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ASSESSMENT OF HEAVY METAL CONCENTRATION IN AFRICAN RIVER PRAWN (*Macrobrachium vollenhovenii -* Herklots, 1857) IN THE LOWER CROSS RIVER SYSTEM, SOUTHEASTERN NIGERIA.

ANIEMA PAUL INYANG-ETOH, SUNDAY UROM ETENG AND EMAEDIONG OKON UDOEKPO

Email: aniemainyangetoh@yahoo.com

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ABSTRACT

The assessment of heavy metals in the tissues of prawns is essential to understand the extent of contamination and potential health risk in human and the aquatic ecosystem. The study aimed to determine the concentration levels of iron, chromium, lead and cadmium in the tissues of *Macrobrachium vollenhovenii* of the lower Cross River System comparing it with the permissible consumption limits of Federal Ministry of Environment (FMENV) and the World Health Organization (WHO). Samples of the prawn were collected from artisanal fishermen on landing between November, 2022 and January, 2023. Collected samples were placed on ice and transported to the laboratory for Atomic Absorption Spectrophotometry analysis. The prawn samples had a total length and weight of 18.0cm – 24.0cm and 98.42g – 248.64g, respectively. The results revealed that the toxicity levels of heavy metals in the tissues of *M. vollenhovenii* ranged as follows: Fe: 1.041 - 2.082mg/kg; Pb: 0.173 - 0.618mg/kg; Cd: 0.114 - 0.242mg/kg; Cr: 0.007 - 0.038mg/kg. The results from the analysis indicated that, Fe had the highest concentration, followed by Pb, Cd and least was Cr. The result revealed that, there was no statistically significant difference in the Lead levels ($p=0.266$) and chromium levels ($p=0.882$) across the months (NOV-JAN). There was statistically significant increase in the Iron levels across the months (NOV-JAN) ($p=0.037$). There was statistically significant decrease in the cadmium levels across the months (NOV-JAN) ($p=0.022$). Therefore, this study presents valuable information about heavy metals bioaccumulation in a commercially important prawn caught from the lower Cross River which is consumed by locals in the region. Also the evaluation of possible risks on health associated with contaminated prawn consumption on human is clear. Except for Cr, the heavy metal concentrations in the prawn samples analyzed were above the prescribed limits set by various authorities (WHO/FMENV). The result of this study calls for a close monitoring of anthropogenic activities of the area for safety in seafood consumption by human.

KEYWORDS: Assessment, Heavy Metal Concentration, African River Prawn, lower Cross River system.

INTRODUCTION

Heavy metals are any naturally occurring element having an atomic number and elemental density which is >20 and 5gcm-3, respectively, is regarded as heavy metal. These metals include: Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Zn, and elements within the platinum group.

These elements have a very fine line of toxicity and essentiality to living organisms ((Babel and Kurniawan 2003; Zwolak *et al*. 2019). Although, they may act as enzyme inhibitors at the required levels but at higher concentrations, they can lead to poisoning. However, few metals, such as lead, cadmium and, mercury are poisonous even in small quantities (Onwubiko *et al.,* 2020; Jimoh *et al.,* 2020;

Aniema Paul Inyang-Etoh, Department of Mariculture and Marine Fisheries Resources, Faculty of Oceanography University of Calabar, Calabar, Nigeria
Emaediong Okon Udoekpo, Department of Mariculture and Marine Fisheries Resources, Faculty of Oceanography University of Calabar, Calabar, Nigeria
Sunday Urom Eteng, Department of Fisheries and Aquaculture, Faculty of Agriculture, University of Calabar, Calabar, Nigeria

Omoigberale & Ikponwosa-Eweka, 2010). Heavy metals are one of the most serious pollutants in our aquatic environment due to their toxicity, persistence and bio-accumulation effects. Sources of heavy metals in the environment are: geogenic (rock weathering and leaching), anthropogenic (industrial, agricultural, pharmaceutical, domestic effluents), and atmospheric (acid rain) source (Zhang *et al.,* 1990).

According to literature, any naturally occurring element having an atomic number and elemental density which is >20 and 5gcm-3, respectively, is regarded as heavy metal. These metals include: Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Zn, and elements within the platinum group. These elements have a very fine line of toxicity and essentiality to living organisms. Even though, some elements are non-essential to living organisms at all but the ones of paramount concern include Cd, As, Hg, and Pb amongst others. On exposure, these elements can pose hazardous effects to living organisms due to their toxicity and are listed as priority pollutants by numerous organizations and regulatory entities (Babel and Kurniawan 2003; Fu and Wang 2011; Rai *et al.* 2019; Tangahu *et al*. 2011; Zwolak *et al*. 2019). High production in industries is also linked to an increase generation of effluents rich in heavy metals amongst other pollutants. This poses massive impacts to different receiving environment and their precious resources. As such, environmental contamination by heavy metals has perpetually led to the degradation of the environment hence impairing its ability to foster life and render its intrinsic values (Akinwekomi *et al*. 2017; Masindi 2017a, b; Masindi *et al*. 2018a, 2019; Mavhungu *et al*., 2019).

Most prevalently, heavy metals can be found as hydroxides, oxides, sulphides, sulphates, phosphates, silicates, carbonates, and organic matrix amongst others. They emanate from natural and anthropogenic sources (Selim and Sparks 2001; Selim *et al.,* 2001; Sparks 1995, 2005; Sparks and Sparks 2003; Langmuir 1997; Langmuir *et al.,* 1997). However, anthropogenic sources has been classified as the main source and route in which heavy metals are distribute to different compartments of the environment. Transport media are mainly water, soil and air. Heavy metal laddened fractions will be released to the atmosphere, aqua-sphere and the terra-sphere via those transport media (Langmuir 1916, 1918, 1997; Langmuir *et al*., 1997).

Heavy metals are dangerous because they do not undergo bio degradation but tend to bio-accumulate. Compounds accumulate in living things whenever they are taken up and are stored faster in different organs than they are metabolized (broken down) or excreted (Agah *et al.,* 2009).

Heavy metal poisoning could result, for instance, from drinking contaminated water, inhailing (air), absorbing and eating food. They are considered as major environmental pollutants causing cytotoxic, mutagenic, and carcinogenic effects in animals (Rauf *et al.,* 2009).

Heavy metals have been reported to cause an upsurge of liver and kidney diseases and are responsible for a high proportion of mortality in Nigeria (Ndiokwere, 2004). The acute neurological effects of heavy metal toxicity in human manifest itself in the form of abdominal cramps, bloody diarrhea, queasiness, dizziness and chest pain. Also rheumatic arthritis, muscular pain and osteomalacia in the elderly, are evidence of chronic cadmium exposure (Klaassen, 1999).

Shell-fishes have been widely used as bio-indicators to monitor heavy metal concentrations in the environment due to their wide range of distribution and their importance in food chain (Asuquo and Udoh, 2002).Prawns and shrimps are known to consume sand and mud along with detritus during feeding (Koli et al., 1978; Jimoh and Anetekhai, 2004). This sediment act as sink for the heavy metals in aquatic ecosystems and since these metals are non-biodegradable and can persist in the environment for a very long period (Kumolu-Johnson *et al.,* 2010), they can be taken up by shell-fishes during feeding . Another factor that makes shell fishes suitable for aquatic bio-monitoring is because of their slow mobility. Unlike the fin fishes that will quickly swim away from polluted environment, shellfishes may remain in the polluted environment and this makes them a better bio-monitor than fin fishes (Kumolu - Johnson *et al.,* 2010).

Studies on heavy metal concentrations in African River Prawn (*Macrobrachium vollenhovenii*) in the Nigerian coastal waters include those of Asuquo & Udoh (2002); Banjo *et al.* (2010), Jenyo-Oni & Oladele (2016), Jimoh *et al.* (2011), Oguzie & Achegbulu (2010) and Onwubiko *et al.* (2020). Seafood consumption with high significant level of concentration of heavy metals is a major concern, because metal poisoning is reported in Niger Delta and other part of the world (Chowdhury *et al.,* 2008). *Microbrachium vollenhovenii*, commonly known as freshwater prawn is an excellent source of protein. This makes it highly demanded for consumption by human. (Ugbome and Boma, 2013). It therefore, necessitates this study to assess the concentration level of iron (Fe), cadmium (Cd), chromium (Cr) and lead (Pb) content of *M. vollenhovenii* collected from the lower Cross River and compare them with national and international standards and this will help to ascertain their safety for human consumption.

MATERIALS AND METHODS

The study area is the lower Cross River System, a tropical freshwater fluvial system draining the rainforest belt of Akwa Ibom and Cross River States, Nigeria (King, 1998; Job, 2019). There are two seasons in the area: the dry (November – March) and the rainy (April – October) seasons (Job, 2019). At Ayadehe, itu bridge-end area of the river system located at approximately 05012'N, 007059'E. The river experiences tidal variations which are most pronounced in the dry season (King, 1998; Etim & Bray, 1994; Job, 2019). During the peak of rains (July – October), the river flow becomes unidirectional, with the current velocity increasing from 0.4 – 0.6m/s in the dry season to 0.7 – 1.5mls during the rains (king, 1998; Job, 2019). Etim and King (1998), Etim & Brey (1994) and Job (2019) report that the average depth of the river system ranges between 4m in the dry season to 14m in the wet season.

Sample collection

Samples of the African river prawn (*Macrobrahium vollenhovenii*) was collected on landing from the fishermen in lower Cross River, twice a month for the duration of three months between; November, 2022 - January, 2023.

Samples were washed with clean water, placed on ice and transported the same day to Federal ministry of science and technology Uyo, Akwa Ibom state. Samples were deep frozen at 4°C for analysis in the laboratory.

Plate 1: The African River Prawn (*Macrobrachium vollenhovenii* - Herklots, 1857) from the lower Cross River, Nigeria.

Samples processing

The preserved samples were allowed to thaw at 26°C - 27°C room temperature. The samples were thereafter, washed with deionized water and muscle tissues taken for digestion.

Digestion of sample

Sample tissue was cut and weighed to obtain a certain gram and oven dried at 105°C for one hour. The dried sample was crushed with the aid of a laboratory mortar and pestle and sieved. Thereafter, 0.5g of the sieved sample was taken and dissolved in a 0.5ml of nitric acid in a calibrated round bottom flask. Deionized water was added to obtain a 100ml and heated in a water bath under fume cupboard to reduce it to 20ml.

The Sample on cooling, was filtered with the aid of filtering apparatus. Deionized water was added to the filtered sample in a calibrated round bottom flask to obtain a desire volume of 100ml and thereafter, turned into a digestion bottle for analysis.

Analysis on heavy metal concentrations

Heavy metals were analyzed using Atomic Absorption Spectrometer based on appropriate wavelength of the metals following the method of AOAC (2023).

Statistical analysis

Statistics on data analysis was done using predictive analytical software (PASW) program (version 20) for Windows. Analysis of variance (ANOVA) was used to assessed the significant variation of heavy metal concentration within the study period at $P < 0.05$ level of significance.

RESULTS

The monthly variation (November 2022 to January 2023) in the levels of heavy metals in the tissues of the prawn (*M. vollenhovenii*) is presented in Figure 3 (Appendix I). Lead ranged from 0.173 - 0.618 mg/kg, Iron ranged from 1.041 - 2.082 mg/kg, Cadmium ranged from 0.114 - 0.242 mg/kg, Chromium ranged from 0.007 - 0.038 mg/kg, as depicted in Figure 3 below, Fe had the highest concentration, followed by Pb, next by Cd and least was Cr.

Monthly mean levels of heavy metals in tissues of the prawn (*M. vollenhovenii*) are presented in Table 1 below. Results revealed that Lead had a mean concentration that ranged from 0.23±0.06 mg/kg in January 2023 to 0.48±0.14 mg/kg in December 2022. Monthly mean Iron levels of Iron ranged from 1.05±0.01 mg/kg in November 2022 to 1.88±0.20 mg/kg in December 2022. For Cadmium, mean monthly levels ranged from 0.12±0.01 mg/kg in December 2022 to 0.23±0.02 mg/kg in November 2022, while Chromium ranged from 0.02±0.00 mg/kg in November 2022 to 0.03±0.00 mg/kg in January 2023 (Figure 2). There was no statistically significant difference in the Lead levels (p=0.266) and chromium levels (p=0.882) across the months (Nov-Jan). There was statistically significant increase in the Iron levels across the months (Nov-Jan) (p=0.037). There was statistically significant decrease in the cadmium levels across the months (Nov-Jan) (p=0.022).

Table 1: Monthly variation in the concentration of the heavy metals (mg/kg) in the tissues of *M. vollenhovenii* from the lower Cross River, Nigeria.

Heavy metals	Nov-22	Dec-22	Jan-23	Mean±SEM	F-cal (ANOVA)	P-value
Lead	$0.40±0.02^{a}$	$0.48±0.14^{a}$	$0.23±0.06^{a}$	0.37±0.059	2.129	0.266
Iron	$1.05±0.01^{a}$	$1.88±0.20^{b}$	$1.38±0.06^{ab}$	1.44±0.20	12.037	0.037
Cadmium	$0.23±0.02^{b}$	$0.12±0.01^{a}$	$0.15±0.01^{a}$	0.16±0.026	17.445	0.022
Chromium	$0.02±0.00^{a}$	$0.02±0.02^{a}$	$0.03±0.00^{a}$	0.023±0.002	0.131	0.882

Except indicated, values are mean ± SD; mean values with different superscripts are statistically different at P<0.05.

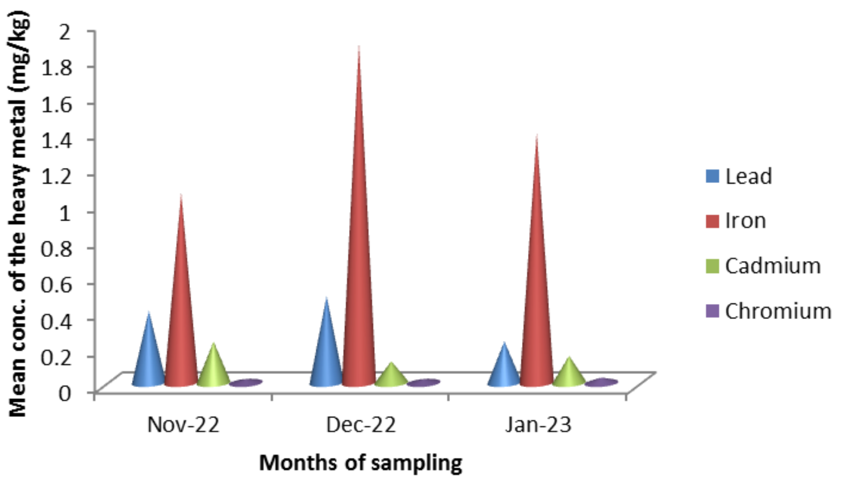

Figure 2: Variation in the levels of heavy metals in tissues of *M. vollenhovenii* from the lower Cross River, Nigeria.

Analysis of variance on the monthly variation levels of heavy metals in the tissues of *M. vollenhovenii*

The results of the analysis of variance (ANOVA) revealed monthly variation in the levels of some heavy metals obtained from the tissues of *M. vollenhovenii* in the lower Cross River System, Nigeria. Lead and Cadmium were not significantly (P>0.05) affected by the months of sampling, while Iron and Chromium showed statistical variation (P<0.05) with months of sampling. The values of Lead (0.173 –0.618 mg/kg) observed in the present study were higher than the World Health Organization (WHO) and Federal Ministry for Environment (FMENV) Nigeria stipulated level of 0.05 mg/kg. The analyzed values of Iron (1.041 –2.082 mg/kg) were also higher than the WHO and FMEV stipulated level of 0.03 mg/kg. More so, Cadmium (0.114 –0.242 mg/kg) levels were also higher than the WHO and FMEV stipulated level of 0.003 mg/kg. Chromium levels (0.007 –0.038 mg/kg) in this study were lower than the WHO and FMEV stipulated level of 0.05 mg/kg and 0.10 mg/kg respectively (Table 2).

Table 2: Summary statistics on variation in levels of heavy metals in the tissues of *M. vollenhovenii,* compared with World Health Organization (WHO) and Federal Ministry of Environment (FMENV) standards.

Heavy metals	Mean±SD	Range	WHO limit	FMENV limit	F-cal (ANOVA)	P-value
Lead	0.37±0.15	0.173 - 0.618	0.05	0.05	2.13	0.27
Iron	1.44±0.40	1.041 - 2.082	0.03	N/A	12.04	0.037
Cadmium	0.16±0.05	0.114 - 0.242	0.003	1.0	17.44	0.022
Chromium	0.023±0.01	0.007 - 0.038	0.05	<0.10	0.13	0.88

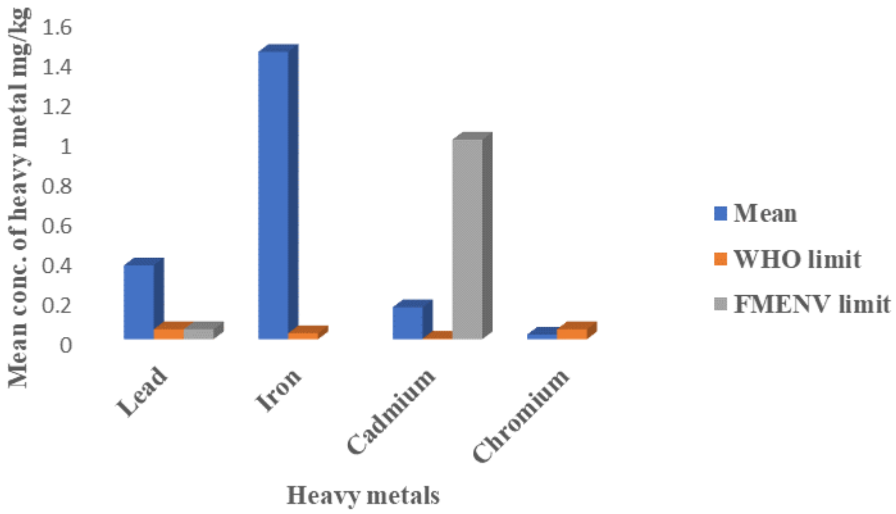

Figure 3: Variation in the levels of heavy metals in tissues of *M. vollenhovenii* from the lower Cross River, Nigeria in relation to WHO and FMENV standard.

DISCUSSION

From the results of the study, heavy metal concentrations in the M. vollenhovenii showed variations with month of sampling an indication of varying levels of contamination, similar variations in the concentration of heavy metals were reported in the species in the Calabar River (Onwubiko et al., 2020). Cadmium concentration level in the prawn samples varied significantly (P<0.05) within the sampling months (November 2022 to January 2023). This implied that the differences in the level of anthropogenic inputs which introduced Cadmium were not the same in the three sampling months and so had a significant influence in the concentration. Although values reported in the present study did not exceed the Federal Ministry of Environment (FMENV) stipulated limit of 1.0mg/kg in seafood, values exceeded the WHO standard of 0.003 mg/kg. In the present study, cadmium levels which ranged from 0.114 - 0.242 mg/kg was higher than 0.01-0.049 mg/kg reported by Asuquo et al. (2018) and lower than 1.12 mg/kg reported by Enuneku et al. (2013). Even at very low concentrations, cadmium can cause both acute and chronic intoxications (Chakraborty et al., 2013). Cadmium is highly toxic to the kidney and it accumulates in the proximal tubular cells in higher concentrations. Cadmium can cause bone mineralization either through bone damage or by renal dysfunction. If cadmium is ingested in higher amounts, it can lead to stomach upset and result in vomiting and diarrhea. On a long exposure time at lower concentrations, it can become deposited in the kidney and finally lead to kidney disease, fragile bones and lungs damage (Bernard, 2008).

Iron ranged from 1.041 to 2.082mg/kg and was higher than the WHO permissible limit of 0.03. This implies that the prawns of the study area have been contaminated with iron. Although iron is the most crucial element for growth and survival of almost all living organisms (Valko et al., 2005), levels higher than stipulated standard implies iron contamination. Fin or shell fish contaminated with iron can cause DNA strand breaks on consumption by human. Iron poisoning can cause gastrointestinal effects such as gastro intestinal bleeding, nausea and diarrhea (Osweiler et al., 1985). Iron poisoning is also characterized by shocks, hypotension, weakness, abnormal rapid heartbeat, liver damage, metabolic acidosis (excess hydrogen ion concentration) and sometimes death (Hillman, 2001). It is also marked by the formation of gastrointestinal ulcerations and development of strictures.

Excess iron uptake is a serious problem in developed countries and it increases the risk of cancer (Nelson, 1992).

As observed in the present study, the levels of Lead and Chromium did not vary significantly within the three sampling months at $P > 0.05$. This implied that the levels of anthropogenic inputs during the time of sampling were in agreement with those of Lead and Chromium levels. Oguzie and Achegbulu (2010) reported lower values of Lead and Chromium in Macrobrachium vollenhovenii and Penaeus notialis. The agreements in the heavy metal levels between the reports and that of the present study could be as a result of anthropogenic activities.

For Lead, the values observed in the present study were lower than the average value of 7.60 mg/kg reported by Sani (2011) but similar to 0.00-0.250 mg/kg reported by Oguzie et al. (2010) in Macrobrachium macrobrachion from Ovia river bank in Edo State. Lead poisoning has been noted to cause neuropathy and other neurological dysfunctions such as mental retardation and brain disorder. Behavioral trauma, lack of assimilation in learning and slow growth, are some of the symptoms that can also be seen in teenagers (Dietrich et al., 1993).

Chromium concentration levels observed in the present study were lower than 0.05 mg/kg limit stipulated by World Health Organization (WHO). The present finding is within the range (0.00-0.350mg/kg) reported by Oguzie et al. (2010) in the tissues of M. vollenhovenii obtained from the Ovia river bank in Edo State. Chromium toxicity causes kidney and liver damage as well as damage to the circulatory and nerve tissues in human (Jackson and Morris, 1989).

Generally; Lead, Iron, and Cadmium concentrations in prawns from lower Cross River system**,** Nigeria were above the WHO and FMENV acceptable limits while Chromium were below the stipulated standards. This implies that the prawns from the study area have high concentrations of Lead, Iron and Cadmium and as such, they may be unsafe for consumption. Similar report was given by Oguzie and Achegbulu (2010) in commercially important prawns of Ovia River in Edo State, Nigeria.

Monthly differences usually influence the accumulation of heavy metals in the tissues of a biological organism. In this study, there were variations in the mean concentrations of the heavy metal in the months of sampling (November 2022 to January 2023). These monthly variations could be as a result of the fluctuations in the number of sewage effluents, run-offs and industrial discharges into the river as previously reported by Zyadah (1995). However, the mean of Lead and Chromium levels did not vary significantly between the months of sampling even though some of the metals had higher concentration during one month as compared to the other. This result is similar to the finding of Onwubiko et al. (2020) who reported that such case could be due to the intense discharges of organic pollutants from surface water run-offs and drainage channels into the river during the sampling period thereby leading to high values of these mentioned metals during sampling. Also, the study period was short as it cut across only three months of the dry season. So statistical similarity in levels of heavy metals could be expected in the present study.

The high concentrations of Pb, Fe, and Cd analyzed in the tissues of M. vollenhovenii obtained from the lower Cross River, Nigeria, could be related to anthropogenic activities such as indiscriminate disposal of untreated wastes in the area which are incessant. The results from this study have valuable information about heavy metal contents in a commercially important prawn consumed as it forms an important protein source in human nutrition of the area. An evaluation of the possible risks associated with prawn consumption can be deduced from this study. Except for Cr, the heavy metal concentrations in the prawn samples analyzed were above the prescribed limits set by various authorities.

CONCLUSION

The present study assessed the levels of heavy metals in the tissues of M. vollenhovenii obtained from the lower Cross River, Nigeria, between November 2022 to January 2023. Results revealed that Fe had the highest concentration, followed by Pb, next by Cd and least was Cr. The results of the analysis of variance (ANOVA) revealed monthly variation in the levels of some heavy metals obtained from the tissues of M. vollenhovenii of the lower Cross River, Nigeria, with Pb, Fe, and Cr being highest in December while Cd was highest in November. The results of the Analysis of Variance (ANOVA) also revealed that Pb and Cd were not significantly ($P>0.05$) affected by months of sampling, while Fe and Cr showed statistical variation ($P<0.05$) with months of sampling. The values of Pb, Fe, and Cd were higher than the WHO and FMENV stipulated levels. However, Hg and Cr levels were lower than the permissible levels in tissues of prawns.

The study therefore concludes that the African River Prawn M. vollenhovenii is contaminated with Pb, Fe, and Cd and therefore points to risk of inclusion in human nutrition and recommends that the government and other relevant agencies should organize sensitization campaign to create awareness on the impact of indiscriminate disposal of wastes in the study areas, enforcement of stringent environmental policy can help reduce the indiscriminate disposal of potentially hazardous wastes, consumption of prawns caught from the river system should be discontinued for now till further studies prove otherwise, more studies should also be funded towards the continuous monitoring of the lower Cross River system to further reveal its ecological stability.

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