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Research Article

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Human Health Risk Assessment of Consuming Heavy Metals in Oyster (*Crassostrea virginica*) from Different Markets in Port Harcourt, Rivers State

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Abstract
Food safety is of great concern in the Niger Delta region of Nigeria because of its pollution levels. Shell fish is one of the major diets of the people of Rivers State and they affect a large market and customer target in the region. This study was aimed at assessing the human health risk from consuming oysters bought from different markets in Rivers State namely; Creek Road market Borokiri, Mile 3 Market and Choba Market while Oysters harvested from ARAC (Buguma) were used as control. Lead (Pb), Cadmium (Cd), Chromium (Cr), Cobalt (Co) and Nickel (Ni) were analysed in all samples using the Atomic absorption spectrometer. Cobalt had the highest concentration in all the samples from the markets except Mile 3 market, it ranged from 0.011-0.014mg/kg, while Lead ranged from 0.007-0.012 mg/kg, Chromium ranged from 0.008-0.010 mg/kg, Nickel ranged from 0.008-0.021 mg/kg and Cadmium ranged from 0.001-0.007mg/kg .The Estimated Daily Intake (EDI) of these metals were calculated, Target Human Quotient (THQ) and Hazard Index (HI) derived. EDI values were within tolerable limit. THQ and HI calculated indicated likely risk from oyster consumption since the values obtained were above the threshold of one (1) for lead, Cobalt and Cadmium, long term consumption may lead to health risk.

Keywords: Estimated Daily Intake, Target hazard quotient, Hazard Index

Introduction
Heavy metals contamination is a major problem in the environment and they are one of the major contaminating agents of food supply [1, 2]. The problem of heavy metal contamination is receiving more attention all over the world, particularly in developing countries because it is of public health concerns. The biological half-lives of these heavy metals are long and have the potential to accumulate in different body organs and thus produce unwanted side effects [3, 4]. Although theses heavy metals can be picked up by living organisms from a variety of sources, the most common and vulnerable source are water bodies which receive effluent discharges containing trace metals from production processes. Excessive accumulation of these heavy metals in human have been implicated in cardiovascular and bone diseases [5]. It is known that serious systemic problems can develop as a result of increased accumulation of dietary heavy metals such as cadmium and lead in the human body [6]. Heavy metals are extremely persistent in the environment; they are non-biodegradable and non-thermo-degradable and thus their accumulation may readily reach to toxic levels. Heavy metals poisoning could result from eating heavy metal contaminated foods, exposure to polluted air and water. It is important to note that not all heavy metals are toxic at low quantities. However, all heavy metals can become and will become toxic if they exceed permissible levels in any living system. Each heavy metal toxicity depends largely on dosage, mode of exposure, route of entry, frequency of exposure, susceptibility of the body, age and exposure duration. Excessive amount of any particular heavy metal may produce cellular and tissue damage leading to a variety of adverse effects and diseases in humans.

Different studies have been carried out in recent years on the effects of heavy metals on aquatic organisms and shell fishes owing to their non-biodegradability, long half-lives, cytotoxic and carcinogenic effects on the human population [7]. However, these studies were not limited to only sea foods as vegetables and general food items are also affected by these heavy metal concentrations and cause problems for the population. Studied heavy metal contamination in some markets in Rivers state and observed that fish from Mile 1 market Diobu had Lead levels higher than the WHO permissible limit of 0.3 mg/kg with a fold range of 0.270 to 0.390 mg/kg [8]. Assessed toxic trace metals in selected fish species and parts of domestic animals and reported mean Lead concentrations (mg/kg) in fishes species as (3.5 ± 0.06), (3.43 ± 0.02), (4.6±0.43) and (4.78 ± 0.045) which exceeded the recommended limits specified by most food regulatory bodies [9]. Studied Bioaccumulation of heavy metals in oysters from the Southern Coast of Korea, the Cd concentration was the highest of the three hazardous metals (Cd, Pb, and Hg); However, the concentrations of the harmful metals in the study were within the regulatory limits set by Korea and other countries [10]. Studied heavy-metal contents in oysters (*Crassostrea gigas*) cultivated on the southeastern coast of the Gulf of California and showed the following rank order of accumulation: Zn>Cu>Cr>Cd>Ni>Pb>As>Hg in oysters and observed that mean values of Cu and Cd burdens were higher when compared to other studies in Mexico but lower when compared to other countries [11]. Revealed that oysters (*Crassostrea rhizophorae* and *C. virginica*) has the ability to bioaccumulate copper and zinc in their tissues Health risk assessment is an indelible tool for the evaluation of possible impacts of pollutant to man when food materials, especially from un-trusted areas are consumed [12, 13]. It is necessary to know a probable adverse health hazard to humans that might arise as a result of exposure to toxic levels of metals. Methods employed are thorough and are compared to stipulated standards. It also helps to ensure the quality assurance of food items. For a highly industrialized city like Port Harcourt, it is necessary to evaluate the human health risk of consuming oysters sold in the markets by assessing the heavy metal content in Oyster (*Crassostrea virginica*) from the different Markets in Port Harcourt, Rivers State.

Materials and Methods

Study Areas

Three major large open air markets were selected and oysters were bought at random from the sellers. These markets were Choba market which has the coordinates latitude 4.87°N and longitude 6.91°E, Creek road market Borokiri with latitude 4.7386°N, longitude 7.0337°E and Mile 3 market, Diobu with latitude 4°47´24´N and longitude 6°59´36´E. These markets were chosen because they contained a wide range of people patronizing them, different classes and ages and due to their very open nature are likely markets susceptible for heavy metal residues. Samples were also collected from Africa Regional Aquaculture Centre (ARAC) located in Buguma with latitude 4°73N and longitude 6°86E as control. ARAC is a research outfit where expected pure breed of fish can be obtained for research purposes.

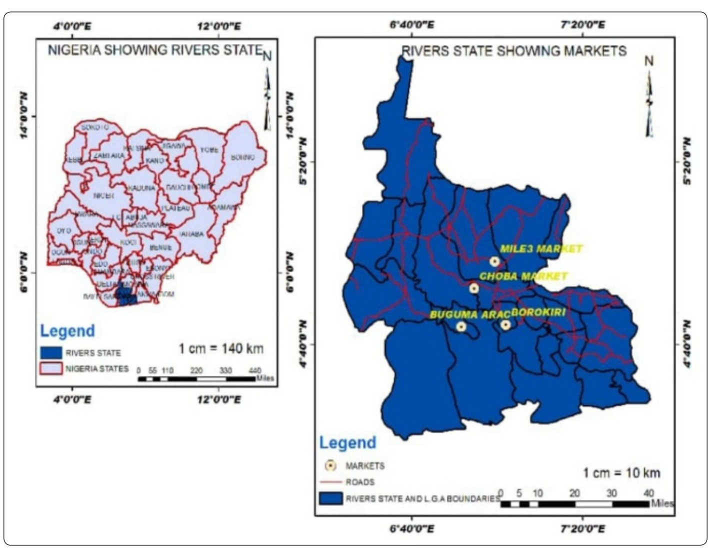

Figure 1: Map of the study Area

Sample Collection

Fresh Oyster (*Crassostrea virginica*) sample were collected at random from the different markets in Port Harcourt, kept in polythene bags and taken to the laboratory the same day for further analysis. Samples were collected in one day excluding the sample from Buguma. These samples were stored in a refrigerator until preparation.

Sample Preparation

Tissues of the samples collected were removed from its shell and thoroughly washed and oven dried. After drying, samples were grounded using a mortar and pestle and sieved through a 500µm-mesh. The samples were all prepared individually based on the location of each sample. Samples were then labelled accordingly and stored in a polyethene bag until digestion.

Digestion

3 grams of each of the samples were measured using a very sensitive weighing balance (0.0001 g) and were digested using 10 ml of hydrogen peroxide. Although this process is exothermic, it was set on a hot plate for about 10-12 minutes until all the fumes had disappeared and then allowed to cool. Hydrogen peroxide was added to digest the organic content of the samples and to enable a clear sample digestion process. 10 ml of HCl was added with the use of a measuring cylinder into 250 ml beakers containing each of the samples. 30 ml of $HNO3$ was also added to the sample mixture after which it was set on a hot plate to heat up until the fumes had disappeared and a clear sample was obtained. The digested solutions were then cooled at room temperature, filtered using 11mm filter paper and transferred to a conical flask containing 100 ml with distilled water. Samples were then labelled and kept in clean plastic containers before metal analysis. The total metal concentrations were determined by flame atomic absorption spectrometer type A 200 AA.

Quality Assurance and Quality Control

All samples were collected randomly and in a representative manner. Samples were stored in a polythene bag and transported to the laboratory the same day they were collected on ice chest to avoid any external form of contamination or spoilage. These samples were then stored in the refrigerator for preservation. All representative samples were analysed to allow for statistical variation if any. The sampling and processes of analysed samples was carried out as described by American Public Health Association 1020QC [14].

Health Risk Assessment

The potential health risks of heavy metal consumption from oyster were assessed based on the Estimated Daily Intake of metals (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI).

Estimated Daily Intake

The estimated daily intake of metals is the amount of a substance in food expressed on a body mass basis (usually in mg/kg body weight), which can be ingested daily over a lifetime by humans without appreciable health risk. The EDI of trace metals were estimated from the average concentrations of trace metals in the samples studied and estimated daily average consumption rate of 34.5 g for fish was used for an adult with average body weight of 60 kg as reported by [15].

$$EDI = \frac{C_{metal} \times D_{Food\ intake}}{BW_{average}} \quad (1)$$

Where C is the concentration of heavy metal in the tissues of oysters, $D_{Food\ intake}$ is the daily average intake of oysters in the study region and BW is the average body weight in kg.

Target Hazard Quotient

The Target Hazard Quotient (THQ) has a bench mark of 1 was calculated using equation 2

$$THQ = \frac{EFr \times ED \times FIR \times C}{RfD \times BWaverage \times ATn\ (EFr \times ED)} \times 10^{-3} \quad (2)$$

EF is the exposure frequency 365 days/year, ED is the exposure duration, equivalent to average life expectancy of a Nigeria 55 years, Rfd is reference dose, Table 1 contains the reference dose used, FIR is the shellfish ingestion rate (Kg/person/day) which is considered to be 0.0345kg/person/day in Nigeria C is the trace metal concentration in food stuffs mg/kg BW average = average body weight (halfway between the 70 kg male and 50 kg female normally taken as the standard 60kg) and ATn is the average exposure time for non-carcinogens [15, 16].

Table 1: Reference Dose stipulated by USEPA, [17]

Heavy metal	Cr	Pb	Cd	Co	Ni
RfDo (mg/kg/day)	1.5	0.0035	0.001	0.0003	0.02

Hazard Index

Hazard index (HI) is used to calculate the potential risk to human health for more than one heavy metal. It is the sum of hazard quotients (HQs). Different pollutants can cause similar adverse health effects, it is often appropriate to combine HQs associated with different substances.

$$HI = \Sigma THQ\ (THQ_1 + THQ_2 + THQ_3 \ldots\ldots\ldots\ THQ_n)\ \ldots\ldots \quad (3)$$

Results

The values of heavy metal concentration in the studied samples is represented in Table 2. The values ranked as Co > Pb > Cr > Ni > Cd for Samples from Choba Markets and ARAC in Buguma while Co > Pb =Cr > Ni > Cd for Samples from Creek Road Market and Ni > Co > Cr > Pb > Cd for Samples from Mile 3 Market. Cobalt had the highest concentration in all the samples from the markets except in Mile 3 market. Cobalt ranged from 0.011-0.014mg/kg, while Lead ranged from 0.007-0.012 mg/kg, Chromium ranged from 0.008-0.010 mg/kg, Nickel ranged from 0.008-0.021 mg/kg and cadmium ranged from 0.001-0.007mg/kg

Table 2: Heavy metal concentrations (mg/kg) in oysters from 3 different markets and ARAC in Rivers State [18].

Heavy metal	Choba market	Creek road market	Mile 3 market	ARAC Buguma	FAO/WHO, 2011, 2015
Chromium (Cr)	0.009	0.009	0.008	0.010	0.1
Lead (Pb)	0.012	0.009	0.007	0.011	0.3
Cadmium (Cd)	0.001	0.004	0.001	0.007	2.0
Cobalt (CO)	0.014	0.011	0.011	0.013	-
Nickel (Ni)	0.008	0.008	0.021	0.008	0.2

Estimated Daily intake (EDI)

The values of the Estimated Daily Intake (EDI) is represented in Figure 2. Oyster from Choba market had an EDI value in ascending order of 5.75E-07, 4.60E-06, 5.18E-06, 6.90E-06 and 8.05E-06 for Cd, Ni, Cr, Pb and Co respectively, while Oyster from Creek road market had an EDI values in ascending order of 2.30E-06, 4.60E-06, 5.18E-06, 5.18E-06 and 6.33E-06 for Cd, Ni, Cr, Pb and Co respectively. Oyster from Mile 3 had EDI values of 5.75E-07, 4.60E-06, 4.03E-06, Cobalt 6.33E-06 and Nickel 1.21E-05 for Cd, Cr, Pb, Co and Ni respectively. Oyster from ARAC in Buguma had EDI values in an ascending order of 4.03E-06, 4.60E-06, 5.75E-06, 6.33E- 06 and 7.48E-06 for Cd, Ni, Cr, Pb and Co respectively

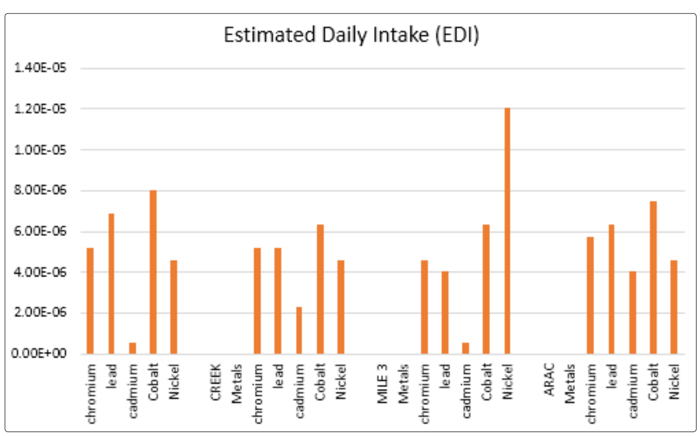

Figure 2: Estimated Daily Intake (EDI) of heavy metals for Adult in Oyster

Target Hazard Quotient (THQ)

The values of the Target Hazard Quotient is shown in Figure 3. Cobalt has the highest THQ with a THQ of 26.833, 21.0833, 21.0833 and 24.916 from Choba, Creek road, Mile 3 Markets and ARAC respectively, followed by Lead which has THQ of 1.971, 1.478, 1.15 and 1. 807 from Choba, Creek, Mile 3 Markets and ARAC respectively, while Cadmium has a THQ of 0.575, 2.3, 0575 and 4.025 from Choba, creek, mile 3 Markets and ARAC respectively, while Nickel had THQ of 0.23, 0.23, 0.603 and 0.23 from Choba, creek, mile 3 Markets and ARAC respectively, while Chromium had the least THQ of 0.00345, 0.00345, 0.00306 and 0.003833 from Choba, creek, mile 3 Markets and ARAC respectively.

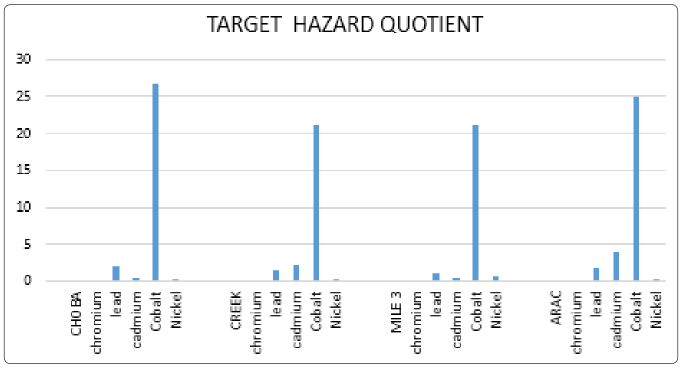

Figure 3: Target Hazard Quotient for adult exposed to heavy metals

Hazard Index (HI)

Hazard index value of 29.613 was calculated for oyster from Choba market while HI value of 25.095 was calculated for Creek road Market, 23.415 for Mile 3 Markets and 30.983 for ARAC in Buguma

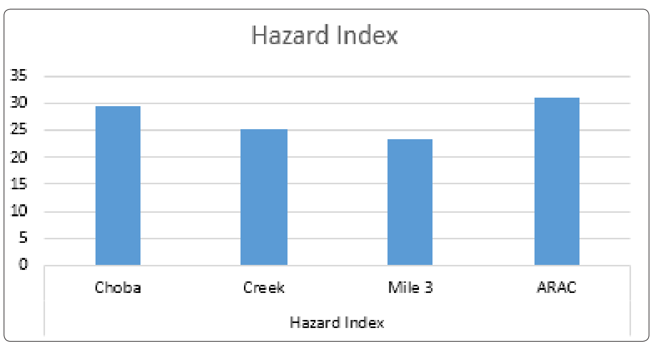

Figure 4: Hazard Index for heavy metal mixture

Discussion

Heavy metals have been implicated in so many disease because of the toxicity which has increasing significance for ecological, evolutionary, nutritional and environmental reasons [19, 20] reported implications of excessive intake of trace metals in pathological events and inflammation [19, 20]. Pb levels in this study did not exceed the maximum permissible limit stipulated by were observed to be lower than those reported by a Pb range of 0.00–61.17 µg/g in different foodstuffs from southeastern Nigeria [15, 21]. Also reported lead concentration of 76.07±161.48mg/g in *Citharinus citharus* and 23.16±26.30 mg/g in *Tilapia zilli* while reported mean Pb level of (0.150 ± 0.062 µg/g) in oyster from Korea which is higher than levels found in this study Lead causes toxicity in living cells by following ionic mechanism and that of oxidative stress, the ionic mechanism of lead toxicity causes significant changes in various biological processes such as cell adhesion, intra- and inter-cellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters [19, 22]. Lead can substitute calcium even in picomolar concentration affecting protein kinase C, which regulates neural excitation and memory storage [19, 23].

Cobalt levels reported in this study had the highest values than any of the other metals analysed, there are no permissible limit for Cobalt. It is an essential part of Vitamin B12 which is beneficial. However, exposure to high levels of cobalt can result in lung and heart effects and dermatitis, reported cobalt concentrations in the range of 0.02 - 0.67 mg/kg for muscles of fish from the fish markets in India, while reported 0.006 - 0.244 µg/g concentration for muscles of fish from the coastal waters of the Caspian Sea which is within the range of the concentration found in this study [24, 25]. Reported a higher concentration of 0.113 mg kg in fresh and marine water fishes [26]. The result of the current study showed that the level of cobalt in fish may not pose any health risks. Nickel level in the present study indicate very low concentration and are below the stipulated permissible limit by WHO/FAO. Reported Ni values of 1.64 – 3.58ppm from fishes from Olomoro water bodies and values of 0.24 and 0.36ppm in *Mormyops deliciosus* and *Mormyrus mactrophthalmus* has been reported by Ikpoba river dam which is higher than the one reported in this study [27, 28]. Nickel is believed to contribute to physiological process as a co-factor in the absorption of Iron from the Intestine. However, at a higher level in the body Nickel can become toxic or act as a carcinogen [29]. Nickel can cause respiratory problems, its acute toxicity arises from competitive interaction with five major essential elements namely: calcium, cobalt, copper, iron and zinc [30]. Values of Cadmium were below the permissible limit of and the concentration reported in this study ranged from 0.008-0.01mg/kg which is much lower than the concentrations recorded in tissues of fish species *Scomber scombrus* Species Sold in Nigeria ranged between 2.133mg/kg and 19.758mg/kg [21, 31]. Reported Cd concentrations of 0.11-1.03ug/g in the flesh of *Clarias anguillaris* and *Synodontis budgetti* from the River Benue in Nigeria [32]. Several reports were found which shows that cadmium are usually accumulated in the tissues of fish [33, 34, 35, 36]. Cadmium and zinc have the same oxidation states and hence cadmium can replace zinc present in metallothionein, thereby inhibiting it from acting as a free radical scavenger within the cell. Acute cadmium ingestion can also cause gastrointestinal tract erosion, pulmonary, hepatic or renal injury and coma, depending on the route of poisoning [37, 38]. Chronic exposure to cadmium has a depressive effect on levels of norepinephrine, serotonin, and acetylcholine [37].

Values of chromium fell below the Maximum permissible limit stipulated by [21]. The concentration of Chromium in this study ranged from 0.008-0.01 which was lower than the concentration of 0.05- 0.12ppm in Fishes from Kiri Dam and River Gongola, North eastern Nigeria reported by [39]. The biological usable form of chromium plays an important role in Glucose, Lipid, Protein metabolism and Insulin action though beneficial to some extent it has also been reported that long term exposure to Cr can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation [40, 41].

The Estimated Daily Intake (EDIs) of Cr, Pb, Cd, Co and Ni were evaluated according to the average concentration of each metal in each food item and the respective consumption rates for adults are presented in figure 2. The EDI values were also compared with the oral reference dose (RfD) to assess potential health risks. EDI values were very low and when compared with the tolerable daily intake the values were below the tolerable daily intake THQ greater than one indicates a likely risk, in this study Cobalt values were highest in all the markets followed by Lead, with THQ greater than one (1), Cadmium had THQ greater than one (1) in oysters from Creek Road Market and ARAC in Buguma, while Nickel and Chromium had values less than one (1) The THQ values for Cr and Ni were all below 1 in all the markets and ARAC. Therefore, the daily intakes for Chromium and Nickel derived from a real meal at levels of assumed exposure may not likely cause any adverse effect during a human lifetime. However, THQ values of Pb and Cd indicate the presence of a likely health risk, this means that there is a potential risk of developing chronic systemic effects due to Pb and Cd intake. There was a high concentration of Cobalt in all the markets when the THQ was calculated. This is due to the fact that Cobalt is an essential element that supplies the body with Vitamin B12 and once Cobalt makes its way through the environment it cannot be destroyed [42]. It is safe to say that the Cobalt levels are not any cause for alarm as they are essential body mineral needed by the body but must not pass the threshold required by the body. It was observed that oyster from ARAC had the highest HI followed by Choba, Creek road and then Mile 3 Markets. Humans are exposed to more than one heavy metals and hence the interactive effect of heavy metal mixture. The HI index was above the recommended threshold of HI<1. Therefore population exposed to Oyster consumption from this market might be at greater risk of heavy metal toxicity of Pb, Cd, Cr, Co and Ni. HI values reported in this study were greater than one (1). Reported HI values above the recommended threshold of one (1) in their studies [43, 44].

Conclusion

The study showed that Oysters collected from Choba, Mile 3, Creek road markets and Buguma have low concentrations of heavy metals which were all below the Permissible limit when compared to WHO/FAO regulatory limits. EDI values were also within tolerable limit. THQ and HI calculated indicated likely risk from Oyster consumption since the values were above the threshold of 1 for lead, Cobalt and Cadmium, long term consumption may lead to health risk. Hence, stringent environmental laws should be reinforced and compliance should be adhered to in order to protect humans from consuming contaminated fish and continuous monitoring of Fish sold in the markets should be implement by policy makers.

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