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Bioaccumulation of heavy metals in some tissues of croaker fish from oil spilled rivers of Niger Delta region, Nigeria

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ABSTRACT

Objective: To investigate the level of heavy metals (Cu, Zn, Fe Mn Ni, Pb and Cd) in muscles, gills and liver tissue of Croaker fish from oil spilled rivers of Bonny and Finima, Niger Delta region, Nigeria.

Methods: Twenty four Croacker fishes (*Genyonemus lineatus*) weighing between 250 and 260 g and 24–26 cm length were collected from each sampling sites (Bonny and finima rivers). The frozen fishes were thawed and dried at 105 °C until they reach a constant weight. The dried samples were homogenized and digested with 10 ml tri-acid mixture (HNO_3: $HClO_4$:H_2SO_4), and then the digested samples were diluted to 100 ml with deionized water. Heavy metals (Cu, Ni, Zn, Pb, Mn, Iron Fe, and Cd) concentrations were determined by atomic absorption spectrophotometer.

Results: Our results revealed that concentrations and distribution patterns of all heavy metals studied varied significantly ($P < 0.05$) amongst the fish tissues analyzed and sampling sites. Moreover, liver tissue of fish caught from Finima creek accumulated the highest concentrations of Cu (52.64 ± 3.01 μg/g dry wt), Zn (166.50 ± 6.45 μg/g dry wt) and Fe (801.50 ± 14.15 μg/g dry wt) in comparison to the liver of fish caught from Bonny river in which the levels of Cu, Zn and Fe were (45.00 ± 2.79), (49.90 ± 2.91) and (216 ± 6.11 μg/g dry wt), respectively. In addition, Mn, Ni, Pb and Cd exhibited their highest concentrations in gills from both locations. As expected, muscle tissue contained the least concentrations of all metals investigated from both sampling sites. Furthermore, all the metals investigated exhibited highest concentration in fish collected from Finima creek. These abnormal high level heavy metals accumulation observed in this location could be linked to the frequent crude oil spills as well as industrial activity around the area which might get discharged into Finima creek. In general, the mean concentrations of some toxic heavy metals investigated exceed the recommended maximum permissible limits set by the Joint FAO/WHO committee. However, some metals are within the acceptable limits.

Conclusions: In conclusion, our data showed the abnormal higher concentration of these metals and this might be toxic to the fish and other aquatic organisms directly or by extension to humans that frequently consumed such contaminated fishes.

1. Introduction

Pollution can be caused by many sources, including agricultural drainage, industrial effluent discharge, sewage discharge, accidental chemical wastes disposal, oil spills, and gasoline from fishing boats [1,2]. In addition, global rapidity in the development of crude oil exploration and transportation has increased the tendency of oil spill incident which release heavy metals including Pb, Ni, V, Zn, Cd as the major contaminants into the environments which might caused many adverse health effects to humans [1,3]. The need for agricultural growth and industrialization in Nigeria has resulted in the increase of heavy metals pollution particularly in the Niger Delta region in which oil exploration, transportation and refining activities are conducted [4]. Oil spill is the uncontrolled release of crude oil into the environment and a major contributor for the higher levels of heavy metals

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in different types of environment such as soil, seawater and freshwater in oil producing areas of Niger Delta region [5,6]. An oil spill incident in various parts of the region has been reported at such a frequent rate in the country [7]. Crude oil through spills often spread out over a wide area, destroying crops and aquaculture through contamination of rivers, lakes, ground water and soils, making the environment uninhabitable for living things [5,8]. Moreover, these pollutants from crude oil tend to spread faster and wider when in aquatic environment as most moving water bodies are connected. Crude oil and its refined petroleum products have been reported to contain several toxic organic and inorganic components such as polycyclic aromatic hydrocarbon compounds and molecules of sulfur, nitrogen, oxygen as well as metals such as iron, vanadium, sodium, nickel, chromium and other metals [9], which constitutes a significant health risk to both marine organisms and people. Excessive levels of heavy metals are a global concern, due to their potential toxic effects and ability to bioaccumulate in aquatic ecosystems and food chains [10–13]. Moreover, consumption of fish in the world has increased especially due to the available information regarding their nutritional and beneficial health effects in humans. For instance, fish is considered as an important source of animal proteins and other essential minerals, vitamins and unsaturated fatty acids [14]. However, fishes are relatively situated at the top of the aquatic food chain and normal metabolism of fishes can bioaccumulates several heavy metals from contaminated food, water and sediments [15,16]. The contents of toxic heavy metals in fish can counteract their beneficial health effects in humans [17]. Because of serious threats associated with consumption of heavy metals contaminated fish such as renal failure, liver damage, cardiovascular diseases and even death [18,19]. In Nigeria, fish and other aquatic organisms constitute a large part of daily meal of human population, consumption of heavy metal-contaminated fish for prolonged period results in accumulation of heavy metals in human. Bonny River and Finima creek are the major sources of fish and other sea foods for the people of the region. However, incidence oil spills and environmental pollution of nearby rivers and lakes posed a serious health risk to the people of the region due to crude oil spillage and natural gas exploration and refining activities. Although, several studies demonstrated that fishes are important biological indicator for investigation of heavy metal contamination and health risk to both animals directly and human upon consumptions of aquatic foods in different parts of the world [20–24]. However, most of these studies focused mainly to the fish muscle tissue, which is the main part consumed by humans [25,26]. Therefore, herein the present study, our focus is to investigate the level of some heavy metals (Cu, Zn, Fe Mn Ni, Pb and Cd) in muscles, gills and liver tissue of Croaker fish caught from oil spilled rivers of Bonny and Finima which is commonly consumed by the people of Niger Delta region and also assess the quality and safety of the fish for human consumption.

2. Materials and methods

2.1. Study sites and sample collection

Bonny River and Finima creek are located in Rivers state Niger Delta region. Bonny river lies between Latitude 4° 26′ 0″, Longitude 7° 10′ 0″ (Figure 1). The river expands along the coast from the river's basin in the west. The area is characterized by extensive inter-connection of creek. Whereas Finima, is a small town near Bonny Island surrounded by long stretches of creek. Finima's creek is situated 4.8 km away from Bonny river. Bonny river is among the Niger Delta rivers that drain into the Atlantic Ocean and is connected to other rivers via creek. Twenty four Croacker fishes (*Genyonemus lineatus*) weighing between 250 and 260 g and 24–26 cm length were collected from each sampling site described above. The fishes were immediately preserved in an ice box and transferred to the laboratory where they were identified and stored at −80 °C until analysis.

2.2. Sample preparation, digestion and heavy metals determination

Prior to analyses, Croacker fishes were thawed at room temperature, washed with deionized distilled water, then liver, gills and muscles were removed using stainless knives. Each organ was dried at 105 °C until they reach a constant weight. The dried samples were homogenized to fine powder using ceramic mortar and pestle. One gram of each of the ground fish tissues were transferred to a porcelain basin and put into a Thermicon P muffle furnace at a temperature of 550 °C for 5 h. Samples were digested with 10 ml tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4) in a ratio of 6.5:6:2. The samples were heated at 105 °C until a clear colorless solution was obtained. The digested samples were allowed to cool and then diluted to 100 ml with deionized distilled water and then filtered through Whatman filter paper No. 42 and the filtrate was diluted to 100 ml with deionized water for determination of heavy metals (Cu, Ni, Zn, Pb, Mn, Iron Fe, and Cd) concentration by atomic absorption spectrophotometer (Shimadzu Model 6800 with graphite furnace Model GFA 7000). Heavy metals concentrations were expressed as μg/g dry weight.

2.3. Statistical analysis

Data were expressed and presented as mean values ± SEM from triplicates of independent experiments ($n = 3$) using GraphPad Prism 5.01 version (GraphPad Software Inc., San Diego, CA, USA). Comparisons were made using one-way ANOVA. Turkey's Post hoc test was employed to test the significance of difference between metal species in single organ. Student's *t*-test was used for comparison of data between Bonny and Finima rivers and $P < 0.05$ was considered as statistically significant.

3. Results

This study revealed significant concentrations of Cu, Ni, Zn, Pb, Mn, and Fe in the liver, gills and muscles of fishes sampled from Finima creek and Bonny River, River State Nigeria. The results of fishes from Finima Creek presented in Table 1, showed the highest mean concentrations of heavy metals compared to fishes from Bonny River (Table 2). For instance the mean concentrations of Cu, Zn Fe Mn Ni, Pb, and Cd in fish tissues from Bonny river were in the range of 3.50–45.00 μg/g, 14.00–49.90 μg/g, 102.00–216.03 μg/g, 9.34–11.02 μg/g, 5.33–9.60 μg/g, 0.20–0.50 μg/g and 0.00–1.50 μg/g, respectively. The distribution ranking of Cu, Zn, and Fe were in the order of liver > gills > muscles whereas Mn, Ni, Pb and Cd distributions

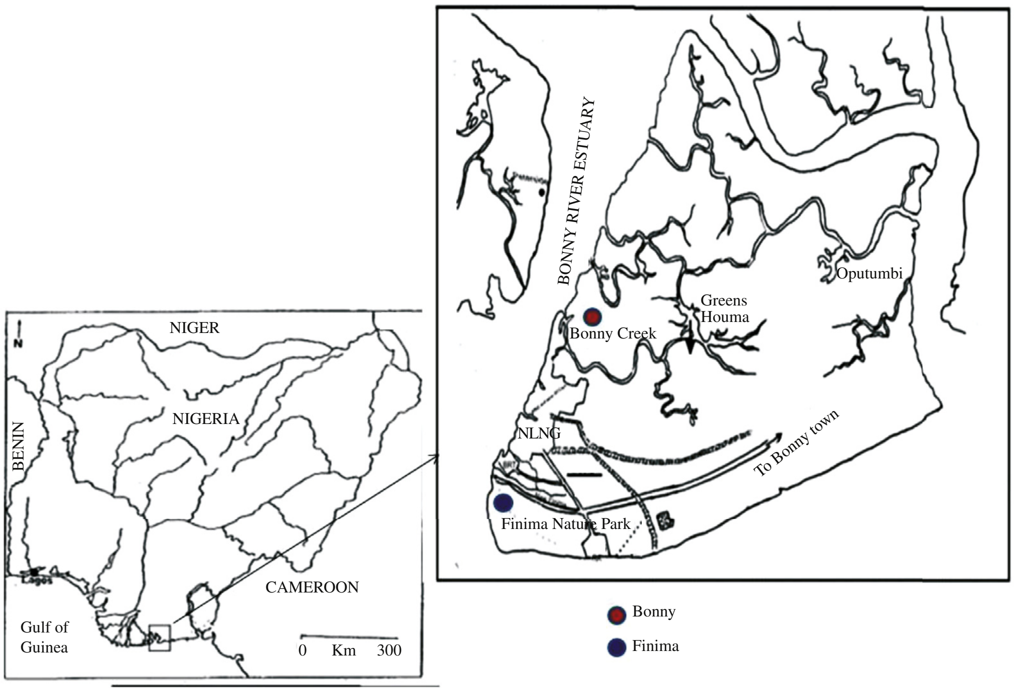

Figure 1. Map of Nigeria showing sampling sites (Bonny and Finima).

Table 1
Concentrations of heavy metals (μg/g dry wt.) in some organs of fish sample collected from Bonny River.

	Cu	Zn	Fe	Mn	Ni	Pb	Cd
Muscles	3.50 ± 0.77[c]	14.00 ± 1.55[c]	102.00 ± 4.20[c]	9.34 ± 1.27[a]	5.33 ± 0.96[c]	0.20 ± 0.05[b]	ND
Gills	14.00 ± 1.55[b]	24.90 ± 2.07[b]	139.90 ± 4.91[b]	11.02 ± 1.30[b]	9.60 ± 2.57[a]	0.50 ± 0.09[a]	1.50 ± 0.04
Liver	45.00 ± 2.79[a]	49.90 ± 2.91[a]	216.03 ± 6.11[a]	9.55 ± 1.28[a]	7.51 ± 1.13[b]	0.30 ± 0.07[b]	ND

Mean ± SEM (n = 8) bearing different superscripts down the column are significantly ($P < 0.05$) different in heavy metal concentration.

Table 2
Concentrations of heavy metals (μg/g dry wt.) in some organs of fish sample collected from Finima River.

	Cu	Zn	Fe	Mn	Ni	Pb	Cd
Muscles	15.75 ± 1.65[c]	124.50 ± 4.34[b]	565.60 ± 11.89[c]	43.72 ± 3.42[c]	30.00 ± 2.27[b]	5.00 ± 0.62[a]	ND
Gills	33.92 ± 2.42[b]	134.00 ± 4.18[b]	646.25 ± 12.71[b]	323.00 ± 7.47[a]	76.50 ± 3.64[a]	6.38 ± 0.75[a]	1.50 ± 0.50
Liver	52.64 ± 3.01[a]	166.50 ± 6.45[a]	801.50 ± 14.15[a]	202.50 ± 5.91[b]	37.00 ± 2.53[b]	3.63 ± 0.53[b]	ND

Mean ± SEM (n = 8) bearing different superscripts down the column are significantly ($P < 0.05$) different in heavy metal concentration.

were in the order of gills > liver > muscles (Table 1). Similarly, the mean concentrations of Cu, Zn Fe Mn Ni and Pb, and Cd respectively from fish caught from Finima creek were in the range of 15.75–52.64 μg/g, 124.50–166.50 μg/g, 646.25–801.50 μg/g, 202.50–323.00 μg/g, 30.00–76.50 μg/g, 3.63–6.38 μg/g and 0.00–1.50 μg/g. Furthermore, the trend of distributions of Cu, Zn, and Fe were in the order of liver > gills > muscles while the highest concentration of Mn, Ni, Pb and Cd was in the order of gills > liver > muscles (Table 2). Also, our data demonstrated that gills contained the highest concentrations of toxic heavy metals such as Mn, Ni, Pb and Cd in comparison to other tissues investigated. The mean concentrations of some toxic heavy metals investigated exceed the recommended maximum permissible limits set by the Joint FAO/WHO committee (Table 3). However, some metals are within the acceptable limits.

Table 3
Maximum permissible limits of heavy metals in fish (μg/g) according to WHO/FAO.

References	Cu	Zn	Fe	Mn	Ni	Pb	Cd
[36]	30	30	–	–	–	0.5	0.05
[37]	30	92.70	186	12.97	8.97	0.05	2.00
[38]	30	100	100	1.00	–	2.00	1.00

4. Discussion

Elevation of heavy metals concentration in fish tissues from aquatic environment is a good indicator of pollution status of the environment [27]. The present study, investigated the concentrations of heavy metals (Cu, Zn, Fe Mn, Ni, Pb and Cd) in muscles, gills and liver tissues of Croaker fish from Bonny and Finima rivers. The results of this study, demonstrated that all the heavy metal species investigated have been detected to some extent, except Cd which was detected only in the gills from the two sampling sites. Our data indicated that the mean concentrations of heavy metals in muscle, liver and gills differed significantly ($P < 0.05$). Moreover, the concentrations of some metals analyzed in different organs varied significantly ($P < 0.05$) amongst the sampling sites. Also, our data demonstrated that gills contained the highest concentrations of toxic heavy metals such as Mn, Ni, Pb and Cd in comparison to other tissues investigated. Our data were consistent with previous study which also reported that muscle contains lower concentration of heavy metals when compared to the gills and liver [28–30].

Copper is an essential element play vital role in enzymes activity and is necessary for the synthesis of hemoglobin [31]. However, when accumulated to higher amounts could pose health hazards to both animals and humans [32]. The present study reveals Cu accumulated in all fish tissues examined; the highest mean concentration of 52.64 ± 3.01 μg/g dry wt. was obtained in the liver tissue of sample from Finima creek. Whereas muscles contained the lowest concentration of 3.50 ± 0.77 μg/g dry wt. in fish caught from Bonny River. The accumulation of Cu amongst the fish organs investigated in both locations was in the order liver > gills > muscles. The higher levels of Cu obtained in this study correlates with the previous study which also demonstrated high concentrations of these metals in liver [33]. These higher accumulations of essential metals such as Cu in the liver may be attributed to the binding proteins such as metallothioneins and its role in storage, metabolism and detoxification which may increase its tendency to accumulate essential metals at higher concentrations [15,34,35]. Thus, our data clearly demonstrated that concentrations of Cu in fish tissues were significantly higher than maximum permissible limits set by the Joint FAO/WHO committee [36–38].

Zinc being an essential metal, is required in certain amount for normal metabolic functions and is involved in many cellular processes either as structural component of regulatory proteins or catalytic part of enzymes. When in excess amounts, Zn can be toxic to all living organisms including fish [39]. Our data showed quite variations in mean concentration of Zn in the studied fish tissues as well as sampling sites. For instance, the highest mean concentration of 166.50 ± 6.45 μg/g dry wt. was in the liver tissue of fish caught from Finima creek. The least concentration of 124.50 ± 4.34 μg/g dry wt. and 14.00 ± 1.55 μg/g dry wt. was detected in muscles while the accumulation pattern was in the order of liver > gills > muscles. The level of Zn obtained in the present study was higher than maximum permissible limit set by the Joint FAO/WHO committee.

Iron (Fe) is considered as essential metal because of its biochemical and physiological role in blood cells and hemoglobin synthesis and cofactor of many enzymes [34,40]. However, high amount of Fe above the physiological level in living organisms may result in iron overload [41]. Our results indicated that highest mean concentration of Fe was 801.50 ± 14.15 μg/g dry wt. in fish caught from Finima creek. As expected, muscle tissues which is the major part consumed by humans contained the lowest mean concentrations of 565.60 ± 11.89 μg/g dry wt. and 102.00 ± 4.20 μg/g dry wt. for Finima creek and Bonny River respectively. Fe accumulation in both sampling sites was in the following order of liver > gills > muscles. Our results were in agreement with previous study which demonstrated higher concentrations of Fe in the liver tissue of fish from the Egyptian Red Sea [42]. Also, previous studies covering different fish species reported similar observations [43–45].

Manganese is an essential metal, and low level is necessary for human health, however, excess amount can induced oxidative stress and toxic effects in aquatic organisms [46]. In this study, Mn concentration fluctuates between organs and the locations of sample collections. The highest mean concentration of Mn was 323.00 ± 7.47 μg/g dy wt. was detected in gills from fish caught in Finima creek. The lowest mean concentration of 43.72 ± 3.42 μg/g and 9.34 ± 1.27 μg/g dry wt. was detected in muscle tissues from Finima creek and Bonny River respectively. Moreover, the accumulation pattern was in the order of gills > liver > muscles. The mean Mn concentration recorded from tissue samples in this study was to some extent similar with previous studies [16,47].

Aquatic environments generally have low concentration of nickel. However, excess Ni can cause variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema and tumors [48]. Our data showed variation in mean concentration of Ni among the studied fish tissues as well as sampling sites. The highest mean concentration was 76.50 ± 3.64 μg/g dry wt. and was observed in gills of fish caught from Finima creek. While the mean concentration in muscles and liver tissue fluctuates amongst the sampling sites. The pattern accumulation of Ni was in the order of gills > liver > muscles. The mean concentration of Ni in the edible parts of fish recorded in the present study was above the established safe level of 5.5 mg/kg by Western Australian Food and Drug Regulations [49].

Lead (Pb) is one of the most ubiquitous metals with no known biological function in humans and it is detectable in all phases of the inert environment and biological systems [17]. In addition, Pb has been reported to cause neurotoxicity in humans [50], nephrotoxicity, hepatotoxicity and many others adverse health effects [51]. The obtained results showed the highest mean concentration of Pb was 6.38 ± 0.75 μg/g dry wt. in gills from Finima creek. The mean concentrations of Pb in muscles and liver fluctuate between the sampling sites. For instance, the mean concentrations of Pb in muscles and liver from Finima creek were 5.00 ± 0.62 μg/g dry wt. and 3.63 ± 0.53 μg/g dry wt, respectively. Whereas the mean concentrations in muscles and liver from Bonny River were 0.20 ± 0.05 μg/g/dry wt. and 0.30 ± 0.07 μg/g dry wt, respectively. Pb accumulation in both sampling sites was in the following order of liver > gills > muscles. It is well established that Pb concentration in fish should not exceed 2 mg/kg as fresh weight basis [52]. Similarly, the maximum permissible level of Pb proposed by Australian National Health and Medical Research Council (ANHMRC) is 2.0 mg/kg as wet weight basis [53]. Also, Spanish legislation limits the concentration level for Pb at 2 mg/kg [54]. The present study indicated the level of Pb in fish caught from Finima Creek was relatively higher than the proposed acceptable limit of 0.5 μg/g [36]. The higher concentration of Pb in Finima creek could be as a result of frequent discharge of industrial effluents from various industries including oil spills.

Cadmium (Cd) exposure is regarded as being lethal and capable of producing chronic lung disease and testicular degeneration [55]. Our results revealed that Cd was detected only in the gills from both locations of sample collection. The mean concentrations of Cd for fish caught from Finima creek and

Bonny River respectively were 1.50 ± 0.50 μg/g dry wt. and 1.50 ± 0.40 μg/g dry wt. respectively. The probable explanation could be that, the concentration of Cd was below detection limits. The Spanish legislation limits the levels for Cd at 1 mg/kg [54]. The Australian National Health and Medical Research Council (ANHMRC) standard for Cd in seafood is set at 2.0 mg/kg [49]. Cd concentration in gills obtained in the present study was within the acceptable limits.

In conclusion, this study investigated some heavy metals accumulation in some organs of croaker fish. Our data demonstrated that fish organs analyzed contained higher concentrations of some toxic heavy metals. Moreover, liver and gills accumulated higher concentrations of these metals in both sampling sites when compared to the muscles. This abnormal high level of heavy metals in fish tissues could be attributed to the frequent crude oil spills as well as other industrial discharge around the region particularly, Finima river which contained highest concentrations of all the metals investigated. The concentrations of some of the heavy metals in fish organs analyzed were above the recommended maximum permissible limits set by the joint FAO/WHO standards. However, some are within the acceptable limits. Altogether, the abnormal higher concentrations of these metals might be toxic to the fish and other aquatic organisms and by extension to humans that frequently consumed such contaminated fishes.

Conflict of interest statement

We declare that we have no conflict of interest.

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