

## Original Research Article

### **Heavy metals in shrimps and water from the Great Kwa River in Cross River State, Nigeria.**

#### **Abstract**

**Background:** The consequence of heavy metal pollution can be hazardous to man, based on the level of consumption of shellfish in riverine area, which is relatively high. There is increasing concern about the quality of aquatic food (e.g. periwinkle and shrimp) in several parts of the world. The determination of toxic elements in food has prompted studies on toxicological effects of heavy metals in foods. Due to man's industrialization activities such as oil exploration, the levels of toxicants in marine environment is increased.

**Objectives:** This study aimed at evaluating heavy metals content (lead, cadmium, nickel, manganese, chromium and copper) in shrimp and water obtained from Great Kwa River, Calabar, Cross River State, Nigeria.

**Methodology:** Fresh shrimp and water samples were collected from the river in Calabar metropolis, Cross River State. In the preparation, the soft tissue (edible part) of the shrimps were extracted, dried in the oven (heat drying oven at 80°C) for 72 hours then the weights of the shrimps and water from each sampling were measured. Digestion of the samples were carried out before an Atomic Absorption Spectrophotometer was used to determine the concentrations of the heavy metals. Graph pad Prism Version 7 was used for statistical analysis and Microsoft Excel Application.

**Results:** The results indicate contamination of both the shrimps and water from the Great Kwa River by some heavy metals, which also pose a health risk to aquatic ecosystem and humans. Among the heavy metals studied, chromium had the highest concentrations in both shrimps ( $30.07 \pm 0.67$ ppm) and water ( $27.68 \pm 0.34$ ppm). The heavy metals were found in higher concentrations in the shrimp than in water, except for manganese which had a significantly ( $p < 0.05$ ) higher concentration in water ( $15.05 \pm 0.67$ ppm) as against  $14.02 \pm 0.93$ ppm in shrimp).

**Conclusion:** Certain microelements have biological uses, but concentration above recommended levels becomes detrimental to consumers. The levels of some of these heavy metals studied call for more investigation and regulation of their anthropogenic sources in order to reduce risk of public health issues.

**Keywords:** *Bryophyllum pinnatum*, Liver disease, Antioxidants, Aqueous leaf extract.

#### **1.0 Introduction**

Various types of toxicities arisen from the pollutants including heavy metal, microplastics, pesticides etc., have made the aquatic faunal communities one of the most unvoiced victims. Oil exploration and docking activities, industrial and domestic waste water as well as natural processes, (Oluowo and Isibor, 2016). In general, non-degradable heavy metals even in trace amount can cause toxicities in aquatic ecosystems through assimilation, deposition, or

**Comment [FG1]:** Redaction is not clear and confuse

incorporation at a specific concentration into abiotic components and finally, adopting the path of bio-accumulation into aquatic animals (Ullah *et al.*, 2017).

Nigeria is among the protein and minerals deficient nations in the world. Most of the animal protein sources (cattle, goat, chicken, etc.) are inadequate and costly due to drought, disease, exorbitant cost of feeds etc. The high cost of beef and high-class protein sources and the negative health complication of beef and meat such as cardiovascular diseases, cancer, obesity, and diabetes are collectively responsible for more than 80% of the disease-related mortality (Albracht-Schulte *et al.*, 2020). However, consumption of seafood is on the increase, as it has been known for its essential nutrients content (Andreea *et al.*, 2022).

**Comment [FG2]:**

**Comment [FG3]:** Are talking about health in humans or cattle?

Aquatic food is a broad component with major categories, finfish and shellfish, aquatic foods most especially Sea-foods are nutritionally important in the supply of protein, especially the nine essential amino acids. The lipid content of sea foods is primarily in the form of triglycerides or triacylglycerols and is the only major source of highly unsaturated fatty acids (Adeyeye, 2002). Seafood ingests and accumulates omega-3 fatty acids through the food chain algae and phytoplankton, the primary producers of omega-3 fatty acid. Carbohydrate content in sea foods is known to be very small. Sea foods are best known as sources of fat-soluble vitamins; although they are sumptuous provider of some B vitamins and little or no vitamin C. They are better known nutritionally for the dietary minerals they supply (Adeyeye, 2002).

**Comment [FG4]:** Shrimp have cholesterol high level

Hosomiet *al.* (2012) reported that the consumption of seafood on regular basis will result in good health among consumers. Shellfishes are consumed by the inhabitants of the Calabar River and other coastal areas because the meat is tasty, contain high nutritional value and are abundant in nature. Shellfish meat are popular due to their high protein content, low carbohydrate values, low fat/cholesterol profile, significant amounts of omega-3-fatty acids, good lipids profile, essential amino acids, vitamins and vital minerals such as copper, calcium, sodium, zinc, iron, and manganese (Davies and Jamabo, 2016). Seafood is a healthful choice for people of all ages; growing children, pregnant women, active adults, and the elderly (Kris-Etherton *et al.*, 2011). Seafood is an excellent source of lean, high quality, easily digested protein. A 3.5-ounce serving of seafood provides almost half of an adult's daily protein needs for only 100 to 200 calories (Kris-Etherton *et al.*, 2011). Seafood is low in saturated fat and sodium and is a rich source of many essential vitamins and minerals. Seafood also is one of the few foods that contain long-chain omega-3 fatty acids, which have many beneficial health effects and are essential for the development of the nervous system and retina (Malden and Yaktine, 2011). Seafood includes fish (such as catfish, salmon, tuna, trout and tilapia) and shellfish (such as shrimp, crab, clams and oysters). Shrimps and periwinkles are some of the cheaper seafoods popularly consumed in Nigeria especially by people living in the coastal areas like Cross River state.

**Comment [FG5]:** Comment reply several times

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Chiocchietti *et al.*, 2017). Heavy metals have long been recognized as serious pollutants of the aquatic system. The heavy metals

that are toxic to many organisms at very low concentrations and are never beneficial to living beings are Hg, Cd and Pb (Duralet *et al.*,2010). The consequence of heavy metal pollution can be hazardous to man, based on the level of consumption of shellfish in riverine area, which is relatively high. There is increasing concern about the quality of aquatic food (e.g. periwinkle and shrimp) in several parts of the world. The determination of toxic elements in food has prompted studies on toxicological effects of heavy metals in foods (Indrajit *et al.*, 2011). Consequently, this study aimed at evaluating heavy metals content (lead, cadmium, nickel, manganese, chromium and copper) in shrimp obtained from great Qua river, Calabar, Cross River State, Nigeria.

## 2.0 Materials and methods

### 2.1 Apparatus and equipment

Grinding machine, electronic weighing balance, beakers, test tube, plastic buckets, measuring cylinder, refrigerator, thermo-regulated water bath, syringes, studded needles, spectrophotometer, pipette, cuvettes, centrifuge.

#### Reagents/materials

Reagents used in the study include: Trioxonitrate(V) acid, Sodium hydroxide (NaOH), Potassium iodide, Perchloric acid, Potassium borohydride, Acetylene, Argon gas, Hydrochloric Acid (HCl), distilled water, Nitric Acid. All reagents used in this work were of analytic grade. Cotton wool, filter paper, beam balance, beakers, test tubes, measuring flasks, dropper, heating cylinder were among the laboratory materials used.

**Comment [FG6]:** Argon gas ? or Argon gas

### 2.2 Sample Collection, identification and preparation

Fresh shrimp and water samples were collected from Qua River, Calabar metropolis, Cross River State. Total size and weight of the samples were measured; shrimp length ranged between 20-22 cm and the wet weight ranged between 220-240g. The shrimps were carefully conserved with ice in clean poly ethylene bags differently for easy identification on standard taxonomic. Ice was used to minimize the tissue decay and to maintain moist conditions during transportation. Shrimps were placed in an isolated container during transportation and immediately transported to the laboratory of the Department of Biochemistry, University of Calabar for analysis. The shrimps were then washed with deionized water and wrapped separately in acid washed polyethylene bags.

**Comment [FG7]:** Perhaps would be convenient a map or coordinate; nobody knows where is this place

### 2.3 Experimental procedure

### Preparation of samples for analysis

In the preparation, the soft tissue (edible part) of the shrimps were extracted, dried in the oven (heat drying oven at 80°C) for 72 hours then the weights of the shrimps and water from each sampling were measured using m-metlar weighing balance. The grams of each sample taken are as follows:

**Comment [FG8]:** How do you know the humid and dry weight?

For shrimps:

1. S1 – 1.09g
2. S2 – 0.20g
3. S3 -- 0.48g
4. S4 -- 1g

For Water:

1. W1 -- 100g
2. W2 -- 100g
3. W3 -- 100g
4. W4 -- 100g

After the weight of the above samples was taken, the samples were digested using concentrated hydrochloric acid (HCl) and nitric acid (HNO<sub>3</sub>); 20ml of nitric acid was then taken and diluted with 10ml of hydrochloric acid in a beaker containing the sample (shrimps) and placed on a wire gauze on top of the cooking gas for the digestion to take place. Standard laboratory procedures were followed. This lasted for about five to 10 minutes and the digestion was taken and filtered using the Whatman filter paper to remove impurities. The same process was also applied with water. Later, the filtrate of each sample was turned into clean sample bottles and locked. The recommended and required volume of the filtrate (50ml) was used to measure the heavy metal levels in these samples using Air / Acetylene flame Atomic Adsorption spectrophotometer. At each step of the digestion, acid blanks were prepared in order to ensure that the samples and chemicals used were not contaminated. Similarly, water samples collected from Calabar Qua river were acidified with 5ml of hydrochloric acid and nitric acid in a beaker. The sample beaker was then covered with a watch glass and kept on a hot plate to almost evaporate. The sample was removed from the hot plate and allowed to cool at room temperature. The filtered solution was then transferred to a volumetric flask, and deionized water was added to obtain the required volume for analysis using Atomic Absorption Spectrometer (AAS). Each set of digestion has its own acid blank and was corrected by using its blank. A blank solution of distilled water was used to check the accuracy of the standard solutions. The metal content was calculated using the formula below:

**Comment [FG9]:** Diluted or mixed?

**Comment [FG10]:** Standard procedures, proposed by who?

$$C = CAAS \cdot V$$

where C is the concentration (mg kg<sup>-1</sup> ppm dry weight)

CAAS - concentration of the element using AAS (ppm)

V - Volume made up.

## 2.4 Heavy Metal analysis

The metal concentration in the digests was determined by atomic absorption spectrophotometry. The calibration curve was prepared by running different concentrations of the standard solutions.

### 2.4.1 Analytical quality assurance

Appropriate quality assurance procedures and precautions were taken to ensure the authenticity of the results. Samples were carefully handled to avoid cross-contamination. Glassware was properly cleaned and distilled deionized water was used throughout the study. Reagents used – nitric acid and concentrated hydrochloric acid. In order to check the reliability of the analytical method employed for metal determination, one blank and combined standards were run with every batch of samples to detect background contamination and monitor consistency between batches. The results of the analysis were validated by digesting and analyzing standard reference materials.

**Comment [FG11]:** who supplied the reference standards?

### 2.5 Statistical analysis

Test for normality was carried out using the Shapiro–Wilks test and the Z-score test was used to check for outliers. Having passed the test for normality and outliers, data collected were subjected to statistical analyses. P-value < 0.05 was considered statistically significant. Graph pad Prism Version 7 (Statistical Package) was used for statistical analysis and Microsoft Excel Application Software 08. Data were expressed as mean  $\pm$  standard error of mean (SEM).

## 3.0 Results

### Heavy metal content of shrimp and water from Great Kwa River

The result of the evaluation of lead (Pb), cadmium (Cd) and nickel (Ni), chromium (Cr), copper (Cu) and manganese (Mn) levels of shrimp and water from the Great Kwa River is presented in Table 1. The Pb level in shrimp was  $9.36 \pm 0.55$  ppm, while in water it was slightly lower -  $8.62 \pm 0.32$  ppm. Cadmium level in shrimp was  $11.13 \pm 1.19$  ppm, also slightly higher than the concentration in water -  $10.75 \pm 1.13$  ppm. In the shrimps, the Ni concentration was  $9.61 \pm 0.47$  ppm, but in water it was  $8.75 \pm 0.50$  ppm. The Cr level ( $30.07 \pm 0.67$  ppm) in shrimp was significantly ( $p < 0.05$ ) higher than the level in water ( $68 \pm 0.34$  ppm). The Cu level in shrimp was  $33.17 \pm 0.79$  ppm which was significantly different ( $p < 0.05$ ) from the level in

water(17.65±0.61ppm). The Mn level in shrimp was 14.02±0.93ppm, which was significantly lower ( $p<0.05$ ) than the Mn level in water(15.05±0.67ppm).

**Table 1 : Heavy metal concentrations in shrimp and water from the Great Kwa River**

Heavy metals	Shrimps	Water
Lead (ppm)	9.36 ± 0.55	8.62 ± 0.32
Cadmium (ppm)	11.13 ± 1.19	10.75 ± 1.13
Nickel (ppm)	9.61 ± 0.47	8.75 ± 0.50
Chromium (ppm)	30.07 ± 0.67*	27.68±0.34
Copper (ppm)	33.17 ± 0.79*	17.65±0.61
Manganese (ppm)	14.02 ± 0.93*	15.05±0.67

Values in the same row with no superscripts are statistically similar ( $p>0.05$ )

**Comment [FG12]:** How do you explain the similar values in water and shrimp samples? There is not bioaccumulation?

#### 4.0 Discussion

The results of this research indicate significant Lead, Cadmium, Nickel, Chromium, Copper and Manganese content in shrimps and water from the Great Kwa River. It is a matter of great concern for both the environment and public health. Heavy metals are known to be toxic and can cause a range of health problems in humans, including neurological disorders, cancer, and kidney damage. Lead, Cadmium, Nickel and Chromium are particularly dangerous because they can accumulate in the body over time and cause chronic health effects. Moreover, exposure to these heavy metals is associated with an increased risk of cardiovascular diseases, hypertension, and impaired cognitive function.

The result of the study shows that Lead was present in both water and shrimp sample collected from the Great Kwa River, the mean lead concentration in water sample was 0.0145mg/l which is higher than the WHO guideline of 0.01mg/l in water, and that of shrimp was above WHO standard of 1.50mg/kg. Lead can enter water bodies from a variety of sources, including industrial discharge, atmospheric deposition, and agricultural runoff. Industrial activities such as mining, smelting, and battery manufacturing are significant contributors to lead contamination in water bodies. Lead can also enter the water through the corrosion of pipes and plumbing fixtures that contain lead. Once lead enters the water, it can be transported through various pathways, including absorption by aquatic plants, ingestion by aquatic animals, and sedimentation in the

**Comment [FG13]:** WHO (year) reference incomplete

bottom of the water body. Shrimps, like other aquatic organisms, can absorb lead through their gills, skin, and digestive tract, and can also ingest it through the food chain. Lead toxicity can have severe consequences on the health and survival of shrimps. Lead can disrupt their growth, reproduction, and immune system, making them more susceptible to diseases and infections (Ahmed *et al.*, 2016). Exposure to high levels of lead can also cause neurological and behavioral changes, leading to decreased mobility, altered swimming behavior, and reduced foraging abilities. Moreover, lead can accumulate in the tissues of shrimps over time, leading to chronic exposure and bioaccumulation. This can result in biomagnification, a process where the concentration of lead increases as it moves up the food chain, ultimately affecting humans who consume contaminated seafood. Lead contamination in water can also have significant environmental and public health impacts. High levels of lead in water can cause a range of health problems in humans, including developmental delays in children, decreased IQ, and an increased risk of cardiovascular disease in adults (Ahmed *et al.*, 2016).

Similarly, the mean cadmium concentration in both shrimp and water samples were above the WHO guideline value of 0.003 mg/L for cadmium in drinking water and the Codex Alimentarius Commission's maximum limit of 0.05 mg/kg. Same with the Nickel concentrations. Cadmium is a toxic element that can cause various health problems, including kidney damage, respiratory problems, and cancer (Haider *et al.*, 2021). Nickel is a naturally occurring element that can be toxic at high concentrations. Exposure to high levels of nickel can cause skin irritation, respiratory problems, and lung cancer.

Globally, the human health risk due to the consumption of food from aquatic ecosystems contaminated with hazardous chemicals including heavy metals has increased (Oguguah *et al.*, 2017). Chromium is an essential element that potentiates insulin action and enhances carbohydrate, lipid and protein metabolism at a maximum intake level of 0.25 mg/day, equivalent to 0.0041 mg/kg body weight per day for an average adult of 60.7 kg (Adedokun *et al.*, 2016). However, exceeding this limit leads to bioaccumulation and toxicity that can result in hepatitis and ulcers (Orisakwe *et al.*, 2015). The value recorded for shrimp in this study was higher than the mean value (1.07 mg/kg) reported by Adedokun *et al.* (2016); 1.57 mg/kg reported for shrimp obtained from the upper reaches of the Bonny River (Moslen *et al.*, 2017), and 0.001-0.003 mg/kg found for shrimp harvested from a perturbed tropical mangrove forest in the Niger Delta (Freeman and Ovie, 2017).

Copper is considered to be an important part of many enzymes but occur in very low levels in food (Akpanyung *et al.*, 2015). From this study, the highest concentration of Cu was found in the shrimp compared to the water obtained from the Great Kwa River. This was above the 1.0-3.0 mg/kg recommended limits for food fish by FAO/WHO (2004); thus, indicating that both the shrimp and water samples examined could pose copper related hazards to consumers. The high level of copper in the shrimp could be due to the fact that Copper in fish is taken up directly from the water via gills and stored in the shrimp. This finding is in agreement with the report of Akan *et al.* (2012) and Azaman *et al.* (2015) who revealed that metals accumulate in high concentrations in the seafood. Also, the reason for the high accumulation of these heavy metals in the shrimp, compared to the water may be as a result of high exposure of bottom feeding shrimp to the sediment which contained high concentrations of these metals (Azaman *et al.* 2015).

Comment [FG14]: (year) incomplete reference

The results from the present study, further validate the fact that, contaminants bioavailability increases with increasing water volume from their anthropogenic sources. In addition, concentration of manganese in both the water and shrimp were relatively high and this was in line with the earlier report of Okorafor *et al.*, (2015), who reported a high level of manganese in seafood. However, manganese is essential to the formation of bone and amino acid, lipid, protein, and carbohydrate metabolism. It is also needed for normal immunity system, regulation of blood sugar and cellular energy, reproduction, digestion, and for the defense mechanisms against free radicals. This trace element is required for the proper function of several metalloenzymes such as arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese superoxide dismutase (Li and Yang, 2018).

#### 4.0 Conclusion

The results indicate contamination of both the shrimps and water from the Great Kwa River by some heavy metals, which also pose a health risk to aquatic ecosystem and humans. It was also observed that human activities (industrial, domestic and agricultural activities) have contributed greatly to heavy metal contamination in that area and except these activities are curtailed, water pollution and sea food contamination may not be curtailed. The bio-accumulation of this heavy metals in water and shrimps exceed International regulatory standards, therefore consumers of water and shrimps from this river are at risk of public health disaster. Regular monitoring and testing of different regions of the river to identify contaminated areas and ensure only safe seafood are consumed, government policies should influence industrial, domestic and agricultural practices in this area, mitigation measures should also be taken such as phytoremediation, bio-remediation and chemical precipitation to reduce heavy metal concentration in these polluted areas.

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