Heavy Metals Analysis in Crab (*Callinectes pali*), Tilapia (*Tilapia queneesis*) and Catfish (*Clarias gariepinus*) Species from Crude Oil Polluted River (Escravos River).

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Abstract

A freshly caught species of crabs, tilapia and cat fishes were bought from fishermen at the point of fishing in Kurutie, Okerenkoko and Kurukunama communities. Samples were oven dried at 105˚C for 24hours and ground to powder. Samples were digested following a modified digestion method of AOAC. Digested samples were analysed with AAS for concentrations of Cu, Fe, Cd, As, Cr, Se, Pb, Zn, Ni and Mn. Results showed that the concentrations of Cu and Pb were below detectable limit, concentrations of Cd and Zn were within the WHO and EU acceptable limit and the concentrations of Fe, As, Se, Ni, Mn and Cr were above the WHO and EU acceptable values. Escravos River is a major oil mining site in Nigeria and as a result have the tendency of bioaccumulating these heavy metals over long period of time which can be bioaccumulated by the aquatic organisms, hence finding their ways into human food chains causing so much ailments associated to a particular heavy metal.

Key words: Heavy metal, Sea food, Crude oil, Pollution, Bioavailability, Escravos river

1.0 Introduction

At the point of sampling for this research work, it was observed that there was no water management or monitoring scheme put in place within the river as there was indiscriminate waste disposal, ecological degradation as a result of industrial activities, bunkering and crude oil theft. Most residence of the environment are not educated and as such may not have known the negative impacts of discharging all their waste materials into the river body, both wastes that are degradable and non-degradable are disposed into the water. The presence of heavy metal in most rivers could be natural or anthropogenic processes. crude oil exploration and drilling is a major cause of increase in heavy metal content in a marine environment. Escravos River is a major oil mining site in Nigeria and as a result have the tendency of bioaccumulating heavy metals for years which can be bioaccumulated by the aquatic organisms, hence finding their ways into human food chains causing so much ailments associated to a particular heavy metal.

Seafood is generally considered to be low in total fat and saturated fat. Most fish and shellfish contain less than 5 percent total fat, and even the fattiest fish, such as mackerel and king salmon, have no more than 15 percent fat. A large proportion of the fat in seafood is polyunsaturated, including omega-3 fatty acids, which have added health benefits. *Omega-3 fatty acids* are essential fatty acids that are required for healthy human development. These organic compounds cannot be produced by the human body and therefore need to be obtained through food. Scientific evidence suggests that the marine-derived omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can help reduce the risk of heart disease and contribute to brain and vision development in infants. Fish and shellfish are the main dietary sources of EPA and DHA. The plant-derived omega-3 fatty acid, alpha-linolenic acid (ALA), is a precursor to EPA and DHA and is only converted at rates of about 0.1-9% in the human body [1, 2].

Heavy metals are highly toxic and non-biodegradable which can easily bioaccumulate in soil and water. High level of heavy metals in any impacted area results in severe pollution of the environment [3]. Most of these heavy metals are needed by living organisms but in a minute quantity such as Zn, Mn, Fe, Cu and Co while other heavy metals are toxic even at lower concentrations [4]. Literature has it that more than 4000 different crude oil spillage have been reported around this area of research since 1960 [5, 6]. Accumulation of heavy metals in living organism causes inhibition of various cellular processes and their effects to a target organ is concentration dependent and varies in their individual toxicity [7]. Heavy metal content in an environment are dependent on the physiology of species, site sample process, soil mineral composition and metal uptake capacity from the polluted source [8, 9].

Fish demand and consumption is relatively high as compared to red meats, this is because a research conducted at Harvard School of Public Health stated that high and frequent consumption of red meats causes high risk of a shortened life span [10]. Crabs, Cat fish and tilapia fish are the most commonly available sea foods found in Escravos river with crab and tilapia dominating and cat fish being seldomly found in a secluded areas of the river because of the salt content of the river. Both fishes in study are highly rich in proteins, vitamins, healthy fats and fatty acids with omega-3 and omega-6 fatty acids. These contents are vital to heart and good health [11]. Chronic effects of heavy metals among living organisms has been in literature. Copper causes brain damage and oxidative stress, Cadmium inhalation impairs the functions of pulmonary arteries, poisoning with Iron causes heart, liver and lung corrosion, Selenium contamination leads to fatigue, nausea, foul breath odour and poor irritability [12-15].

Fish is a natural source of B-complex vitamins, vitamin D and vitamin A (especially oily fish). B-complex vitamins have been associated with healthy development of the nervous system. Vitamin A is needed for healthy vision as well as for healthy skin, while vitamin D is essential in bone development. Fish is also a good source of minerals such as selenium, zinc, iodine and iron. Selenium is a potent antioxidant that protects against cell damage and may help to counter the negative effects of mercury. Zinc is needed for cell growth and immune system health. Iodine helps maintain thyroid gland function, while iron is important in red blood cell production. Small fish eaten whole, such as sardines and anchovies, are an important source of calcium needed for bone development.

Several studies have revealed the presence of heavy metals and hydrocarbons in marine organisms. There is limited information about the heavy metal content in the most consumed aquatic foods from Escravos river hence the need to access quantitatively the amount of these heavy metals in the three most commonly available and consumed sea foods in the study area.

2.0 Sample Collection

A freshly caught species of crabs, tilapia and cat fishes were bought from fishermen at the point of fishing in Kurutie, Okerenkoko and Kurukunama communities. They were transported in an ice container and refrigerated before laboratory analysis.

2.1 Sample Preparation

Fish samples were all dried in oven at 105˚C for 24hours and ground to powder with mortar and pestle.

2.2 Digestion Process

The digestion process of AOAC [16] was modified and process applied. 5.00grams of each fish sample well homogenized was weighed and digested with a 20mL of concentrated nitric acid and 10mL concentrated sulphuric acid until a clear and colourless solution was observed. The colourless solutions were filtered and diluted with a distilled water into a 100mL volumetric flask, made up to mark and stored in a clean container for metal analysis.

2.3 Spectrophotometric Analysis

For each of the metal Atomic absorption spectrophotometer calibration was carried out using a standard prepared from the metal salt. Metal concentrations of Cu, Fe, Cd, As, Cr, Se, Pb, Zn, Ni and Mn were determined from each sample using Atomic Absorption Spectrophotometer (Varian AA240). This involves a direct aspiration of the aqueous clear solutions into an air-acetylene flame. For metals (Fe, Mn and Zn) which were present at high concentrations, the solutions were diluted several times with distilled water and their concentrations were obtained from right calibration curves.

3.0 Results

Table 1: Heavy metal concentrations (mean ± S.E.M, mg/Kg dry wt.) in *Callinectes pali* from the study areas (Kurutie, Okerenkoko and Kurukunama).

Heavy metals	Kurutie	Okerenkoko	Kurukunama
Cu	*BDL*	*BDL*	*BDL*
Fe	201.00 ± 4.82^{a}	196.00 ± 3.77^{b}	204.00 ± 4.70^{a}
Cd	0.05 ± 0.01^{a}	$0.07.\ \pm0.01^{a}$	0.03 ± 0.01^{a}
As	0.77 ± 0.10^{a}	0.90 ± 0.04^{b}	0.74 ± 0.10^{a}
Se	11.46 ± 0.53^{a}	12.30 ± 0.30^{b}	12.10 ± 0.40^{b}
Pb	*BDL*	*BDL*	*BDL*
Zn	48.60 ± 0.12^{a}	44.30 ± 0.77^{b}	46.10 ± 1.20^{a}
Ni	0.88 ± 0.01^{a}	0.84 ± 0.02^{a}	0.99 ± 0.01^{b}
Mn	34.30 ± 0.33^{a}	36.00 ± 0.66^{b}	31.40 ± 0.40^{c}
Cr	0.84 ± 0.03^{a}	0.13 ± 0.05^{b}	0.31 ± 0.09^{c}

Reported values were expressed as mean ± standard error of mean (S.E.M) of triplicate values (n=3). The values with different superscript (a,b,c) in same column showed significant difference at p≤0.05. BDL = Below Detectable Limit

Table 2: Heavy metal concentrations (mean ± S.E.M, mg/Kg dry wt.) in *Tilapia queneesis* from the study areas (Kurutie, Okerenkoko and Kurukunama).

Heavy metals	Kurutie	Okerenkoko	Kurukunama
Cu	*BDL*	*BDL*	*BDL*
Fe	126.70 ± 3.77^{a}	131.30 ± 6.34^{b}	132.00 ± 4.81^{b}
Cd	0.06 ± 0.01^{a}	0.03 ± 0.01^{a}	0.04 ± 0.00^{a}
As	1.26 ± 0.02^{a}	1.30 ± 0.01^{a}	1.67 ± 0.02^{b}
Se	13.48 ± 0.01^{a}	13.22 ± 0.01^{a}	17.61 ± 0.03^{b}
Pb	*BDL*	*BDL*	*BDL*
Zn	22.70 ± 0.01^{a}	25.30 ± 0.46^{b}	30.80 ± 0.06^{c}
Ni	1.88 ± 0.06^{a}	0.84 ± 0.09^{b}	2.30 ± 0.20^{c}

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Mn	40.21±1.89^{a}	36.19±0.90^{b}	41.07±0.90^{a}
Cr	0.48±0.03^{a}	0.40±0.05^{a}	0.66±0.06^{b}

Reported values were expressed as mean ± standard error of mean (S.E.M) of triplicate values (n=3). The values with different superscript (a,b,c) in same column showed significant difference at p≤0.05. BDL = Below Detectable Limit

Table 3: Heavy metal concentrations (mean ± S.E.M, mg/Kg wet wt.) in *Clarias gariepinus* from the study areas (Kurutie, Okerenkoko and Kurukunama).

Heavy metals	Kurutie	Okerenkoko	Kurukunama
Cu	*BDL*	*BDL*	*BDL*
Fe	124.85±2.66^{a}	130.22±1.86^{b}	123.23±2.60^{a}
Cd	0.070±0.02^{a}	0.04±0.01^{a}	0.03±0.01^{a}
As	1.36±0.01^{a}	1.34±0.01^{a}	1.40±0.02^{a}
Se	13.40±0.30^{a}	13.60±1.33^{a}	15.64±0.01^{b}
Pb	*BDL*	*BDL*	*BDL*
Zn	20.49±0.02^{a}	27.81±0.30^{b}	33.04±0.16^{c}
Ni	0.94±0.09^{a}	0.82±0.09^{a}	1.84±0.03^{b}
Mn	38.81±0.66^{a}	42.00±0.05^{b}	47.00±0.05
Cr	0.28±0.01^{a}	0.40±0.03^{b}	0.40±0.04^{b}

Reported values were expressed as mean ± standard error of mean (S.E.M) of triplicate values (n=3). The values with different superscript (a,b,c) in same column showed significant difference at p≤0.05. BDL = Below Detectable Limit

Table 4: World Health Organization (WHO) and European Union (EU) permissible values of Heavy Metal in Consumable Aquatic Animals in mg/Kg

Heavy metal	WHO, 1993	EU, 1998
Cu	2.00	2.00
Fe	0.50-50	0.20
Cd	0.003	0.005
As	0.01	0.01
Se	0.01	0.01
Pb	0.01	0.01
Zn	3.00-50.00	50.00
Ni	0.02	0.02
Mn	0.50	0.05
Cr	0.05	0.05

3.1 Discussion

Table 1, 2 and 3 shows the heavy metal concentrations (mg/Kg dry weight) in *Callinectes pali*, *Tilapia queneesis* and *Clarias gariepinus* from the three study locations. Table 4 is the standard of World Health Organization (WHO) and European Union (EU) for permissible values of heavy metals in all consumable aquatic animals (mg/kg). The concentrations of Lead and Copper were below detectable limits in both samples from the three study locations. This agrees completely with other study [17], but completely in contrast to other findings, where the concentration of Pb in tilapia fish were 12.90±0.11, 16.20±0.13 and 15.70±0.30 mg/kg (dry wt) from three different locations [18]. Copper and Lead metals which are below the detectable limit means that both heavy metals are absent or their presence are infinitesimal which must have caused their inability to be detected by the spectrometer.

The Iron concentration in the three samples were significantly higher than the WHO and EU (0.20 – 50 mg/kg) Fe permissible limit in aquatic foods. *Callinectes pali* showed the highest amount of Fe of 201.00±4.82, 196.00±3.77 and 204.00±4.70 while *Clarias gariepinus* and *Tilapia queneesis* recorded the least amount of Fe 124.85±2.66, 130.22±1.86, 123.23±2.60 and 126.70±3.77, 131.30±6.34, 132.00±4.81 respectively from the three locations. Iron is a mineral vital for the proper function of haemoglobin, a protein needed to transport oxygen in the blood. Iron also has a role in a variety of other important processes in the body. The recommended daily allowance (RDA) of Iron varies between ages, infants between the age of 0-12 months (0.27 – 11mg/day), children at the age 1-8 years (7 – 10mg/day), males at the age of 9years and above (8-11mg/day) while females between the age 9years and above (8-20mg/day) but women who are pregnant require the most (27mg/day) [1, 2]. Iron promotes healthy pregnancy, increased energy, and better athletic performance. Iron deficiency is most common in female athletes. Too much iron can increase the risk of liver cancer and diabetes. Iron supplements can be helpful when people find it difficult to take in enough iron through only dietary measures, such as in a plant-based diet. It is better to try to consume enough in the diet alone by removing or reducing factors that may hinder iron absorption and consuming iron-rich foods. This is because many iron-rich foods also contain a range of other beneficial nutrients that work together to support overall health. Iron helps to preserve many vital functions in the body, including general energy and focus, gastrointestinal processes, the immune system, and the regulation of body temperature. The benefits of iron often go unnoticed until a person is not getting enough. Iron deficiency ‘‘anaemia’’ can cause fatigue, heart palpitations, pale skin, and breathlessness.

The observed concentrations of Cadmium in the three samples were within the WHO and EU (Table 4) permissible limit. *Callinectes pali* Cadmium content was 0.05±0.01, 0.07±0.01, 0.03±0.01 for the three locations, *Tilapia queneesis* recorded 0.06±0.01, 0.03±0.01, 0.04±0.00 whereas *Clarias gariepinus* recorded 0.07±0.02, 0.04±0.01, 0.03±0.01 for the three locations. These results were in contrast to the findings other findings [19] which recorded that the Cadmium concentration in a sea food analysed was 2.00mg/kg, also the result is in agreement with other findings which recorded that the Cadmium concentration in a fish sample from crude oil contaminated river was 0.050mg/kg [20]. The levels of Chromium concentration in *Callinectes pali, Tilapia queneesis* and *Clarias gariepinus* ranged from 0.13±0.05 to 0.84±0.03, 0.40±0.05 to 0.66±0.06 and 0.28±0.01 to 0.40±0.03 respectively. These were all above the WHO and EU Chromium permissible limit (Table 4). Both Cadmium and Chromium are elemental hazards to human health even at trace quantity. Cadmium is a mobile element and can easily be distributed across an affected area. Other research work showed that high concentration of Cadmium in consumable foods could cause death, bone fracture, diarrhoea, stomach pains, reproductive failure, cancer development, DNA damage and central nervous system [3, 21, 22].

The concentration of arsenic may be higher in a particular geographical region. This could be as a result of human activity, such as mining of metals or the use of pesticides. Natural conditions can also lead to a higher concentration. The concentration of As measured from *Callinectes pali, Tilapia queneesis* and *Clarias gariepinus* varied from 0.74±0.10 to 0.90±0.04, 1.26±0.02±1.67±0.02 and 1.34±0.01 to

1.40±0.02 respectively from the three sampling locations. The Arsenic concentration from both samples were above the WHO and EU permissible limit (Table 4). There are trace amounts of arsenic in nearly all of the foods and beverages we consume, including vegetables, fruits, juices, rice, grains, seafood, meat, and wine. Increased exposure of humans to Arsenic causes excess saliva, convulsions, hair loss, blood in the urine, problems of swallowing, cramping of muscles and stomach. Arsenic poisoning, or Arsenicosis, happens when a person takes in dangerous levels of arsenic [23]. Arsenic is a natural semi-metallic chemical that is found all over the world in groundwater. Ingestion of Arsenic poses health problems if a dangerous amount of arsenic enters the body. Then, it can lead to cancer, liver disease, coma, and death. Inorganic arsenic compounds are more harmful than organic ones. They are more likely to react with the cells in the body, displace certain elements from the cell, and change the cell's function.

The concentration of Se from *Callinectes pali*, *Tilapia queneesis* and *Clarias gariepinus* varied from 11.46±0.53 to 12.30±0.30, 13.22±0.01 to 17.61±0.03 and 13.40±0.30 to 15.64±0.01 respectively from the three sampling locations. These concentrations were very far greater than the WHO and EU acceptable limit (Table 4). Selenium is an essential element, and therefore various national and international organizations have established recommended daily intakes of selenium. The joint World Health Organization (WHO)/Food and Agriculture Organization of the United Nations (FAO) consultation on preparation and use of food-based dietary guidelines [24] listed recommended intakes of 6–21 µg of selenium per day for infants and children, according to age, 26 and 30 µg of selenium per day for adolescent females and males, respectively, and 26 and 35 µg of selenium per day for adult females and males, respectively. However, WHO has recommended Selenium to be a potential cause of hair and fingernail changes; damage to the peripheral nervous system; fatigue and irritability. Most people obtain virtually all of their selenium from the foods they eat. In plant and animal tissues, selenium is found mostly bound to proteins. Therefore, the most important food sources of selenium are meats and seafood (0.3–0.5 mg/kg), because of their high protein contents, and cereals (0.1–10 mg/kg), because they tend to be consumed in large amounts. In contrast, foods with relatively low protein levels, such as vegetables and fruits, tend to have relatively low selenium contents (<0.01mg/kg). WHO [24] noted that global selenium intakes vary significantly; average intakes were relatively high in North America (85–150 µg/day), moderate in Europe (40–90 µg/day) and low in parts of China (10–20 µg/day). In Europe, dietary selenium intakes have declined in recent decades: 29–39 µg/day in the United Kingdom and 30–80 µg/day in the Nordic countries in 1997 [25], compared with earlier intakes of 40–90 µg/day[24]. This decline has been attributed to reductions in the importation of higher-selenium wheat grown in North America. Very low selenium status in humans has been associated with a juvenile, multifocal myocarditis called Keshan disease and a chondrodystrophy called Kaschin-Beck disease [26-28]. While Kaschin-Beck disease has not been well characterized, it is clear that selenium supplementation can prevent Keshan disease. Still, the etiology of Keshan disease has been difficult to understand. Studies by Levander and Beck [29] and Beck, Levander and Handy [30] have shed light, however; they suggest a role of a cardiophilic virus, the virulence of which increases in selenium-deficient hosts.

The concentration of Zinc in *Callinectes pali*, *Tilapia queneesis* and *Clarias gariepinus* varied from 44.30±0.77 to 48.60±0.12, 22.70±0.01 to 30.80±0.06 and

20.49±0.02 to 33.04±0.16 (Table 3, 2 and 1) respectively from the three sampling locations. These concentrations are within the WHO and EU Zn permissible value (Table 4). Zn takes part in body reactions such as human immune system booster, protein and DNA production, healing of wound, growth, cell metabolism and good sense of smell and taste. Excess zinc include nausea, vomiting, loss of appetite, stomach cramps, diarrhoea, and headaches.

The concentration of Nickel in *Callinectes pali, Tilapia queneesis* and *Clarias gariepinus* varied from 0.84±0.02 to 0.99±0.01, 0.84±0.09 to 2.30±0.20 and 0.82±0.09 to 1.84±0.03 respectively from the three sampling locations. The concentrations of Ni from the samples were above the WHO and EU Nickel concentration permissible value (Table 4). The body needs nickel, but in very small amounts. Nickel is a common trace element in multiple vitamins. Nickel is used for increasing iron absorption, preventing iron-poor blood (anemia), and treating weak bones (osteoporosis). Much exposure to Nickel also causes blisters and draining fluids, skin patches, severe itching, redness of the skin and rashes or bumps on the skin. The concentration of Manganese in *Callinectes pali, Tilapia queneesis* and *Clarias gariepinus* varied from 31.40±0.40 to 36.00±0.66, 36.19±0.90 to 41.07±0.90 and 38.81±0.66 to 47.00±0.05 respectively for the samples from both locations. The recorded concentrations exceeded the WHO/EU Manganese permissible limit. Taking more than 11 mg per day by mouth is highly risky for most adults. Manganese is unsafe when inhaled by adults for long periods of time. Excess manganese in the body can cause serious side effects, including symptoms resembling Parkinson's disease, such as shaking (tremors).

4.0 Conclusion and Recommendations

The presence of heavy metals in this Escravos River environment is attributed to petroleum prospecting and mining. Crabs, Cat fish and tilapia fish are the most commonly available sea foods found in Escravos river with tilapia and crab dominating and cat fish being seldomly found in a secluded areas of the river because of the salt content of the river. This research established a fact that the concentrations of Cu, Fe, Cd, As, Cr, Se, Pb, Zn, Ni and Mn with exception to Pb, Cu, Zn and Cd from Escravos River sea foods are of high range which implies that consumption of these samples analysed could cause the risk of different ailments as discussed in the literature. The concentrations of Pb, Cu, Zn and Cd are not health threatening from the samples. It will be of environmental benefits if more work on quantifying both the concentrations of heavy metals, total hydrocarbon content and Polycyclic aromatic hydrocarbons present in the sea foods from Escravos river. This check/monitoring should be regularly carried out from time to time because crude oil pollution does not regularly occur in the environment. The community should also enforce proactive measures that could put an end to the most causes of crude oil pollution in the environment such as bunkering and theft. It can also be inferred that the contamination of this river leading to the bioaccumulation by the aquatic animals come from improper waste disposal of environmental wastes from the area. It was observed that this river is the sink for waste disposal for these three communities in study, as a result, most house wastes contains larger amount of these heavy metals which could have not been bioaccumulated in the environment through crude oil pollution. Proper waste management should be put in place to check the main sources of these heavy metals.

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