1}) recorded the highest mean concentration while cadmium (0.03 – 0.18 mgkg^{-1}) recorded the lowest mean concentration in both seasons. The biota, *C. amnicola* has greater levels of trace metal concentrations than water, which can be attributed to biological buildup. Similarly, Lawal-Are *et al.* [17] found elevated levels of zinc (0.74 ± 0.13 mgkg^{-1}), copper (1.21 ± 0.03 mgkg^{-1}), chromium (0.28 ± 0.10 mgkg^{-1}), lead (0.26 ± 0.07 mgkg^{-1}) and nickel (0.28 ± 0.03 mgkg^{-1}) in *C. amnicola* from Igbese River. According to Ezemonye *et al.* [42], elevated heavy metal concentrations in aquatic organisms imply cumulative exposure through water and/or food.

3.3 Trace metal bioaccumulation

The bioaccumulation factors of trace metals in the sampled crabs are presented as Bio-water accumulation factor (BWAF) and Bio-sediment accumulation factor (BSAF) in Fig. 6 and Fig. 7 respectively. All investigated trace metals were observed to bioaccumulate in measurable concentrations in the organism across seasons. Significant differences of BWAF and BSAF were not recorded for any of the investigated trace metals for both seasons. The recorded BSAF values were greater than one for zinc (1.32±0.00) and lead (6.79±0.01) while other investigated metals were less than one.

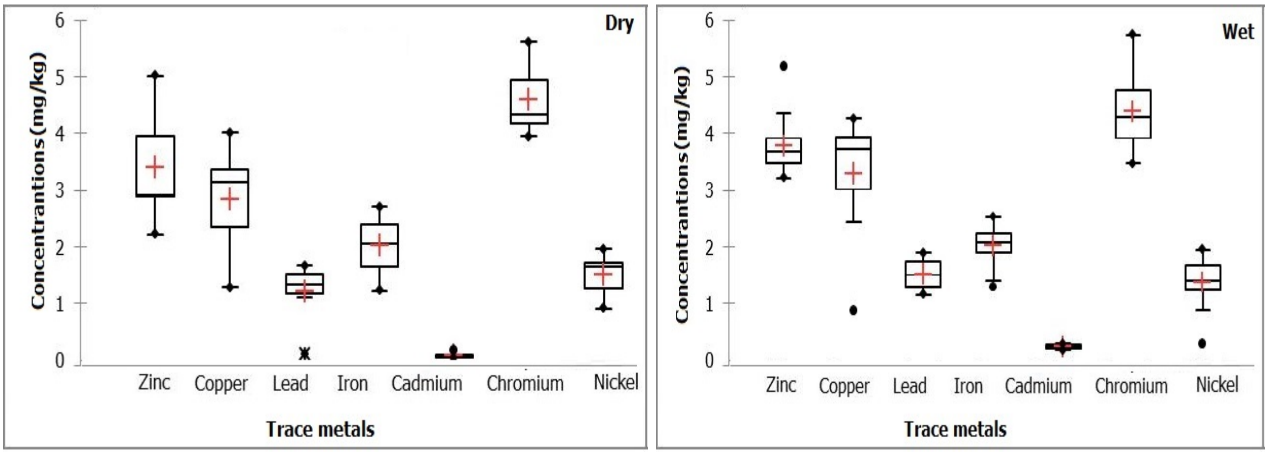

Figure 5. Box plot for seasonal distribution of trace metals in *Callinectes amnicola* (see Fig. 3 caption for explanation of box-plot)

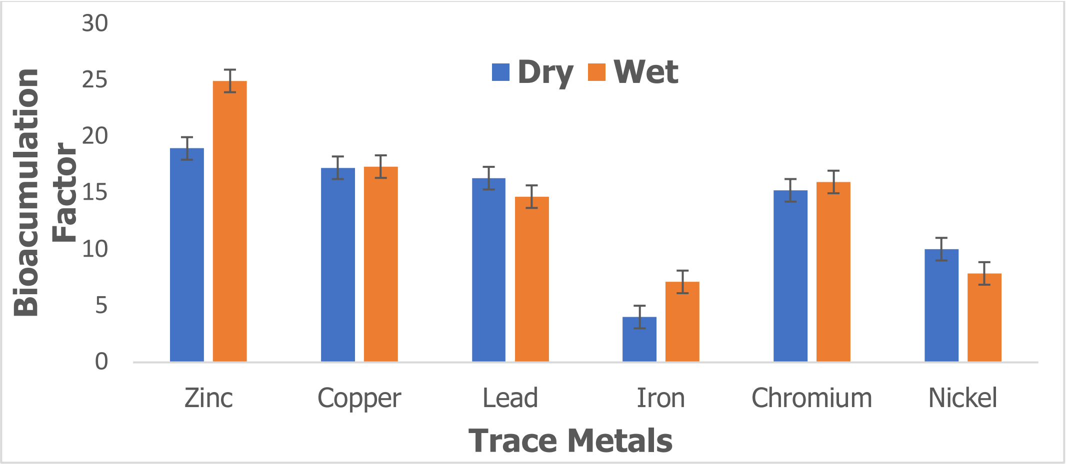

Figure 6. Bio-water accumulation factor of *Callinectes amnicola* in Epe Lagoon

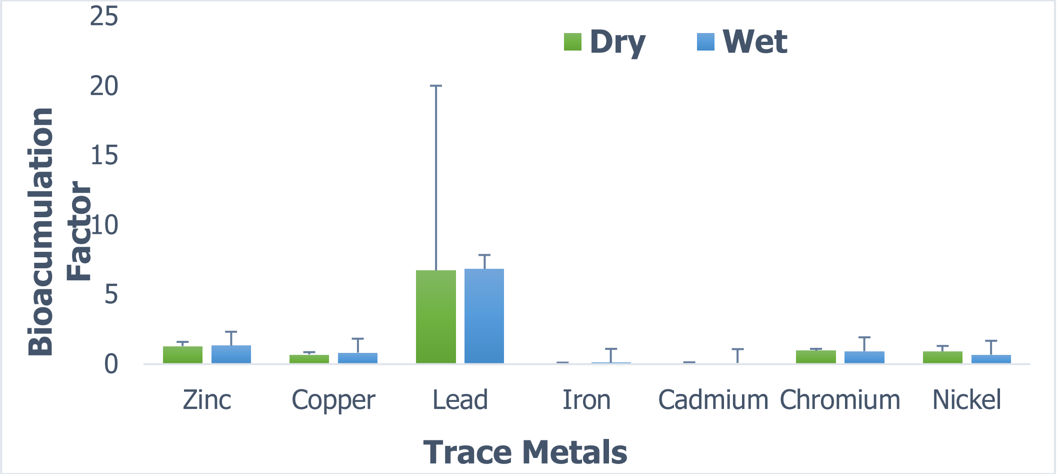

Figure 7. Bio-sediment accumulation factor of *Callinectes amnicola* in Epe Lagoon

The high BAF values of these two metals in spite of their low concentration in sediment reveal their high bio-magnification abilities. The seasonal variation in the levels of BWAF and BSAF in this study might be ascribed to a number of factors that influence metal uptake in crabs, including age, developmental stages, feeding mode, metabolic activities, and reproductive conditions, as mentioned by Simonetti *et al.* [43]. The findings for BSAF in this study backed up that of Capparelli *et al.* [44], who found that BAF values in mudflat fiddler crabs drop as metal concentration in sediments increases.

3.4 Predictive linear regression models for trace metals in crab and sediment

The prediction of pollutant concentrations in tissue of *C. amnicola* for dry and wet seasons is shown in Table 1 and Table 2 respectively. Higher variation was observed in the dry season (R^2= 0.1559-0.8368) compared to the wet season (R^2= 0.0086-0.8246). In dry season, positive correlations exist between the tissue (*C. amnicola*) and sediment concentrations of zinc, copper, lead, iron, cadmium, chromium and nickel with respective regression coefficient (b) of 0.806, 0.482, 0.226, 0.060, -0.360, 0.443 and 0.381.

Table 1. Regression analysis between metal concentrations in *Callinectes amnicola* and sediment during dry season

Metal	Regression equation	Coefficient of determination (R^2)
Zinc	Y = 1.208+0.806 X	0.450
Copper	Y = 0.723+0.482 X	0.837
Lead	Y = 0.218+0.226 X	0.642
Iron	Y = 0.626+0.060 X	0.725
Cadmium	Y = 0.545-0.360 X	0.343
Chromium	Y = 2.458+0.443 X	0.156
Nickel	Y = 0.753+0.381 X	0.806

Likewise, in the wet season, positive correlation between the tissue concentration and concentration in the sediment was recorded for all the investigated metals. Furthermore, 15.9 % of zinc, 31.3 % of copper, 1.0 % of lead, 61.3 % of iron, 26.2 % of cadmium, 8.5 % of chromium, and 82.5% of nickel in *C. amnicola* were predicted by the concentration of the respective metals in the sediment. This result is comparable to the findings of Ghaeni *et al.* [45], who reported a linear regression models with positive relationships between pollutant concentrations in sediment and blue crab from the from Persian Gulf.

Table 2. Regression analysis between metal concentrations in *Callinectes amnicola* and sediment during wet season

Metal	Regression equation	Coefficient of determination (R^2)
Zinc	Y = 2.673+0.307 X	0.159
Copper	Y = 1.462+0.392 X	0.313
Lead	Y = 1.300+0.043 X	0.009
Iron	Y =1.033+0.042 X	0.613
Cadmium	Y = 0.221-0.104 X	0.262
Chromium	Y = 2.293+0.410 X	0.085
Nickel	Y = 0.166+0.568 X	0.825

3.5 Human risk assessment of trace metals via the consumption of C. amnicola

The EDI of the trace metals via the consumption of *C. amnicola* in both wet and dry is presented in Table 3. The result in milligram per body weight per day revealed that the EDI of the investigated metals for both seasons were lower than their respective oral reference dose (RFD). The result corroborated the findings of Moslen and Miebaka [46], who reported the EDI of Cr, Ni, Cu, Pb, Ag and

Cd below the reference oral doses for *C. amnicola* from an estuarine creek in the Niger Delta. In the present study, the EDI values were within the recommended range of FAO/WHO [47].

Table 3. Estimated Daily Intake (mg/kg/day) of trace metals in *Callinectes amnicola*

Metal	**Wet**			**Dry**			**Oral reference dose (RfD)**
	Min	**Max**	**Mean + SD**	**Min**	**Max**	**Mean + SE**	
Zinc	0.001558	0.002552	0.0018±0.00	0.001161	0.00262	0.0018±0.00	0.3
Copper	0.000371	0.002086	0.0016±0.00	0.000669	0.002097	0.0015±.00	0.04
Lead	0.000512	0.000889	0.0007±0.00	0.000052	0.000873	0.0006±0.00	0.004
Iron	0.000586	0.001213	0.0010±0.00	0.000643	0.001412	0.0011±0.00	100
Cadmium	0.0000	0.000073	0.0000±0.00	0.0000	0.000094	0.00004±0.00	0.001
Chromium	0.001684	0.002839	0.0022±0.00	0.00206	0.002933	0.0024±0.00	1.5
Nickel	0.000068	0.000915	0.0006±0.00	0.000476	0.001025	0.0008±0.00	0.05

The THQ of individual metal are presented in Table 4. The highest THQ value was recorded for chromium both in wet season (0.0538±0.00) and dry season (0.0600±0.00) while the least value was recorded for cadmium (0.0011±0.00) across seasons. THQ trend for both seasons was chromium > zinc > copper > iron > lead > nickel > cadmium.

Table 4. Target hazard quotient (THQ) and Total hazard index (THI) of trace metals via the consumption of *Callinectes amnicola* from Epe Lagoon, Southwest, Nigeria

Metal	**Wet**			**Dry**		
	Min	**Max**	**Mean + SD**	**Min**	**Max**	**Mean + SE**
Zinc	0.038953	0.063789	0.0462±0.00	0.029019	0.065488	0.0445±0.01
Copper	0.009281	0.052155	0.0398±0.00	0.016731	0.052416	0.0372±0.01
Lead	0.01281	0.022221	0.0175±0.00	0.001307	0.021829	0.0158±0.00
Iron	0.01464	0.030326	0.0238±0.00	0.016078	0.035293	0.0263±0.00
Cadmium	0.0000	0.00183	0.0011±0.00	0.0000	0.002353	0.001±0.00
Chromium	0.04209	0.070978	0.0538±0.00	0.051501	0.073331	0.0600±0.00
Nickel	0.001699	0.022875	0.0155±0.00	0.011895	0.02562	0.0146±0.00
Total hazard index			0.1976			0.0196

In the work of Moslen and Miebaka [46], reported THQ was in the order of Pb > Cr > Cd > Cu > Ni with all values <1. In the present study, the THI for all the investigated metals were less than one (1); 0.198 for wet season and 0.0196 for dry season, suggesting no considerable health hazard according to USEPA [48] via the consumption of *C. amnicola* from the study area.

Conclusion

The physicochemical factors of Epe Lagoon vary with the seasons, but not significantly. The study found different levels of trace metals in Epe Lagoon water/sediment, as well as different levels of accumulation in *C. amnicola* across seasons. The greater trace metal concentrations in *C. amnicola* compared to water can be attributed to biological buildup, with the sediment serving as a main metal depository. Across seasons, all the studied metals were shown to bioaccumulate in measurable amounts in the organism. The ability of this crab to accumulate metals from water (Bio-water accumulation) was greater than that of sediment accumulation (Bio-sediment accumulation). Furthermore, the linear regression models revealed positive relationships between the tissue and sediment concentrations of all the investigated trace metals for both seasons. Estimated Daily Intake of the investigated metals for both seasons were lower than the oral reference dose while the target hazard quotient and the total hazard index of individual metal via the consumption of *C. amnicola* by average Nigeria adults were less than 1, suggesting no considerable health hazard in consuming *C. amnicola* from the Epe Lagoon.

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Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest. *Compliance with Ethical Standards:* This article does not contain any studies involving human subject.

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