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ZOOLOGY

Target hazard quotient evaluation of selected trace elements in highly consumed crustacean species in Lagos, Nigeria

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ABSTRACT. Crustaceans are known for their trace element bioaccumulation abilities. Muscle tissues of lagoon crab, marine crab, pink shrimp and mantis shrimp marketed for consumption in Nigeria were analyzed for Mn, Fe, Cu, Zn, Hg and Cr levels using standard methods. Muscle levels in mg kg^{-1} of Mn (0.03±0.00 in lagoon crab), Fe (0.072±0.01 in mantis shrimp), Cu (0.344±0.01 in lagoon crab) and Zn (0.073±0.00 in mantis shrimp) were significantly different ($p<0.05$) from their corresponding values in other examined crustaceans. The mean values of Cr and Hg were not significantly different across samples. The estimated daily intake of trace element (mg person^{-1}day^{-1}) revealed that Hg (0.000001) in marine crab contributed the lowest daily intake while Zn (0.000226) in lagoon crab contributed the highest daily intake. Total hazard quotient (THQ) values for the trace elements followed descending order of Hg > Cu > Zn > Fe > Mn > Cr. Lagoon crab showed the highest total hazard index among the organisms with 44.80 %, while the least was observed in mantis shrimp with 13.30 %. It is concluded that, as far as human health is concerned, the mean elemental levels in the muscle tissues of the organisms examined pose no danger (THQ < 1).

Keyword: crab; shrimp; estimated daily intake; hazard index; food safety.

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Introduction

Crustaceans are invertebrates with segmented bodies, protected by chitinous shells and include barnacles, crab, crayfish, krill, lobster, prawn, shrimp, and woodlice (Moruf, 2020). The production of crustaceans typically farmed in coastal aquaculture is dominated by marine shrimp, which is an important source of foreign-exchange earnings for a number of developing countries in Asia and Latin America (Food and Agriculture Organization of the United Nations [FAO], 2020). Dieticians have recommended the need to consume aquatic crustaceans regularly because these edibles contain various high quality proteins, minerals, essential amino acids, and fatty acids such as omega^{-3} that lowers risk of various kinds of cancer, type 2 diabetes, and heart attack (Guérin et al., 2011; Moruf, Ogunbambo, & Moruf, 2020). However, invertebrates accumulate trace elements in their tissues whether or not these elements are essential to their metabolism. Different invertebrates accumulate different trace elements to different degrees and accumulated concentrations vary greatly at tissue, organ and body levels (Wang, Xu, Sun, Liu, & Li, 2013). To take one taxon as an example, tissue and body concentrations of trace elements vary greatly in crustaceans, even in the absence of anthropogenic input of trace metal contaminants (Wang et al., 2013).

The Lagos Lagoon is one of the major lagoon ecosystems along the Atlantic and Gulf coasts that foster valuable commercial and recreational fisheries. Due to the preponderance of anthropogenic activities around the coastlines, the water quality is deteriorating with adverse impacts on fisheries and coastal communities (Sogbanmu, Fatunsin, Echebiri, Otitoloju, & Olayinka, 2020). These anthropogenic activities include industrial effluent discharge, sawmilling activities, wood burning and transportation, petroleum tank farms, coastal solid waste dumpsites, shipping and port activities (Sogbanmu et al., 2020). Among animal-based foods, aquatic animals (crustacean inclusive) are in direct contact with and accumulate anthropogenic chemical contaminants to higher concentrations in their tissues (Rodríguez-Hernández et al., 2017).

In recent years, the presence and the amount of trace elements in food and their effects on human health are becoming more important. According to Gu et al. (2015), crustacean consumption has been reported as an important route of human exposure to a variety of chemical contaminants. Some trace metals (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) are essential elements for the organisms being constituents of several key enzymes and playing important roles in various oxidation-reduction reactions (FAO, 2005). However, an excess amount of these elements may produce cellular and tissues damage (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Others metals, such as, Hg, Pb, Cd, As have no established biological functions and are considered as non-essential and potentially toxic at relatively low concentrations (Bonsignore et al., 2018).

With the exception of occupational exposure, fish/shellfish are acknowledged to be the single largest source of trace elements for man. According to Moruf and Durojaiye (2020), concerns have been raised concerning health problems associated with the consumption of shellfish due to the presence of certain trace elements in quantities exceeding the maximum permissible limit. Hence, it is important to investigate the levels of trace elements in these organisms to assess whether the concentration is within the permissible level and will not pose any hazard to the consumers (Palaniappan & Karthikeyan, 2009; Afolayan, Moruf, & Lawal-Are, 2020). Considering the above facts, the present study was undertaken to estimate the health risks of trace elements, such as Manganese (Mn), Iron (Fe), Zinc (Zn), Copper (Cu), Mercury (Hg) and Chromium (Cr) via consumption of lagoon crab (*Callinectes amnicola* De Rochecbrune, 1883), marine crab (*Portunus validus* Herklots, 1851), pink shrimp (*Farfanpenaeus notialis* Pérez Farfante, 1967), and mantis shrimp (*Squilla aculeata calmani* Holthuis, 1959) to the general public in the above district by using the target hazard quotient (THQ) concept. These crustacean species were selected because they are highly consumed locally, regionally, nationally or globally (Guérin, et al., 2011; Jumbo, Wegwu, Belonwu, & Okerenta, 2015; Bonsignore et al., 2018). This study will provide observation regarding the safety levels of crustaceans to consumers.

Material and methods

Sample collection

Samples of fresh aquatic crustacean species; Lagoon crab, marine crab, pink shrimp and mantis shrimp were obtained from point of sales with source from domestic waste contaminated sites (6°31′ 44.35″N, 3°24′ 00.28″E) of the Lagos Lagoon in Nigeria (Figure 1).

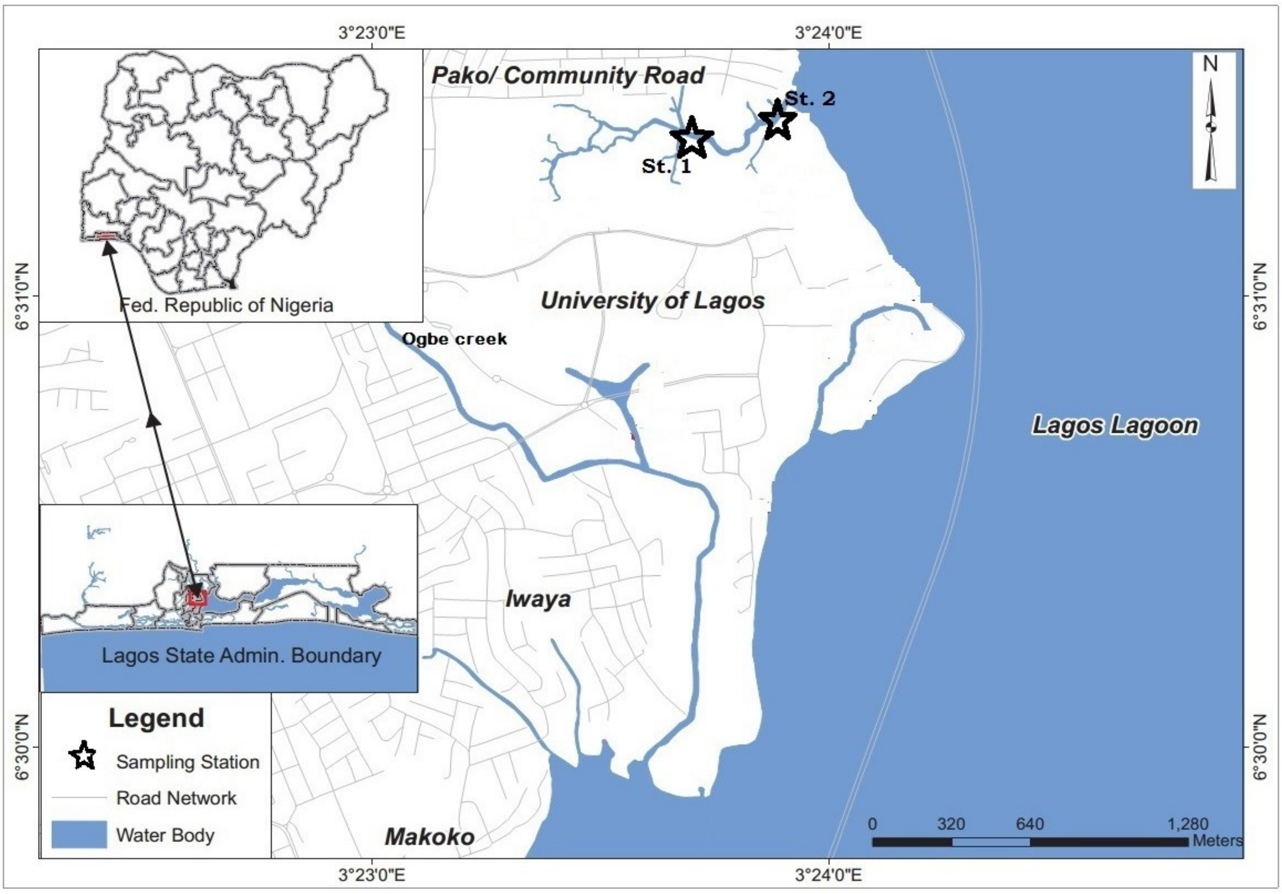

Figure 1: Map showing the Lagos Lagoon with the sampling sites.

Six samples per species were collected between October 2019 and March 2020, totaling 144 specimens. After the identification according to FAO guide (Schneider, 1990), samples were taken to laboratory in cold chain, 0.5 g muscle tissues were dissected, and kept at -4 °C until analysis. The length and weight of experimental materials used are given in Table 1.

Table 1. Sizes of sampled crustacean species highly consumed in Lagos, Nigeria (October 2019 - March 2020).

	Lagoon Crab	Marine Crab	Pink Shrimp	Mantis Shrimp
Length (cm)	9.06±0.50	14.60±0.30	8.15±0.05	10.03±0.50
Weight (g)	55.11±0.04	90.43±0.52	23.7±1.00	30.72±0.31

Keys: Mean ± standard error

Determination of trace elements

Tissue samples were analyzed for manganese, iron, copper, zinc, mercury and chromium using the acid digestion method described by Turkmen and Ciminli (2007). Specimens were transferred to Petri dishes and oven dried at 150 °C for 72 hours and brought to a constant weight. After determining the dry weights, they were transferred to experimental tubes and 2 mL nitric acid (HNO_3, % 65, S.G.: 1.40, Merck®). One (1) mL perchloric acid ($HClO_4$, % 60, S.G.: 1.53, Merck®) was added on to each sample, wet burned at 120 °C for 8 hours. Samples were then transferred to polyethylene tubes and their volumes were made up to 10 mL with deionized water (Korkmaz, Ay, Çolakfakıoğlu, & Erdem, 2019). Samples were passed through a 0.45 µm membrane filter before analysis. Metal levels in tissues were determined using an ICP-MS (Agilent 7500ce, Octopole Reaction System, Agilent Technologies, Japan) on three replicates. Metal content of the tissues were calculated on dry weight basis (mg kg^{-1} d.w.) and converted to wet weight (mg kg^{-1} w.w.) taking the water content of each tissue into account (El-Moselhy, Othman, El-Azem, & El-Metwally, 2014).

Health risk assessment

Health risk estimates were based on the data from trace metal analysis and Environmental Protection Agency (EPA, 2005) guidelines. To assess the health risk of the consumers due to trace element intake from the aquatic crustaceans, the estimated daily intake (EDI), target hazard quotient (THQ) and target hazard index (THI) were calculated from equations 1, 2, and 3 respectively while making the following assumptions:

The hypothetical body weight for adult Nigrian was 70 kg (Agwu, Okoye, Okeji, & Clifford, 2018).

The bioavailability factor is practically 1 (100%) following an oral administration. The maximum absorption rate is 100% because human exposure is usually by dermal/oral absorption.

Estimated daily intake: The exposure dose caused by ingestion of crustacean was calculated using the method proposed by Agency for Toxic Substances and Diseases Registry (ATSDR, 2004):

$$Estimated\ Daily\ Intake, EDI(mg/kg/day) = \frac{\text{C x CR x AFx EF}}{BW} \quad (1)$$

Where:

C = Concentration of the contaminant in the exposure pathway (mg kg^{-1}) of food

CR= Contact Rate; Nigeria crustacean taken day^{-1}, 0.0366 Kg day^{-1} =13.359 kg y^{-1} (Agwu et al., 2018)

AF= Bioavailability factor (100%)

EF = Exposure Factor= 1

BW = Body weight (70kg)

Target Hazard Quotient: The target hazard quotient (THQ) is used to quantify the amount of trace element taken in through ingestion. The target hazard quotient is the average daily dose divided by reference dose, calculated based on the formula by Wang, Sato, Xing, and Tao (2005). If THQ is less than 1, there is no obvious risk from the substance over a lifetime exposure, while if THQ is higher than 1, the toxicant may produce an adverse effect. The higher the THQ value, the higher the probability of the hazard risk on human body.

$$Target\ Hazard\ Quotient, THQ = \frac{\text{EDI}}{\text{RfD}} \quad (2)$$

Where:

EDI= Estimated Daily Intake,

RfD = the oral reference dose (mg $kg^{-1}day^{-1}$), For the risk assessment of multiple trace elements in the crustacean, a total hazard index (THI) was employed by summing all the calculated THQ_i values of trace elements as described below:

$$THI = \sum_{i=1}^{n} \text{THQ} \quad (3)$$

Where THQ_i is the target hazard quotient of an individual element, THI is the total hazard index for all the 6 trace elements in the present study, hence n is 6.

Statistical analysis

With the aid of SPSS statistical software version 22, mean with standard deviation were derived by subjecting data to descriptive analysis while Duncan Multiple Test Range (DMTR) was used to determine critical values for comparisons between means at a significant level of $p < 0.05$.

Results and discussion

Level of trace elements in the crustacean species

Crustaceans bioaccumulate trace elements in minute amounts over time, and this contamination is then concentrated higher up the food chain. The results obtained from the sampled crustaceans for Mn, Fe, Zn, Cu, Hg and Cr concentrations are shown in Table 2. In the samples, Zn (in marine crab) accumulated the most, followed by Fe (in marine crab), Cu (in lagoon crab), Mn (in lagoon crab), Cr (in lagoon crab) and Hg (in lagoon crab). Comparing crustaceans, crab had the highest mean contents of most trace elements. According to Raknuzzaman et al. (2016), crab is a typical benthic organism that resides above or in the sediment that might be good indicators reflecting the contamination levels in surface sediment. Unlike shrimp tissue, crab legs are often buried in surficial sediments and might adsorb metals from sediment more easily. Thus, it is more susceptible to sediment and is expected to possess high metal levels (Zhao et al., 2012). Besides, crab has capability to accumulate more trace elements by the hepatopancreas, one of the most vital organs that play important roles in metal detoxification (Liu, Liao, & Shou 2018). Moreover, crab is known as a scavenger that tends to feed on detritus, which is the most contributing factor to the high pollution in crabs (Leung et al., 2014). Unlike some other benthic organism, crabs often bury themselves in the sediments, making them closer to sediments and thus expose to higher concentration and more metal species (Zhao et al., 2012). Accumulation of trace elements in tissues of other crustaceans has been reported from the region (Lawal-Are, Adekugbe, & Odusoga, 2018).

In the present study, muscle was particularly selected for trace elemental analysis because it is the only edible tissue and thus concentration of toxicants in it was of concern. Statistical analysis showed that mg kg^{-1} concentrations of Mn (0.03±0.00 in lagoon crab), Fe (0.072±0.01 in mantis shrimp), Cu (0.344±0.01 in lagoon crab) and Zn (0.073±0.00 in mantis shrimp) were significantly different ($p < 0.05$) from their corresponding values in other examined crustaceans. The mean values of Cr and Hg were not significantly different across the samples ($p > 0.05$).

Table 2. Trace element in highly consumed crustacean species in Lagos, Nigeria

Metal	Lagoon Crab (mg kg^{-1})	Marine Crab (mg kg^{-1})	Pink Shrimp (mg kg^{-1})	Mantis Shrimp (mg kg^{-1})
Manganese	0.03±0.00a (0.024-0.034)	0.004±0.00b (0.003-0.005)	0.006±0.00b (0.002-0.009)	0.003±0.00b (0.002-0.005)
Iron	0.311±0.04a (0.239-0.351)	0.423±0.02a (0.402-0.455)	0.400±0.00a (0.392-0.409)	0.072±0.01b (0.06-0.095)
Copper	0.344±0.01a (0.333-0.354)	0.028±0.01b (0.016-0.051)	0.020±0.00b (0.011-0.027)	0.016±0.00b (0.012-0.020)
Zinc	0.272±0.02a (0.232-0.302)	0.433±0.00a (0.432-0.435)	0.369±0.04a (0.292-0.424)	0.073±0.00b (0.069-0.078)
Mercury	0.003±0.00a (0.002-0.004)	0.002±0.00a (0.001-0.003)	0.002±0.00a (0.002-0.004)	0.002±0.00a (0.001-0.003)
Chromium	0.007±0.00a (0.006-0.008)	0.007±0.00a (0.006-0.007)	0.006±0.00a (0.002-0.009)	0.006±0.00a (0.005-0.008)

Keys: Mean ± standard error, Range in bracket, Values with different superscripts across row are significantly different at ($p < 0.05$).

Manganese

Daily intake of low doses of Mn is necessary for the normal development and growth in humans (Rajeshkumar & Li, 2018). US EPA reported that oral Reference Dose (RfD) of Mn is 140 μg kg^{-1} day^{-1} and that doses above this value might cause health problems (EPA, 2005). Mean Mn level in the present work is comparable with the results of the studies carried out in the coasts of Bangladesh, Takway Bay and Ogoniland on the levels of Mn in muscle tissues of various crustacean species ranging between 14.80– 15.20 mg kg^{-1} (Raknuzzaman et al., 2016), 0.76 - 13.57 mg kg^{-1} (Lawal-Are & Babaranti, 2014) and 0.12±0.021 to 0.23±0.006 mg kg^{-1} (Jumbo et al. 2015).

Iron

World Health Organization (WHO) reported that provisional tolerable weekly intake limit (PTWI) for iron is about 5600 μg $kg^{-1}week^{-1}$ and intake above this value cause health problems in humans (FAO/WHO, 2004). The concentration of Fe in the present study is similar to what was previously reported for crustacean species in different parts of Nigeria. Mean levels of Fe in *Portunus validus, Farfantepenaeus noitialis, Callinestes amnicola* and *Macrobrachium macrobrachion* in Lagos were reported as 0.661±0.01, 0.597±0.1, 0.329±0.01, and 0.451±0.1 mg kg^{-1} respectively (Lawal-Are et al., 2018) and mean value of 9.73±1.30 mg kg^{-1} in *Macrobrachium rosenbergi* of Niger River (Nsofor et al., 2014).

Copper

Crustaceans store copper in tissues, a structural part in synthesizing blood pigment hemocyanin for gas exchange, which may be associated with high levels of copper, found in muscle tissues (Korkmaz et al., 2019). It was reported that the PTWI value of copper is 3500 μg kg^{-1} $week^{-1}$ (FAO/WHO 2004) above which result in liver and kidney damages in humans (Ali, Khan, & Ilahi, 2019). The highest level of copper (0.344±0.01 mg kg^{-1}) in this study was determined in muscle tissue of lagoon crab while the lowest (0.016±0.00 mg kg^{-1}) in mantis shrimp. Squid, cuttlefish and prawn species marketed in France was reported to have mean copper levels of 2.56, 2.57 and 9.22 mg kg^{-1} (Guerin et al., 2011). Moruf and Akinjogunla (2019) reported the mean Cu level of 1.05±0.09 mg kg^{-1} in *Farfantepenaeus notialis* that seems to be higher than the Cu levels measured in muscle tissue of the crustacean species under the present study.

Zinc

Oral reference concentration of Zn was given as 2100 μg kg^{-1} $week^{-1}$ (EPA, 2005) and reproductive and developmental disorders result in Zn intake over this level (Rajeshkumar & Li, 2018). In a similar study, mean Zn levels were reported to vary between 0.35 -1.24 mg kg^{-1} in *Tympanotonus fuscatus* var *radula* (Moruf & Akinjogunla, 2019) being about the same levels found in the muscle tissues of the crustacean species under the present study.

Mercury

Mercury is a non-essential heavy metal and cannot be excreted easily. It could be retained in the tissues for long periods resulting in behavioural and cognitive changes, neurological impairment and lesions (Authman, Zaki, Khallaf, & Abbas, 2015). The minimum and maximum mercury contents were found as 0.002±0.00 mg kg^{-1} in shrimps and 0.003±0.00 mg kg^{-1} in lagoon crab respectively. The European Commission Regulation stated permitted mercury concentration of 0.50 mg kg^{-1}, which was higher than the values found in the sampled crustacean species.

Chromium

Chromium depending on the valent state can be beneficial or harmful; the hexavalent state of chromium is harmful. The most widespread human effect is chromium allergy caused by exposure to chromium (especially Cr (VI) compounds), and they are assumed to cause cancer (Wilbur, 2000). The species of crab had higher Cr mean values (0.007 mg kg^{-1}) than the shrimps (0.006 mg kg^{-1}). However, the chromium levels obtained from this study are higher than that of Lawal-Are et al. (2018) where they reported a mean value of 0.002 mg kg^{-1} chromium found in imported and local crustacean species in Nigeria.

Human health risk assessment

Estimated Daily Intake of trace element in the species of crustacean

The Estimated Daily Intake (EDI) of trace elements through the consumption of four crustacean species by inhabitants of Lagos and the environs is given in Table 3. The result (mg $person^{-1}day^{-1}$) revealed that Hg (0.000001) in marine crab contributed the lowest daily intake while Zn (0.000226) in lagoon crab contributed the highest daily intake, which agreed well with the earlier result (Lawal-Are et al., 2018; Korkmaz et al., 2019). The Reference Dose (RfD) represents an estimate of the daily exposure to which the human population may be continually exposed over a lifetime without a considerable risk of deleterious effects. According to Raknuzzaman et al. (2016), the RfD represents an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. It is useful as a reference point from which to gauge the potential effects of the chemical at other doses. Usually, doses less than the RfD are not likely to be associated with adverse health risks and are therefore less likely to be of regulatory concern. As the magnitude of the exposures exceeding the RfD increase, the probability of adverse effects in a human population increases. However, it should not be categorically concluded that all doses below the RfD are "acceptable" (or will be risk free) and that all doses in excess of the RfD are "unacceptable" (or will result in adverse effects) (US EPA 2008). There are several approaches of human exposure to trace elements such as breathing and dermal exposure. However, food consumption is often regarded as one of the most important approaches.

In the present study, the EDI was calculated by considering that a 70 kg person consumes 0.0366 Kg per day. It is revealed that the EDI values for the examined crustacean samples were within the recommended values and indicated no risk to people's health associated with the intake of trace elements through the consumption of the selected crustacean samples.

Table 3. Estimated Daily Intake (mg person^{-1}day^{-1}) of trace elements in highly consumed crustacean species in Lagos, Nigeria.

	Manganese	Iron	Copper	Zinc	Mercury	Chromium
Lagoon Crab	0.000016	0.000163	0.000180	0.000226	0.000002	0.000004
Marine Crab	0.000002	0.000221	0.000015	0.000142	0.000001	0.000004
Pink Shrimp	0.000003	0.000209	0.000010	0.000193	0.000002	0.000003
Mantis Shrimp	0.000002	0.000038	0.000008	0.000038	0.000001	0.000003
FAO/WHO (2004) Reference Dose	0.14	0.7	0.04	0.3	0.0005	1.5

Target Hazard Index of crustacean species

Target hazard quotient (THQ) of individual trace element through crustacean consumption by average Nigeria adults are presented in Table 4 while Target hazard index (THI) for all the 6 trace elements is shown in Figure 1.

Table 4. Target hazard quotient of trace element in highly consumed crustacean species in Lagos, Nigeria

	Manganese	Iron	Copper	Zinc	Mercury	Chromium
Lagoon Crab	0.000112	0.000232	0.004497	0.000755	0.003137	0.000002
Marine Crab	0.000015	0.000316	0.000366	0.000474	0.002091	0.000002
Pink Shrimp	0.000022	0.000299	0.000261	0.000643	0.003137	0.000002
Mantis Shrimp	0.000011	0.000054	0.000209	0.000127	0.002091	0.000002

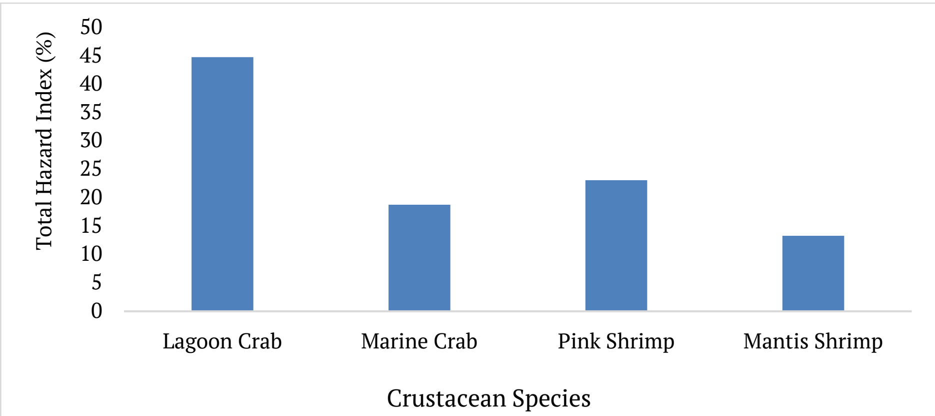

Figure 1. Total hazard index in highly consumed crustacean species in Nigeria.

Table 4 indicated that the THQ value of each metal was less than 1, suggesting that people would not experience significant health risks if they only take individual trace element through the consumption of examined crustacean species. The THQ values for the targeted trace element followed the descending order of Cu > Hg > Zn > Fe > Mn > Cr, which agreed well with the earlier report (Lawal-Are et al., 2018). Generally, Cu and Zn, which are important nutrients for humans, are considered a much lower health risk to humans than Pb, Cd, and As (Zhou et al., 2016). Higher THQ for Cu, Zn and Fe were reported by Korkmaz et al. (2019) in edible crustacean and mollusc species marketed in Mersin.

In this study, the major risk contributor (Figure 1) in terms of organism was lagoon crab with 44.80%, followed by pink shrimp (237.10%), marine crab (18.80%) and mantis shrimp (13.30%) which agreed well with the earlier report (Lawal-Are et al., 2018; Korkmaz et al., 2019). For Lagos populace, food consumption, air pollution, drinking water are the important pathways for human exposure to toxic metals (Njoku, Rumide, Akinola, Adesuyi, & Jolaoso, 2016). Consequently, the potential health risks for the residents were actually higher than the results from this study.

Conclusion

This study provide baseline information on the concentrations of some trace elements in highly consumed crustaceans in coastal areas of Nigeria. The mean trace element levels in muscle tissues of the crustacean species do not pose any threat as far as human health is concerned. However, crabs showed the highest total hazard index among the organisms while the least was observed in the shrimps. It can be recommended that trace elemental analysis should be carried out as frequent as possible in edible parts of aquatic organisms in order to create consumption advisory for consumers against any potential health risks.

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