Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(1): 2923 – 2950 (2025)
www.ejabf.journals.ekb.eg

Bioaccumulation and Human Health Risk Implications of Heavy Metals in the Swimming Blue Crab (*Callinectes Amnicola*) from Calabar and Great Kwa Rivers, Nigeria

Udiba Udiba U. [*1], Nta Abo I.[1], Ekwu Alice O.[2], Ama John[1], Ekpenyong Joseph F., Beshel Solomon B., Ogbin Innocent M.[1], Akpan Ekom R.[3]

[1]Dept of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria
[2]Dept of Fisheries and Aquatic Environmental Management, University of Uyo, Uyo, Nigeria
[3]Institute of Oceanography, University of Calabar, Calabar, Nigeria

[*]Corresponding Author: udiba.udiba@yahoo.com; udibaudiba@unical.edu.ng

ARTICLE INFO

Article History:
Received: June 22, 2024
Accepted: Jan. 26, 2025
Online: Feb. 26, 2025

Keywords:
Toxic metals,
Callinectes Amnicola,
Bioaccumulation,
Health implications,
Calabar River,
Great Kwa rivers

ABSTRACT

The bioaccumulation potential and human health risk implications of heavy metals in *Callinectes Amnicola* from Calabar and Great Kwa rivers were addressed between October 2021 and August 2022 using Atomic Absorption Spectrophotometer (Shemadzu, model 6800, Japan) after wet digestion. Mean lead, cadmium, mercury and arsenic concentrations (mg/kg) in sediment were determined with ranges of 5.826±0.99-10.541±0.59, 0.337±0.03-0.887±0.08, 0.037±0.04-0.079±0.01, 0.291±0.12-0.640±0.09, respectively. Metal concentrations were below US-EPA sediment quality guidelines. Total lead, cadmium, mercury and arsenic concentrations (mg/kg) in *Callinectes amnicola* ranged from 0.511-0.899, 0.317-0.612, 0.001- 0.003 and 0.012-0.089, respectively. Mean lead and cadmium content of *C. amnicola* exceeded FAO/WHO maximum levels (ML). Metal contents of crab followed the same trend as sediment metal concentrations. The strong positive correlations observed between each metal in sediment and in crab, at 99% confidence level, suggests sensitivity of the organism to changing loads of studied metals, hence its suitability as bio-monitors. Bioaccumulation factor (BAF) of cadmium ranging from 0.627 to 1.015 indicates that crabs are accumulating cadmium at moderate to high levels. The average estimated daily intake (EDI) for lead, cadmium and mercury were above their recommended daily intake (RDI) but lower than the upper tolerable intake. The average target hazard quotient (THQ) computed for the metals were less than unity, but the hazard index for Calabar River was above unity. Incremental Lifetime Cancer Risk (ILCR) for cadmium and mercury exceeded the carcinogens' standard tolerable regulatory risk (10^{-4}). Consumption of *C. amnicola* poses both carcinogenic and non-carcinogenic risk. Continued monitoring is recommended to ensure environmental and human health protection.

INTRODUCTION

The Niger Delta region of Nigeria has experienced widespread contamination of its land and water resources due to the combined effects of intense pressure from onshore and offshore oil exploration and exploitation activities, as well as the accompanying developments **(Ayuba, 2012; Chinedu & Chukwuemeka, 2018; Udiba *et al*., 2022)**.

The area was listed as one of the world's top five most contaminated ecosystems **(Ayuba, 2012)**. Water makes up more than half of the Delta which is home to an enormous network of rivers, tributaries, streams, and estuaries that support a varied mangrove swamp ecology with large mudflats that act as sink for these contaminants. Although chemical analysis of environmental matrix elements like water and sediment provides the most direct means of determining the level of pollution in the environment, it is not as effective in revealing the cumulative effects and potential toxicity of such pollution on organisms and ecosystems as the use of the biotic components of an ecosystem to assess periodic changes in the environmental quality of the ecosystem **(Zhuo *et al*., 2008; Lutgen *et al*., 2020; Tiwari, 2020; Nkopuyo & Everard, 2021)**.

Biomonitoring offers an interesting tool for assessing metal pollution in aquatic environments. It is science that uses sampling and analysis of the tissues and fluids of the organisms that inhabit an ecosystem to infer the ecological state of the ecosystem, including human exposure to natural and artificial substances **(Zhuo *et al*., 2008)**. This method makes use of the percepective dwelling with substances that have infiltrated the organisms leaving behind markers that would indicate their exposure. The chemical itself could serve as the marker. It might also be a by-product of the chemical's breakdown or a consequence of biological alterations brought about by the substance's effect on a particular organism **(Kamrin, 2004; Udiba, *et al*., 2014)**. The method most frequently employed in biomonitoring is bioaccumulation, and is defined as the degree to which an organism takes up and retain contaminants from all relevant exposure routes **(Kamrin, 2004; Udiba *et al*., 2014)**. Biomonitoring can therefore be used to determine the amount of natural or manufactured chemicals that are currently or have previously been present in the environment. It is particularly effective at identifying contaminants that persist in the ecosystem. Heavy metals and several synthetic organic compounds, including furans, Dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), poly aromatic hydrocarbons (PAHs), and dioxins are common examples of persistent environmental contaminants.

Metals are one class of environmental pollutants that are of special concern due to their widespread presence, inability to biodegrade, potential for toxicity, and ability to accumulate in aquatic environments. While some of the metals are needed at trace levels, others do not have any bio-significance and are toxic even at minute concentrations. The levels of heavy metals in Nigerian coastal waters, especially in the Niger Delta region, are quite concerning **(Adekola & Mitchell, 2011; Ewa *et al*., 2013; Ephraim & Ajayi, 2015; Ubiogoro & Adeyemo, 2017; Chijioke *et al*., 2018; Ekpo *et al*., 2021)**. This means that regular surface water, sediment and aquatic resource sampling and analysis is necessary to monitor the pollution trend and safety of the marine ecosystems. This study was designed to assess bioaccumulation of lead, cadmium, mercury and arsenic in the swimming blue crab (*Callinectes amnicola*) obtained from the lower reaches of Calabar and Great kwa rivers, evaluate suitability of the organism as a bioindicator of the metals and the potential human risk health due to its consumption.

Fish, bivalves, crayfish, crabs, prawns, and other marine bio-diversities have heavy metal accumulations **(Anyanwu *et al*., 2023)**. Consequently, the risk posed to human health via consuming heavy metals through the contaminated sea foods has increased. *C. amnicola* is one of the Portunidae family's most frequently consumed species in the Niger Delta **(Bakker *et al*., 2016)**. They are native to the Niger Delta region and are typically found along the coasts of tropical, subtropical, and temperate countries. *C. amnicola* is a valuable and affordable source of animal protein, and many people rely on this species for a living **(Uwa *et al*., 2018; Ihumwoa *et al*., 2022)**. Bioactive nutraceuticals are abundant in *C. amnicola*. Nutraceuticals are referred to as naturally occurring substances found in foods, which have been shown to be beneficial in preventing or treating diseases in humans or in enhancing physiological performance above and beyond the effects of adequate nutrition in a way that improves health and wellbeing and lowers the risk of disease **(Ramya & Patel, 2019)**. They are regarded as non-specific biological therapies that are intended to improve overall health and manage symptoms and cancerous processes **(Mestrovic, 2018)**. Crab's meat is rich in many nutrients and is an excellent source of high quality proteins, vitamins, minerals and many therapeutic properties **(Rana, 2018; Uwa *et al*., 2018; Nanda *et al*., 2021)**. The meat contains selenium and foliate that fight off free radicals in body and protect against chronic diseases, moreover it contains calcium and phosphorus that are essential for bone health, as well as zinc and iron that provide special benefits suited to the different requirements of men and women **(Rana, 2018; Nanda *et al*., 2021)**. The synthesis of testosterone, which is essential for the health of male reproductive systems and general well-being, depends critically on zinc. Iron helps the body produce more red blood cells. It is an essential mineral that aids in preventing anemia for women, especially during menstruation. Crab contains chromium, which facilitates the action of insulin, and thus preventing the onset of diabetes. It helps increase the good cholesterol in the body which aids in minimizing the risk of heart attacks, strokes, circulatory and coronary diseases **(Rana, 2018)**. According to **Vijavalingam and Rajesh, (2020)**, only about 40% of crab materials are used for human food, with the remaining 60% being discarded as waste. The wastes are a rich source of minerals and high in protein, making them useful as feed supplements in chicken, fish and other livestock feed

Some metals have the potential to transform into highly toxic and persistent compounds that can accumulate in living organisms and become increasingly more toxic at successively higher levels in the food chain, endangering human health **(Zhou *et al*., 2008)**. Metals including Fe, Cd, Pb, Zn, and Cu have been reported in *C. amnicola* obtained from a River State waterfront **(Davies, 2021)**. *C. amnicola* was collected from a section of the Lagos lagoon, and samples of its gills and hepatopancreas revealed the presence of Cd, Pb, Zn, and Cu **(Ihunwoa *et al*., 2022)**. Significant amount of Pb, Cr, Cd, Ni, and Cu have been reported in liver, gills, and muscles of shellfishes in the Niger Delta **(Anyanwu *et al*., 2023)**. Assessment metals concentrations in shell fish around Niger Delta are available in literature, but evaluation of the potentials of *C. amnicola* from Calabar and Great Kwa rivers for biomonitoring, as well as human health implications for consumers are not available in literature, hence the study was conducted. Moreover, with the continuous activities of the oil and gas industry in the region, periodic monitoring of contaminant levels in the environmental media and biota is necessary to detect changing trends and to safeguard public health.

MATERIALS AND METHODS

1. Study area

The Niger Delta region of Nigeria covers a vast area of 75,000 square kilometers, encompassing nine states, including all the oil-producing states. Cross River State, with its capital in Calabar, is one of the states in this region. Calabar is situated between latitudes 4°.55' and 4°.58'N and longitudes 8°.15' and 8.26'E, and it had an estimated population of 579,000 in 2020 **(Populationstat, 2020; Udiba *et al*., 2020)**. The city is bounded by the Calabar River to the west and the Great Kwa River to the east, both of which discharge into the Cross River estuary, eventually flowing into the Atlantic Ocean at the Gulf of Guinea (Fig. 1) **(Udofia *et al*., 2016)**. These rivers originate from Oban Hill and form an intricate network of tributaries and creeks. The shoreline is characterized by dense vegetation, transitioning from freshwater swamp ecology to mangrove swamp ecology closer to the estuary mouth. The lower reaches of the rivers are tidal, and the region experiences a tropical climate. The river system, formed by the Cross River, Calabar River, Great Kwa River and other tributaries, covers an estimated area of 54,000 square kilometers (21,000 square meters). The mangrove creek system in both rivers serves as spawning grounds for shrimps, crabs, clams, and fish **(Udiba *et al*., 2020)**.

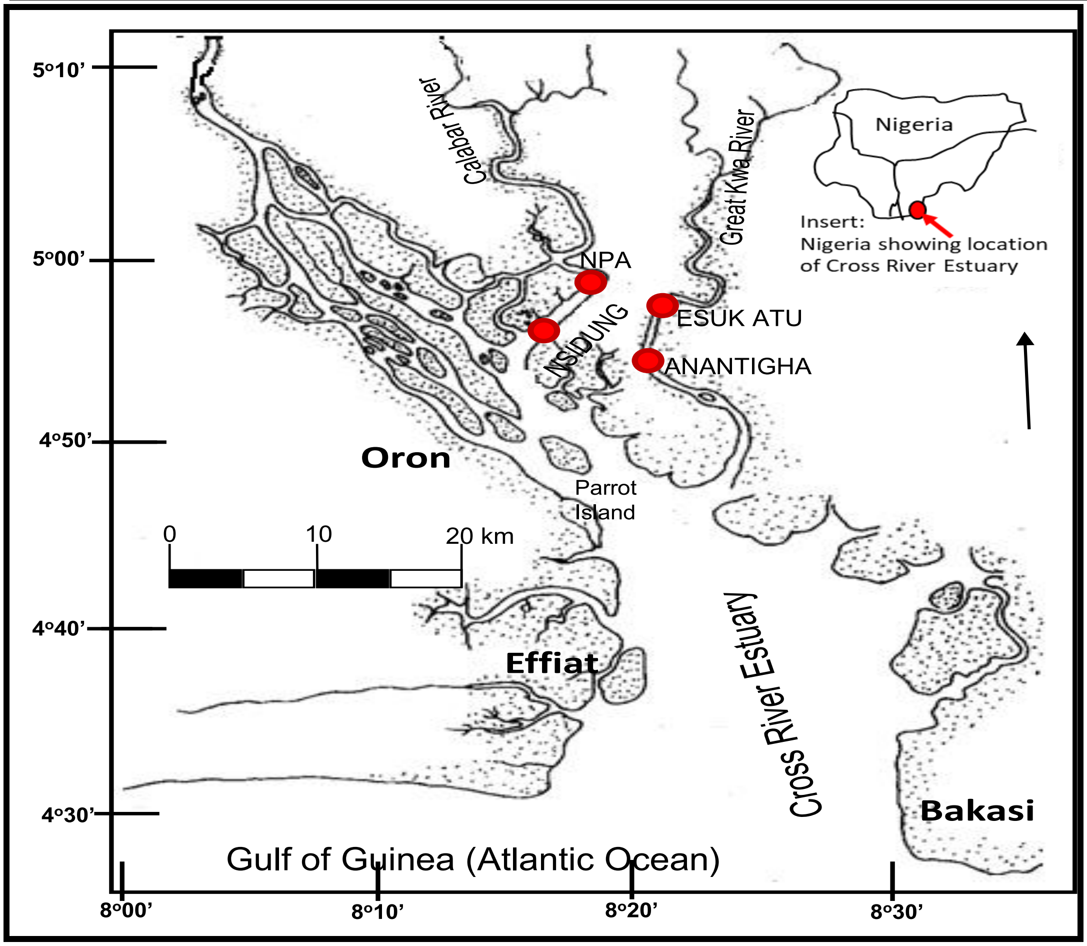

Fig. 1. Map of Cross River estuary showing Calabar River and Great Kwa River with the sampling points

2. Sample collection

The procedure for sample collection and preparation followed the guidelines outlined in **APHA (2005)**. Two sampling points were established at the lower reaches of each river (Esuk Atu and Obufa Esuk for Great Kwa River, and Nigerian Port Authority Jetty and Esuk Nsidung for Calabar River). Sediment samples were collected at each sampling point, using hand auger during ebb tide at a depth between 0-10cm, once a month for six months. Total of twenty four sediment samples (twelve from each River) were used for the study. Five blue crabs (*C. amnicola*) with length ranging from 8.9 - 10cm and weight of 30 - 40g were handpicked at the sampling site, killed and put in black polyethylene bags. A total of one hundred and twenty *C. amnicola* (sixty from each river) were used for the study. The collected samples were placed in coolers packed with ice blocks and transported immediately to LAB 249, Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria, where identification of *C. amnicola* and sample preparation were carried out. The crabs were identified using the identification guides of **Edmunds (1978)**, **Pennak (1978)** and **Durand and Lévêque (1980)**.

3. Sample preparation

The sediment samples were air-dried for five days, ground, and sieved with a 60-mesh sieve (0.3mm). One gram of well-mixed sieved sample was weighed into a 250mL beaker and was digested with a mixture of 20mL nitric acid, hydrofluoric acid, and perchloric acid in a 1:1:3 ratio on a hot plate. After evaporating to near dryness, 10mL of 2% nitric acid was added, filtered into a 50mL volumetric flask, and made up to the mark with distilled deionized water. The sediment digestion procedure was adapted from the method of **Martin (1996)**. The whole crabs from each sampling were oven dried at 50^{o}C for two hours, crushed into powder and mixed thoroughly. 1g of the well mixed sample was digested with perchloric acid and nitric acid mixture (ration 3: 1), and filtered into 50ml volumetric flask made up to the mark.

4. Sample analysis

The concentrations of heavy metals in the digested samples were determined using atomic absorption spectrophotometry (AAS), with a Shimadzu Atomic Absorption Spectrophotometer (model AAS-6800, Japan) at the National Research Institute for Chemical Technology (NARICT) in Zaria, Nigeria. The standard solutions were run at various concentrations to create the calibration curve. The concentration of the metals was then measured after the instrument was reset to zero by running the corresponding reagent blanks. For every determination, the means of three replicates were obtained.

5. Analytical quality assurance

Precautions were taken to avoid cross-contamination of samples. All reagents used, including HNO3 (Riedel-deHaen, Germany), HF (Sigma Aldrich, Germany) and HClO4 (British Drug House Chemicals Limited, England) were of analytical grade. Blank and combined standards were prepared and analyzed with each batch of samples to detect background contamination and to ensure analytical consistency. The accuracy of the analysis was evaluated by concurrently analyzing a standard reference material (Lichen coded, IAEA-336).

6. Statistical analysis

The results obtained were subjected to a statistical test of significance using the independent t-test to compare metal levels between the Calabar River and Great Kwa River and between the wet and dry seasons. Analysis of variance (ANOVA) was used to compare metals levels between the four sampling points. Probabilities less than 5% (P < 0.05) were considered statistically significant. Pearson product moment correlation coefficients was used to determine the association between metals levels in sediment and crab at $\alpha = 0.05$. The following correlation ratings were used to interpret the results: (-0.7) - (-1.0) = strongly negative, (-0.5) - (-0.7) = moderately negative, (-0.2) - (-0.5) = weakly negative, (+0.2) - (-0.2) = no association, (+0.2) - (+0.5) = weakly positive, (+0.5) - (+0.7) = moderately positive and (+0.7) - (+1.0) = strongly positive **(Al-Tamimi, 2018)**. All statistical analyses were performed using IBM SPSS version 23 for Windows.

The bioaccumulation factor (BAF)

Bioaccumulation factor (BAC) of heavy metals in crabs was determined using equation 5:

BAF = CE/(CS)…………………………………………………………… (1)

Where, CS is the heavy metal concentration in the soil and CE is the heavy metal concentration in crabs.

Evaluation of potential human risk

Estimated daily intake (EDI)

Estimated daily intake (EDI) of metals via consumption of crab in this study was evaluated following equation 3 **(Addo *et al*., 2013)**

$$EDI = \frac{EF \times ED \times FIR\ x\ Cm)}{BAW \times AT} \quad \text{…………………………………} (2)$$

Where, EF is the exposure frequency (365 days/year), and ED is the exposure duration (adopted from **Oguguah *et al*., 2012** as 54.5 years, the average life expectancy for a typical Nigerian adult), FIR stands for fish ingestion rate (adopted from **Oguguah *et al*., 2012**, as 0.02kg of fish per person per day. Cm is the concentration of metal in crabs' tissues (milligrams per kilogram). The average adult body weight (WAB) was taken as 60.7kg, and the average exposure time-age (AT) was calculated as EF x ED.

The fish ingestion rate (0.02kg/ person/ day) applies to fresh fish. To ensure consistency between the unit used for fish ingestion rate and measured concentration data, the concentration of metals measured in this study with reference to dry weight as recalculated to fresh weight based on the available information on the mean moisture content of crabs from the area. This was done following **US-EPA (2011)** using equation 2. The conversion of metal concentrations determined in dry weight to wet weight was done using moisture content percentage of 56.00 adopted from **Neji *et al*. (2019)**

$$Cww = Cdw[\frac{100-W}{100}] \quad \text{……………………………………………………..……} (3)$$

Where, W is the moisture content, Cdw is the dry weight concentration, and Cww is the wet weight concentration.

Target hazard quotient (THQ)

The target hazard quotient (THQ) was calculated in order to estimate the possible risk to human health associated with eating edible crab tissues using equation 4.

$$THQ = \frac{EF \times ED \times FIR \times Cm}{RfD \times WAB \times AT} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where, RfD (mg/kg body weight per day), is the oral reference dose for metal. RfD is an estimate of the daily oral exposure of the human population that has no deleterious effects over the course of a lifetime.

Where, RfD is the oral reference dose for metal (mg/kg body weight per day). The RfD value for Pb (0.0035mg/kg per day) was obtained from **ATSDR (2019)**. RfD values for Cd (0.001mg/ kg per day), Hg (0.0003mg/ kg per day), and As (0.0003 mg/kg per day) were obtained from the Integrated Risk Information System **(US-EPA, 2020)**.

Hazard index (HI)

As stated in equation 5, the target hazard quotients of the heavy metals under investigation were added up to create the hazard index **(Guerra *et al.*, 2012)**.

$$HI = \Sigma THQ = THQpb + THQ_{Cd} + THQ_{Hg} + THQ_{As} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Carcinogenic health risk assessment

Carcinogenic risk was calculated as the incremental chance that an individual will get cancer as a result of exposure to carcinogenic or potentially carcinogenic metals via consumption of *C. amnicola* from the study areas using Incremental Life Time Cancer Risk (ILCR). To evaluate the carcinogenic risk resulting from exposure to multiple carcinogenic metals, the Cumulative Cancer Risk (CCR) method was employed.

Incremental lifetime cancer risk (ILCR)

The incremental cancer risk resulting from consuming crab and being exposed to a particular cancer-causing metal was calculated in accordance with **Abba *et al*. (2020)** using equation 6.

$$ILCR_m = EDI_m \text{ x } CSF_m\text{-}oral \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Where, EDI_m is the estimated daily intake for the metal and CSF_m is the cancer slope factor-oral for the metal.

Cumulative cancer risk (CCR)

According to **Liu *et al*. (2013)**, the cumulative cancer risk (CCR) resulting from exposure to multiple cancer-causing metals via human consumption of crab is the total of an individual's metal incremental lifetime cancer risk and was calculated using equation 7.

$$CCR = \sum ILCR = ILCR_{Pb} + ILCR_{Cd} + ILCR_{Hg} + ILCR_{As} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(7)}$$

RESULTS

Analytical quality assurance

The accuracy and precision of the methods used for metal determination are validated by the results of the analysis of certified standard reference materials (Lichen coded IAEA-336) that was conducted concurrently with our samples. The analyzed values fall within the confidence interval of the certified reference values of the metals studied (Table 1).

Table 1. Results of the analysis of the reference material (Lichen IAEA - 336) in comparison with the certified reference values

Metals mg/kg	Pb	Cd	Hg	As	Cr
Analyzed value	4.8	0.105	0.19	0.63	1.00
Reference value	4.3-5.5	0.100-0.134	0.16-0.24	0.55-0.71	0.89-1.23

Concentration metals in sediments of Great Kwa River and Calabar River, Nigeria

Mean concentration (mg/kg) of the metals for dry and wet season were: 8.063±0.96 - 10.541±0.59 and 5.826±0.99 - 8.459±1.30, 0.378±0.02 - 0.887±0.08 and 0.337±0.03 - 0.824±0.05, 0.047±0.01 - 0.079±0.01 and 0.037±0.04 - 0.069±0.07 and, 0.309±0.11 - 0.640±0.09 and 0.291±0.12 - 0.546±0.07 for lead, cadmium, mercury and arsenic, respectively (Table 2). The concentrations of lead, cadmium, mercury and arsenic in Great Kwa River and Calabar River differed significantly ($P \leq 0.05$), with the Calabar River having much greater concentrations than the Great Kwa River in both wet and dry seasons. The two rivers did not exhibit significant seasonal fluctuations in metal concentrations, except for lead concentrations in the Calabar River during the dry season, which were significantly greater than the wet season.

Table 2. Mean concentration of metals in sediments of the Great Kwa and Calabar Rivers, Calabar, Nigeria

Metals (mg/kg)	Dry season				Wet season			
	Great Kwa River		Calabar River		Great Kwa River		Calabar River	
	Esuk Atu	Esuk Anantigha	NPA Jetty	Nsidung Beach	Esuk Atu	Esuk Anantigha	NPA Jetty	Nsidung Beach
Lead	6.844±0.72	8.063±0.96	9.608±0.48	10.541±0.59	5.826±0.99	6.483±1.05	7.896±0.72	8.459±1.30
Cadmium	0.378±0.02	0.511±0.11	0.527±0.16	0.887±0.08	0.337±0.03	0.448±0.12	0.499±0.14	0.824±0.05
Mercury	0.047±0.01	0.055±0.01	0.063±0.01	0.079±0.01	0.037±0.04	0.047±0.05	0.057±0.06	0.069±0.07
Arsenic	0.309±0.11	0.345±0.07	0.404±0.05	0.640±0.09	0.291±0.12	0.323±0.07	0.363±0.03	0.546±0.07

Concentration of metals in Blue Crab (*Callinectes amnicola)* from Great Kwa and labar rivers, Calabar, Nigeria

Table (3) shows that the ranges of metals concentrations (mg/kg) in the blue crab (*Callinectes amnicola)* for dry and wet seasons were: 0.543-0.899 and 0.511-0.847 for lead, 0.332-0.612 and 0.317-0.587 for cadmium, 0.001- 0.003 and 0.001-0.002 for mercury and, 0.023-0.089 and 0.012-0.073 for arsenic, respectively (Table 3). Metals concentration in crab from Calabar River were significantly ($P \leq 0.05$) higher than in the Great Kwa River (Fig. 2), with mercury displaying no significant difference being the only exception. There was no statistically significant ($P \geq 0.05$) seasonal variation in metals content of crabs tissue.

Table 3. Concentration of metals in *C. amnicola* from Great Kwa and Calabar rivers, Calabar, Nigeria

Metal (mg/kg)	Month	WET SEASON				DRY SEASON			
		ESUK ATU	ESUK ANANTIGHA	NPA JETTY	NSIDUNG BEACH	ESUK ATU	ESUK ANANTIGHA	NPA JETTY	NSIDUNG BEACH
Lead	**October**	0.543	0.675	0.765	0.773	0.555	0.758	0.839	0.847
	January	0.557	0.589	0.764	0.771	0.538	0.534	0.739	0.734
	March	0.565	0.767	0.878	0.899	0.511	0.545	0.714	0.726
	Mean±SD	0.555±0.01 [a]	0.677±0.07 [a]	0.802±0.05 [b]	0.814±0.06 [b]	0.535±0.02 [a]	0.612±0.10 [a]	0.764±0.05 [b]	0.769±0.06 [b]
	Range	0.543-0.899				0.511-0.847			
Cadmium	**October**	0.332	0.443	0.476	0.498	0.321	0.482	0.544	0.587
	January	0.348	0.457	0.561	0.586	0.328	0.425	0.521	0.553
	March	0.337	0.543	0.567	0.612	0.317	0.413	0.437	0.452
	Mean±SD	0.339±0.01 [a]	0.481±0.04 [b]	0.535±0.04 [b]	0.565±0.05 [b]	0.322±0.01 [a]	0.440±0.03 [b]	0.501±0.04 [b]	0.531±0.05 [b]
		0.332-0.612				0.317-0.587			
Mercury	**October**	0.001	0.001	0.002	0.003	0.001	0.001	0.002	0.002
	January	0.001	0.002	0.002	0.003	0.001	0.001	0.001	0.001
	March	0.001	0.001	0.002	0.003	0.001	0.001	0.001	0.001
	Mean±SD	0.001±0.00 [a]	0.001±0.00 [a]	0.002±0.00 [a]	0.003±0.00 [a]	0.001±0.00 [a]	0.001±0.00 [a]	0.001±0.00 [a]	0.001±0.00 [a]
	Range	0.001-0.003				0.001-0.002			
Arsenic	**October**	0.023	0.053	0.054	0.048	0.052	0.057	0.073	0.073
	January	0.058	0.078	0.079	0.087	0.042	0.048	0.064	0.065
	March	0.062	0.076	0.087	0.089	0.012	0.038	0.047	0.039
	Mean±SD	0.048±0.05 [a]	0.069±0.07 [a]	0.073±0.07 [a]	0.075±0.07 [a]	0.035±0.02 [a]	0.048±0.01 [a]	0.061±0.01 [a]	0.059±0.02 [a]
	Range	0.023-0.089				0.012-0.073			

Mean with different superscript across the row indicates significant ($P < 0.05$, ANOVA) difference in metal concentration.

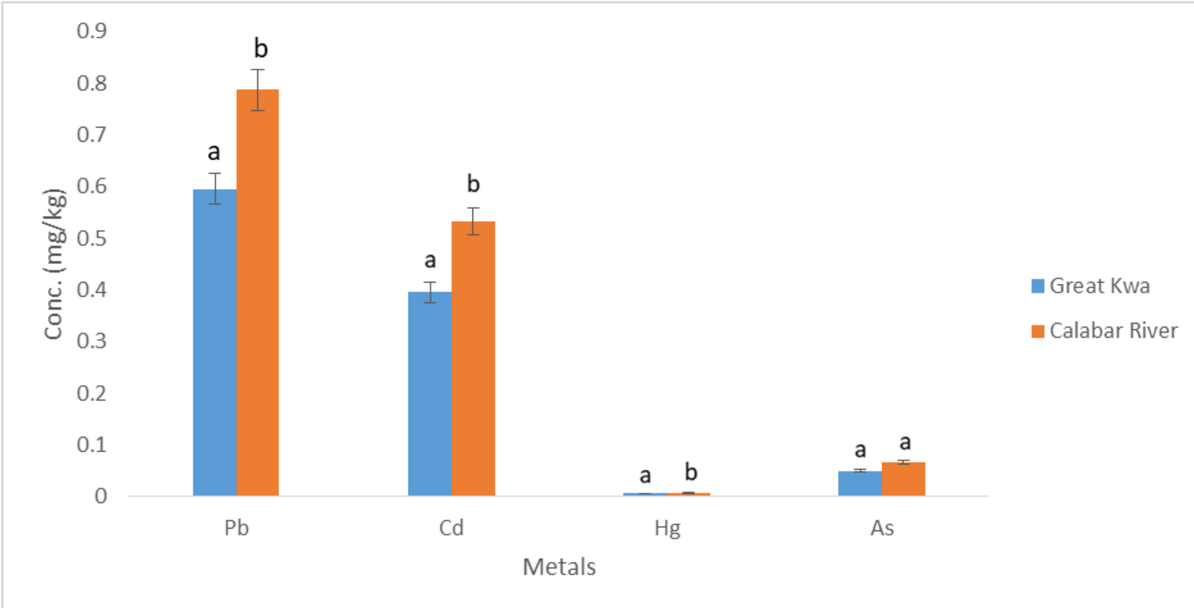

Fig. 2. Comparison of metals contents of *C. amnicola* from Great Kwa and Calabar rivers, Calabar, Nigeria

Relationship between concentration of metals in sediment and blue crab (*Callinectes amnicola)* from Great Kwa and Calabar rivers, Calabar, Nigeria

A strong positive correlations were observed between lead in sediment and lead in *C. amnicola* (r = 0.846), cadmium in sediment and *C. amnicola* (0.784), mercury in sediment and *C. amnicola* (0.734), and between arsenic in sediment and *C. amnicola* (0.726). The correlations were significant at 99% confidence level (Table 4)

Table 4. Relationship between concentration of metals in sediment and *C. amnicola* from Great Kwa and Calabar rivers, Calabar, Nigeria

Metal	Sediment against *C. amnicola*	Inference
Pb	0.846**	Strong positive relationship
Cd	0.784**	Strong positive relationship
Hg	0.734**	Strong positive relationship
As	0.726**	Strong positive relationship

Correlation is significant at the 0.01 level (two tail)

Bioaccumulation factor (BAF)

Bioaccumulation factor of the metals in *Callinectes amnicola* across the two rivers for both dry and wet seasons ranged from 0.073-0.097 for lead, 0.627-1.015 for cadmium, 0.018-0.027 mercury and, 0.120-0.200 arsenic (Table 5).

Table 5. Bioaccumulation factor (BAF) of metals in *C. amnicola* from Great Kwa and Calabar rivers, Calabar, Nigeria

Sample location	**Sampling point**	**Dry season**				**Wet season**			
		Pb	Cd	Hg	As	Pb	Cd	Hg	As
Great Kwa River	Esuk Atu	0.081	0.897	0.021	0.155	0.092	0.955	0.027	0.120
	Esuk Anantigha	0.084	0.941	0.018	0.200	0.094	0.982	0.021	0.149
	Average	**0.083**	**0.919**	**0.020**	**0.178**	**0.093**	**0.969**	**0.024**	**0.135**
Calabar River	NPA Jetty	0.080	1.015	0.032	0.181	0.097	1.002	0.018	0.168
	Nsidung Beach	0.073	0.627	0.038	0.117	0.091	0.644	0.014	0.108
	Average	**0.077**	**0.821**	**0.035**	**0.149**	**0.094**	**0.823**	**0.016**	**0.138**

Estimated daily intake

The estimated daily intake (mg/kg body weight (bw) per day) of the metals due to the consumption of *Callinectes amnicola* obtained from the two rivers for both dry and wet seasons ranged from 0.062-0.094 for lead, 0.037-0.065 for cadmium, 0.000012-0.00036 for mercury and, 0.004-0.0086 for arsenic, respectively (Table 6).

Table 6. Estimated daily intake (mg/kg body weight (bw) per day) of metals via consumption of *C. amnicola* from Great Kwa and Calabar rivers, Calabar, Nigeria

Sample location	Sampling point	Dry season				Wet season			
		Pb	Cd	Hg	As	Pb	Cd	Hg	As
Great Kwa River	Esuk Atu	0.064	0.039	0.000012	0.0056	0.062	0.037	0.000012	0.004
	Esuk Anantigha	0.078	0.039	0.000012	0.0079	0.071	0.051	0.000012	0.0056
	Average	**0.071**	**0.039**	**0.000012**	**0.00675**	**0.0665**	**0.044**	**0.000012**	**0.0048**
Calabar River	NPA Jetty	0.093	0.062	0.00023	0.0086	0.088	0.058	0.000012	0.0069
	Nsidung Beach	0.094	0.065	0.00036	0.0086	0.089	0.061	0.000012	0.0069
	Average	**0.0935**	**0.0635**	**0.000295**	**0.0086**	**0.0885**	**0.0595**	**0.000012**	**0.0069**
Recommended Dietary Intake		**0.00**	**0.00**	**0.00**	**0.5-1**	**0.00**	**0.00**	**0.00**	**0.5-1**
Upper Tolerable Limit		**0.240**	**0.064**	**1.6***	**1-3**	**0.240**	**0.064**	**1.6***	**1-3**

Target hazard quotient

The target hazard quotient of the metals due to the consumption of *Callinectes amnicola* obtained from the two rivers for both dry and wet seasons ranged from 0.176-0.265 for lead, 0.302-0.652 for cadmium, 0.004-0.008 for mercury and, 0.026-0.286 for arsenic respectively (Table 7).

Hazard index

The mean hazard index for dry and wet season were 0.778 and 0.8277 for the Great Kwa River and, 1.063 and 1.082 for the Calabar River (Table 8).

Table 7. Target hazard quotient (THQ)/hazard index (HI) of metals via consumption of *C. amnicola* from Great Kwa and Calabar rivers, Calabar, Nigeria

Sample location	Sampling point	Dry season					Wet season				
		Target Hazard Quotient				Hazard Index	Target Hazard Quotient				Hazard Index
		Pb	Cd	Hg	As		Pb	Cd	Hg	As	
Great Kwa River	Esuk Atu	0.183	0.302	0.004	0.187	**0.676**	0.176	0.372	0.004	0.137	**0.689**
	Esuk Anantigha	0.223	0.389	0.004	0.264	**0.88**	0.201	0.504	0.004	0.1187	**0.8277**
	Average	**0.203**	**0.346**	**0.004**	**0.226**	**0.778**	**0.189**	**0.438**	**0.004**	**0.128**	**0.758**
Calabar River	NPA Jetty	0.265	0.616	0.008	0.026	**0.915**	0.251	0.576	0.004	0.231	**1.062**
	Nsidung Beach	0.261	0.652	0.012	0.286	**1.211**	0.254	0.613	0.004	0.231	**1.102**
	Average	**0.263**	**0.634**	**0.01**	**0.156**	**1.063**	**0.253**	**0.595**	**0.004**	**0.231**	**1.082**

Incremental life time cancer risk (ILCR)

The average incremental lifetime cancer risk the metals due to the consumption of *Callinectes amnicola* obtained from the two rivers for both dry and wet seasons ranged from $5.3x10^{-4}$- $8.0\ x10^{-4}$ for lead, $5.6\ x10^{-1}$-$9.8\ x10^{-1}$ for cadmium, and, $6.0\ x10^{-3}$-$1.2\ x10^{-2}$, respectively (Table 8)

Cumulative cancer risk (CCR)

Cumulative cancer risk for Great Kwa River was $7.8x110^{-1}$ for dry season and $7.6x110^{-1}$ for wet season while that of the Calabar River was 1.063 for dry season and wet season.

Table 8. Incremental life time cancer risk (ILCR) and cumulative cancer risk (CCR) of metals via consumption of *C. amnicola* from Great Kwa and Calabar rivers, Calabar, Nigeria

Sample Location	Sampling point	Dry season					Wet season				
		ILCR				CCR	ILCR				CCR
		Pb	Cd	Hg	As		Pb	Cd	Hg	As	
Great Kwa River	Esuk Atu	0.00054	0.585	-	0.0084	**0.59394**	0.00053	0.555	-	0.006	**0.56153**
	Esuk Anantigha	0.00066	0.585	-	0.0119	**0.59756**	0.0006	0.765	-	0.0084	**0.774**
	Average	**0.0006**	**0.585**	**-**	**0.01015**	**0.59575**	**0.000565**	**0.66**	**-**	**0.0072**	**0.667765**
Calabar River	NPA Jetty	0.00079	0.93	-	0.0129	**0.94369**	0.00075	0.87	-	0.01	**0.88075**
	Nsidung Beach	0.0008	0.975	-	0.0129	**0.9887**	0.00076	0.915	-	0.01	**0.92576**
	Average	**0.000795**	**0.9525**	**-**	**0.0129**	**0.966195**	**0.000755**	**0.8925**	**-**	**0.01**	**0903255**

DISCUSSION

Discussion

Metal concentrations in surface sediments of Great Kwa River and Calabar River

Sediment quality of the Great Kwa and Calabar rivers was assessed using the United State Environmental Protection Agency's sediment quality recommendations **(US-EPA, 1999)**, and the pollution status for each river corresponds to not polluted with respect to the metals under study. The significantly higher metal concentrations in sediment measured for the Calabar River can be linked to the higher level of human activities in the Calabar River basin. The increase in sediment metal content toward the sea may be due to the continuous transportation of contaminated sediments by the rivers and their deposition in downstream locations, as observed by **Udiba *et al.* (2012)**. The two rivers did not exhibit significant seasonal variations in metal concentrations.

Metals concentration in crabs obtained from Great Kwa River and Calabar River

Fish are recognized as the most important single source of high-quality protein **(Anyanwu *et al*., 2023)**. Crayfish, periwinkles and crabs have the potential to lessen protein deficiency in the diet of the common man but the buildup of toxins in aquatic diversities, especially the benthic organisms, present an increase in the danger to human health via consumption of contaminated fishery resources. In order to evaluate the safety of crabs obtained from the lower reaches of the Great Kwa and Calabar rivers for human consumption, the concentrations of studied metals in the organism was first measured (Table 3), and the results were compared with international regulatory criteria.

The mean lead content in *C. amnicola* (Table 3) were above the Commission of European Communities maximum permissible limit of 0.5mg/ kg set for the metal in crustaceans **(EC, 2015)** and the Codex maximum level (ML) of 0.30mg/ kg **(FAO/WHO, 2015)** throughout the investigation. **Neji *et al*. (2019**) recorded similar mean lead levels (0.811±0.0493) in crabs obtained from Esierebom beach, Calabar, Nigeria. Mean concentrations ranging from 0.071±0.013-1.364±0.060 were reported for muscles, gills and hepatopancreas of crabs obtained from Woji Creek, Nigeria **(Ihunwoa *et al*., 2022)**. Lead is one subtle threat that has a considerable risk of permanent health damage **(Kathuria, 2020)**. It predominantly targets the liver, kidney, and central nervous systems. It interferes with the absorption of essential micronutrients due to its propensity to substitute most bivalent metals, including calcium, iron, and magnesium, and monovalent metals, like sodium **(Jaishankar *et al*., 2014)**. Lead toxicity affects the integrity of genetic materials and exhibits genotoxic, carcinogenic, and mutagenic effects **(Szymanski, 2014)**. It has also been linked to reduced cognitive development and intellectual ability in children.

The Commission of European Communities' maximum limits **(EC, 2015)** and the Codex maximum level (ML) for cadmium in crustaceans is 0.5mg/ kg **(FAO/WHO, 2015)**. Mean cadmium concentrations measured in *C. amnicola* obtained from Calabar River were also found to be above the permissible limits throughout the investigation. On the other hand, cadmium content of blue crabs obtained from the Great Kwa River were below the acceptable limit. Cadmium is a known cumulative poison with extreme toxicity. Measured concentrations of cadmium in *C. amnicola* tissues are therefore a serious cause for concern given its long biological half-life (20–30 years in humans), poor rate of elimination from the body, and proclivity for storage in soft tissues (liver and kidney) compounded by its teratogenicity, carcinogenicity, nephrotoxicity, endocrine disruption, and reproductive toxicity **(Jaishankar *et al*., 2014; Rania *et al*., 2014; Alia *et al*., 2015)**. There is no effective therapy for cadmium poisoning **(Rania, 2014)**. Oxidative stress has been reported to play a key role in cadmium poisoning and frequently causes physiological harm to a number of organs, including the kidney, liver, bones, lungs, and reproductive organs **(Jaishankar *et al*., 2014)**. Cadmium content of crabs recorded in the present study are higher than the mean cadmium content (0.1366 ±0.0002mg/ kg) reported for crabs by **Neji *et al*. (2019**). Lower mean concentrations ranging from 0.061±0.009-0.110±0.006 were reported for muscles, gills and hepatopancreas of crabs **(Ihunwoa *et al*., 2022)**.

The mean content of mercury in *C. amnicola* (Table 2) is below Codex maximum level (ML) and the Commission of European Communities maximum levels for crustaceans of 0.5mg/ kg **(EC, 2015; FAO/WHO, 2015)**. A tolerated intake level of 1.6µg/ kg b.w per week for methyl mercury (corresponding to 0.097mg/ kg b.w for an adult of 60.7kg evaluated in this study) was established by the joint FAO/WHO expert committee on food additives (JECFA) in 2004 to protect developing foetus from neurotoxic effects **(JECFA, 2003; WHO, 2007)**. The findings of this study implies that, *C. amnicola* from the study is safe even for pregnant women with respect to mercury intoxication. A lower mean mercury content of 0.0031 ±0.0005mg/ kg was reported for crabs by **Neji *et al*. (2019)**.

The study indicated that the average arsenic content of blue crab (Table 2) was below the maximum standards of the Commission of European Communities for crustaceans **(EC, 2015)** and the Codex maximum level (ML) **(FAO/WHO, 2015)** of 0.50mg/ kg. Lower mean concentration arsenic (0.811±0.0493mg/ kg) was recorded for crabs by **Neji *et al*. (2019)**. Lower mean concentrations ranging from 0.001±0.001-0.003±0.001mg/ kg were reported for muscles, gills and hepatopancreas of crabs **(Ihunwoa *et al*., 2022)**.

Relationship between concentration of metals in sediment and blue crab (*Callinectes amnicola)* from Great Kwa and Calabar rivers, Calabar, Nigeria

Concentration of the metals in crab followed the same trend as sediment metal concentration, with the Calabar River being significantly higher than the Great Kwa River and each river displaying gradual increase toward the estuary, with no significant seasonal fluctuations. This suggests that *C. amnicola* are very sensitive to changing environmental conditions, particularly, loads of the studied metals, hence their suitability as bio-monitors. The strong positive correlations observed between lead in sediment and lead in *C. amnicola*, cadmium in sediment and *C. amnicola*, mercury in sediment and *C. amnicola*, and between arsenic in sediment and *C. amnicola* indicate that an increase in the concentration of each of the metal in sediment is accompanied by a corresponding increase in crab tissues suggesting that same source may be responsible for their presence at the concentrations determined. Appropriate bio-indicators often greatly aid biomonitoring **(Zhuo *et al*., 2008)**. Using *C. amnicola* as a bio-indicator of lead, cadmium, mercury and arsenic could be a plausible approach given that blue crab like many other crustaceans are benthic organisms and are relatively high in the food chain, meaning they can accumulate the metals from their prey and from the environment over time. In contrast to contaminants in surface water, which are more or less transient in lotic systems, sediments are a more appropriate medium for reflecting aquatic ecosystem's level of contamination since they may store periodic inputs **(Lutgen *et al*., 2020; Tiwari, 2020; Nkopuyo & Everard, 2021)**, provide habitats and a food source for benthic organisms. With feeding habit characterized by scavenging and predation, monitoring toxic metals using *C. amnicola* can provide valuable information about the contamination level in the environment and can serve as indicator for environmental and human health.

Bioaccumulation factor (BAF)

A complex interaction between sediment chemistry, crab physiology, and environmental conditions determines the bioaccumulation of heavy metals in crabs. Ascertaining the possible hazards that toxic metal pollution may pose to crab populations, ecosystem which they inhabit and human consumers of the protein rich seafood, requires close observation and comprehension of these variables. Over time, lead, cadmium, mercury and arsenic bio-accumulate in crab tissues especially in organs like the gills, muscle tissues and hepatopancreas **(Bakker *et al*., 2016; Ihunwoa *et al*., 2022)**. High organic matter in sediment boost the rate of bioaccumulation. While low pH enhances bioaccumulation of lead, cadmium and possibly arsenic, the relationship between pH and bioaccumulation of mercury is relatively complex and is influenced by multiple factors **(Uwa *et al*., 2018)**. The following terminologies were used to interpret bioaccumulation factor. BAF < 5, low bioaccumulation potential; BAF 0.5-1, moderate bioaccumulation potential; BAF > 1, high bioaccumulation potentials **(EC, 2019; USEPA, 2019)**. The bioaccumulation factors (0.018-0.200) computed for lead, mercury and arsenic indicate that, *C. amnicola* are accumulating the metals at minimal rate, suggesting the metals are unlikely to accumulate in the organism to harmful levels, hence, minimal risk. However, given the high toxicity of the metals in question, there is still cause for concern. BAF computed for cadmium (0.627-1.015) indicates that, crabs are accumulating cadmium at moderate to high levels. This implies that, cadmium is entering the food chain at moderate levels to high levels and could pose significant risk to other organisms including man.

Evaluation of potential human health risk

Human health risk assessment in this study refers to the approaches used to evaluate the potential health impact that consumption of *C. amnicola* obtained from the lower reaches of the Great Kwa and Calabar rivers could have on consumers with regard to lead, cadmium, mercury and arsenic poisoning. Both carcinogenic and non-carcinogenic risk were evaluated.

Non-carcinogenic risk

To assess the potential non-carcinogenic risk associated with the consumption of crabs obtained from the Great Kwa and Calabar rivers, estimated daily intake (EDI), target hazard quotient (THQ) and hazard index (HI) were computed.

Estimated daily intake (EDI)

To determine the safe levels of the metals that could be consumed from *C. amnicola*, EDI, which combined information on the concentration of metals in the *C. amnicola* and the quantity of *C. amnicola* ingested on daily basis, was used **(Lanre-Iyanda & Adekunle, 2012)**. The study's computed estimated daily intake for lead, cadmium, mercury, and arsenic was compared to the upper tolerable daily intake (UL) of each metal as well as the recommended daily intake (RDI) (Table 6). Recommended daily intake (RDI) is an estimate of the quantity of a chemical pollutant from all available sources that can be consumed daily over the course of a lifetime, without posing a significant harm to one's health **(Guerra, *et al*., 2012)**. The average estimated daily intake values (mg/kg body weight (bw) per day) computed for lead, cadmium and mercury were above their recommended daily intake values but lower than the upper tolerable intake, except cadmium in the Calabar River during dry season, recording an approximately equal value to the UL. The EDI for arsenic was below both the RDI and UL. The study's estimated daily metal intake was expressed in milligrams per kilogram body weight per day (mg/kg b.w/day). For example, an average adult weighing 60.7kg would have an average EDI of, for example, cadmium in *C. amnicola* from theGreat Kwa and Calabar rivers are equivalent to 2.37 and 3.85 for dry season and 2.67 and 3.61 for wet season. Intake of cadmium via consumption of *C. amnicola* is particularly concerning, given that the metal has the tendency to bio-accumulate. Consumption of *C. amnicola* from the study area could pose considerable toxicological risk. Due to the significant health concerns involved, regulatory bodies and health organizations recommend reducing or avoiding exposure to lead, cadmium, and mercury whenever feasible. The recommendation of zero intake reflects the goal of protecting public health by minimizing exposure to these toxic substances.

Target hazard quotient (THQ)

Target hazard quotient is the ratio of a substance's possible exposure to its reference oral dose. It is the level below at which no effects are anticipated **(Guerra *et al*., 2012; Lanre-Iyanda & Adekunle, 2012)**. When the ratio is less than unity, there is no obvious risk. The average THQ computed for all the metals were less than unity during both wet and dry season. The THQ approach used in this study only took into account exposure to the metals under investigation from intake of *C. amnicola* obtained from the study area. It did not take into account exposure through other pathways.

Hazard index (HI)

The hazard index is a tool used to assess the possible risk to human health posed by many contaminants **(Guerra *et al*., 2012)**. The underlying premise is that the degree of harm caused by metal poisoning is directly related to the total amount of metals exposed. It also presupposes analogous operational mechanisms influencing the target organ in a linear fashion **(Guerra, *et al*., 2012)**. When HI is larger than 1, a significant potential health risk is implied. The potential risk could increase when all metals are taken into consideration, even though there was no obvious concern when each metal was examined separately. The hazard risk due to consumption *C. amnicola* was obtained from the Great Kwa River during both dry and wet seasons. The study found out that the average adult weight of 60.7kg was less than unity, but the average adult weight in Calabar was larger than unity. This suggests that there is a considerable toxicological risk associated with consuming *C. amnicola* from the Calabar River. The proportionate shares of the total risk posed by lead, cadmium, mercury and arsenic were: 26.09, 44.47, 0.51 and 29.05%, respectively, for the Great Kwa River, and 24.74, 59.64, 0.94, and 23.34%, respectively, for the Calabar River during dry season. The relative contributions during wet season were 24.94, 57.78, 0.53, and 16.89%, respectively, for the Great Kwa River, and 14.68, 23.38, 0.37 and 21.35%, respectively, for the Calabar River during wet season.

Incremental lifetime cancer risk (ILCR)

According to the **US-EPA (2005)**, the acceptable risk range for carcinogens is between 10-4, where the lifetime risk of developing cancer is 1 in 10,000, and 10-6, where the risk is 1 in 1,000,000. Cancer risk is regarded as insignificant below 10^{-6} and as unacceptable above 10^{-4}. The study's computed cancer risks (Table 5) for lead in both rivers were below the acceptable limit, whereas the ILCR for cadmium and arsenic exceeded the carcinogens' standard tolerable regulatory risk (10^{-4}). This suggests that there is a considerable risk of cancer from cadmium and arsenic in *C. amnicola*.

Cumulative cancer risk (CCR)

The study revealed that, the cumulative cancer risk that could be triggered by lead cadmium and arsenic exposure resulting from consuming *C. amnicola* from the rivers under investigation was higher than the standard tolerated regulation of risk for carcinogens (10–4), indicating a significant carcinogenic risk.

CONCLUSION

Mean lead and cadmium content of *C. amnicola* exceeded the maximum permissible limits for the metals in crustaceans. Metals contents of crab followed the same trend as sediment metal concentrations, with the Calabar River being significantly higher than Great Kwa River. The two rivers displayed gradual increase in metals concentrations towards the estuary with no significant seasonal fluctuations. The strong positive correlations observed between each metal in sediment and in *C. amnicola* at 99% confidence level, suggests same source may be responsible for their occurrence at the determined concentrations, and that *C. amnicola* are sensitive to changing loads of the studied metals, hence their suitability as bio-monitors. BAF indicates that crabs are accumulating cadmium from the environment at moderate to high levels. The average EDI for Pb, Cd and Hg were above their recommended daily intake but lower than the upper tolerable intake. The average THQ computed for all the metals were less than unity but the hazard index for Calabar River were above unity. ILCR for cadmium and arsenic exceeded the carcinogens' tolerable standard regulatory risk (10^{-4}). Eating *C. Amnicola* from the study area, at or above the fish ingestion rate, poses both carcinogenic and non-carcinogenic risk. Continued monitoring is necessary to ensure environmental and human health protection.

REFERENCES

Addo, M. A.; Darko E. O.; Gordon B. J. and Nyarko B. (2013). Heavy Metal Contaminations in soil and cassava harvested near cement processing facility in the Volta Region, Ghana: Implications of Health risk for the Population living in the Vicinity. *e-journal of science and tech.,* 3(8): 71-83

Adekola, O. and Mitchell, G. (2011). The Niger Delta wetlands: threats to ecosystem services, their importance to dependent communities and possible management measures. *International Journal of Biodiversity Science, Ecosystem Services and Management, (*7)1: 50–68.

Alia, N.; Sardar K.; Said, M.; Salma, K.; Sadia, A.; Sadaf, S.; Toqeer, A. and Miklas, S. (2015). Toxicity and Bioaccumulation of Heavy Metals in Spinach (Spinacia oleracea) Grown in a Controlled Environment. *International Journal Environmental Research* 12: 7400-7416

Anyanwu, B. O.; Davies, I. and Chris, B. (2023). Human health hazard implications of heavy metals concentration in swimming crab (Callinectes amnicola) from polluted creeks in Rivers State, Nigeria. *Case Studies in Chemical and Environmental Engineering.* 7 100325: 1-10

APHA (American Public Health Association) (2005). *Standard Methods for the Examination of Water and Wastewater.* Washington, DC.

ATSDR (Agency for Toxic Substances and Disease Registry) (2019). 'Toxicological profile for chromium. U.S. Department of Health and Human Services, Atlanta, Georgia, USA'.

Ayuba, A. K. (2012). Environmental Impacts of Oil Exploration and Exploitation in the Niger Delta of Nigeria. *Global Journal Science Frontier Research*, 12(3):18-28.

Bakker, A.; Dutton, J. and Santangelo, N. (2016). Metal accumulation in horseshoe crab (*Limulus polyphemus*) eggs, embryos, and larvae from potentially contaminated public beaches. *Wilderness Environ. Med.*, 27(3): 430–431. https://doi.org/10.1016/j.wem.2016.06.025.

Chijioke, B. O.; Ebong, H. and Ufomba, I. B. (2018). The Impact of Oil Exploration and Environmental Degradation in the Niger Delta Region of Nigeria: A Study of Oil Producing Communities in Akwa Ibom State. *Global Journal of Human Social Science*, 18(3): 54-70.

Chinedu, E. and Chukwuemeka, C. K. (2018). Oil spillage and heavy metals toxicity risk in the Niger Delta, Nigeria. *Journal of Health and Pollution*, 8(19): 21-32.

Davies, I. C.; Nkeeh, D. K.; Akoko, S. and Ariri, I. P. (2021). Spatial variation of heavy metals in blue crab (*Calinectes amnicola*) harvested from the water front of some fishing communities in River State, Nigeria. *International Research Journal of Applied Sciences, Engineering and Technology*, 7(11): 33–49.

Durand, J. R. and Leveque, C. (1980). *Flore et Fauna aquatiques de l'Afrique Sahelo-Soudanienne*. Editions de l'office de la Recherche Scientifique et Technique Outre-Mer, Collection Initiations. Orstom Press, Paris. pp. 19-681.

EC (2015). Commission Regulation 2015/1005 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs (Text with EEA relevance).

EC (European Commission). (2019). Guidance on the assessment of bioaccumulation in aquatic species.

Edmunds, J. (1978). *Sea shells and other molluscs found on West African coast and estuaries*. Ghana Universities Press, 87-146.

Ekpo, P. B.; Umoyen, A. J.; Akpan, N. G.; Ekpo, I. P. and Abu, G. (2021). Evaluation of Pollution load: Heavy metal contents and physiochemical properties of the Great Kwa River, Calabar, Cross River state, Nigeria. *International Journal of Environment and Climate Change*, 11(2): 19-31.

Ephraim, B. E. and Ajayi, I. O. (2015). Compositional evaluation and quality status of surface waters of Mbat-Abiati and Oberekkai Creeks of the Great Kwa River, Southeastern Nigeria. *Advances in Applied Science Research*, 6(6): 36-46.

Ewa, E. E.; Iwara, A. I.; Offiong, V. E.; Essoka, P. A. and Njar, G. N. (2013). Seasonal variation in the heavy metal status of the Calabar River, Cross River State Nigeria. *Journal of Natural Sciences Research*, 3(11): 78-83.

FAO/WHO (2015). *General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995)*. Adopted in 1995, Revised 2015. The Hague, The Netherlands. 38-48.

Guerra, F.; Trevizam, A. R.; Muraoka, T.; Marcante, N. C. and Canniatti-Brazaca, S. G. (2012). Heavy metals in vegetables and potential risk for human health. *Science Agricultural*, 69(1): 54-60.

Ihunwo, O. C.; Ibezim-Ezeani, M. U.; Shahabinia, A. R.; Onyema, M. O.; Mmoma, P. C. and Bonnail, E. (2022). Metal accumulation in two crab species from the Woji Creek (Nigeria) and human health risk assessment due to crab consumption: *Callinectes sapidus* and *Cardisoma armatum*. *Watershed Ecology and the Environment*, 4: 169–176.

Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B. B. and Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2): 60–72.

JECFA (2003). *Summary and Conclusions of the Sixty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*. JECFA (Joint

FAO/WHO Expert Committee on Food Additives), 2003: 18–22. Available at http://www.who.int/pcs/jecfa/Summary61.pdf.

Kamrin, M. (2004). *Biomonitoring Basics: A Report from biomonitoringinfo.org*. Manassas, VA: Environmental Health Research Foundation. Available: http://www.biomonitoringinfo.org/images/What_is_Biomonitoring.pdf [accessed 18 April 2024].

Kathuria, P. (2020). Lead Toxicity. https://emedicine.medscape.com/article/1174752-overview (Accessed 16 May 2021).

Lanre-Iyanda, T. Y. and Adekunle, I. M. (2012). Assessment of heavy metals and their estimated daily intakes from two commonly consumed foods (*kulikuli* and *Zobo*) found in Nigeria. *African J. Food, Nutrition and Development*, 3: 6157-6169.

Liu, X. M.; Song, Q.; Tang, Y.; Li, W. L.; Xu, J. M. and Wu, J. J. (2013). Human health risk assessment of heavy metals in the soil-vegetable system: A multi-medium analysis. *Sci Total Environ*, 463–464: 530–540.

Lutgen, A.; Jiang, G.; Sienkiewicz, N.; Mattern, K.; Kan, J. and Inamdar, S. (2020). Nutrients and Heavy Metals in Legacy Sediments: Concentrations, Comparisons with Upland Soils, and Implications for Water Quality. *Journal of the American Water Resources Association*, 56(4): 669–691.

Martin, C. W. (1999). Heavy metal concentrations in floodplain surface soils, Lahn River, Germany. *Environ. Geol.*, 30: 119–125.

Mestrovic, T. (2018). What are Nutraceuticals? https://www.news-medical.net/health/What-are-Nutraceuticals.aspx (Accessed 1 April 2021).

Nanda, P. K.; Das, A. R.; Dandapat, P.; Dhar, P.; Bandyopadhyay, S.; Dib, A. L.; Lorenzo, J. M. and Gagaouan, M. (2021). Nutritional aspects, flavour profile and health benefit of crab meat based novel food products and valorisation of processing waste to wealth. A review. *Trend in Food Science and Technology*, 112: 252-267.

Neji, P. A.; Agwupuye, J. A. and Ushie, O. A. (2019). Assessment of Bioaccumulation Tendency of Heavy Metals in Crab and Prawn from Esierebom Beach, Calabar South Local Government Area, Cross River State Nigeria. *Dutse Journal of Pure and Applied Sciences*, 5(2b): 295-299.

Nkopuyo, U. S. and Everard, M. (2021). Assessment of Heavy Metals Pollution in Soft Sediment of the Severn Estuary and Inner Bristol Channel System. *British Journal of Environmental Sciences*, 9(2): 1-30.

Oguguah, N. M.; Onyekachi, M. and Ikegwu, J. (2017). Concentration and Human Health Implications of Trace Metals in Fish of Economic Importance in Lagos Lagoon, Nigeria. *Journal of Health and Pollution*, 7(3): 66-72.

Pennak, E. A. (1978). *Freshwater invertebrates of the United States*. 2nd Edition, John Wiley and Sons, New York. 803 pp.

Population Stat. Calabar, Nigeria population. *Population Stat*. [cited 10 October 2023]. Available from: https://populationstat.com/nigeria/calabar.

Ramya, V. and Patel, P. (2019). Health benefits of vegetables. *International Journal of Chemical Studies*, 7(2): 82-87.

Rana, S. (2018). Ethno-medicinal importance of crab. *International Journal of Zoological Studies*, 3(4): 06-07.

Rania, A.; Kumar, A.; Lal, A. and Pant, M. (2014). Cellular mechanisms of cadmium-induced toxicity: a review. *International Journal of Environmental Health Research*, 24(4): 378-399.

Szymanski, M. (2014). Molecular mechanism of zinc toxicity. *Journal of Biotechnology, Computational Biology and Bionanotechnology*, 95(2): 137-149.

Tiwari, R. N. (2020). *Textures of Sedimentary Rocks*. New Delhi: Indira Gandhi National Open University.

Ubiogoro, O. E. and Adeyemo, O. K. (2017). Heavy metal pollution of aquatic systems in oil-producing communities of delta state, Nigeria. *Journal of Applied Biosciences*, 120: 11993-11998.

Udiba, U. U.; Akpan, E. R.; Ogabiela, E. E.; Magomya, A. M.; Yebpella, G. G.; Apugo-Nwosu, T.; Hammuel, C.; Ade-Ajayi, A. F. and Aina, O. B. (2012). Influence of Oil Exploitation in the Niger Delta on the Water Quality of Calabar River Estuary, Calabar, Nigeria. *Journal of Basic and Applied Scientific Research*, 2(2): 1658-1666.

Udiba, U. U.; Inuwa, B.; Saminu, F.; Bello, G.; Kabir, A. F. and Zakariyya, A. (2014). Biomonitoring of Heavy Metal in selected biomarkers of *Clarias gariepinus* (Burchell, 1822), A Comparative Study of River Galma, River Kubani and Fish Farms in Zaria, Nigeria. *Advances in Applied Science Research*, 5(6): 198-206.

Udiba, U. U.; Odey, M. O.; Udofia, U. U.; Akpan, E. R.; Ama, J.; Antai, E. E. and Dan, M. U. (2022). Dietary Intake, Carcinogenic and Non-Carcinogenic Risk Potentials of Lead, Cadmium, Mercury and Arsenic Exposure via Consumption of

Dried Crayfish in Calabar, Nigeria. *Journal of Geoscience and Environment Protection*, 10: 340-363.

Udiba, U. U.; Udofia, U. U. and Akpan, E. R. (2020). Concentration and Potential Human Health Hazards of Heavy Metals in Periwinkle (*Tympanotonus fuscatus*) Purchased from Major Markets in Calabar, Nigeria. *Journal of Health and Pollution*, 10(28): 1-12.

Udofia, U. U.; Udiba, U. U.; Udofia, L. E.; Ezike, N. N. and Udiba, S. U. (2016). Assessment of the impact of solid waste dumps on groundwater quality, Calabar Municipality, Nigeria. *Journal of Advance Research in Biology and Pharmacy Research*, 1(4): 18-34.

US-EPA (United States Environmental Protection Agency). (2005). *Guideline for Carcinogenic Risk Assessment*. EPA/630/P-03/001B. Available at https://www3.epa.gov/airtoxics/cancer_guidelines_final_3-25-05.pdf.

US-EPA (United States Environmental Protection Agency). (2011). *Exposure Factors Handbook (2011 Edition)*. Final Report. National Centre for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F. Available from the National Technical Information Service, Springfield, VA, and online at http://www.epa.gov/ncea/efh. 10-57.

US-EPA (United States Environmental Protection Agency). (2019). *Bioaccumulation: Frequently Asked Questions*. U.S. Environmental Protection Agency. https://www.epa.gov.

US-EPA (United States Environmental Protection Agency). (2020). *Regional Screening Levels (RSLs) – Generic Tables*. USEPA. May 2020 [accessed 26 May 2024]. Available from: https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables.

Uwa, C. U.; Ugah, U. I.; Ukaegbu, M. C.; Udu-Ituma and Okata-Nwali, D. O. (2018). Bioaccumulation of Heavy Metal Concentrations on the Tissues of Crab (*Cancer pagurus*) Found in Oferekpe River, Ikwo, Ebonyi State, Nigeria. *World Applied Sciences Journal*, 36(1): 171-177.

Vijayalingam, T. A. and Rajesh, N. V. (2020). Analysis of Nutritional Value of crab meal as feed supplement for Livestock and Poultry. *Chemical Science Review and Letters*, 9(35): 773-776.

WHO. (2007). *Exposure to Mercury: A Major Public Health Concern*. Geneva, World Health Organization. Available at https://www.who.int/phe/news/Mercury-flyer.pdf.

Zhou, Q.; Zhang, J.; Fu, J.; Shi, J. and Jiang, G. (2008). Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica Chimica Acta*, 60.