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Comparative Assessment of Heavy Metals Profile on Tilapia Fish (*Oreochromis niloticus)* from three waterbodies (New Calabar River, Elechi and Woji Creeks) in Port Harcourt, Niger-Delta, Nigeria.

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ABSTRACT

Heavy metals concentration was studied in tilapia fishes (*Oreochromis niloticus*) from New Calabar river (Station 1), Elechi creek (Station 2), Woji creek (Station 3) in Port Harcourt, Nigeria using Atomic spectrophotometer. From the results obtained, Zinc showed concentration of 8.38mg/kg, 7.31mg/kg and 9.32mg/kg in the three stations respectively which do not exceed the WHO regulatory limit of 30.0mg/kg. Nickel concentration in the tilapia fish in Station I (0.55mg/kg), exceeded the regulatory limit of 0.5mg/kg, while fishes from Station II and III had 0.43mg/kg and 0.37mg/kg respectively. Cadmium and Chromium were not detected in the fish from three different Stations. Iron was highest with concentration of 54.17mg/kg, 50.10mg/kg, and 35.30mg/kg in the different stations respectively and were lower than WHO regulatory limit of 100.0 mg/kg .Lead showed the lowest concentration of heavy metals in the different stations and were also lower than the WHO regulatory limit of 5.0mg/kg. There was high concentration of (Iron) Fe in the fish species from the three stations while high concentration of Nickel was also observed from species from Station I. Following the high concentrations of metals in these stations, it's imperative therefore that adequate measures should be put in place to reduce activities related to heavy metal contamination to avoid futuristic adverse effects. Finally, *Oreochromis niloticus* may perhaps be a good indicator to monitor heavy metal contamination in the waterbody.

Introduction

Fishes are one of the most widely distributed organisms in the aquatic environment and considered as one of the main protein sources of food for human (Rashed, 2011). Freshwater fishes are similar to sea water fishes which play an important role in determining resident's diet (Ahmad *et al*., 2009).

Tilapia fishes (*Oreochromis niloticus)* are native of Africa and are part of cichlid family, in three aquatic genera and are found in vast varieties within their native lakes. All species of tilapia are tolerant to brackish water and though they thrive better in warmer water they can also survive in colder water above 5^0C. Most varieties of tilapia are omnivores and their ability to adapt to poor water conditions keeps their population flourishing, making them excellent farm fishes.

Tilapia Fish (*Oreochromis niloticus*) as commercially important fish species(Christopher *et al.*,2004) can survive at adverse environmental conditions, their resistance to disease is strong, their respiratory demands are slight so that they can tolerate low oxygen, high ammonia levels and wide range of salinities (Oguzie, 2003).

Tilapia Fishes (*Oreochromis niloticus)* constitute a major source of heavy metals in food, (Kaplan *et al*., 2011), high level of metals in environment may lead to an excessive accumulation which cause problem to human, animal and plants.

Tilapia Fish has been found to be excellent indicator for the heavy metal contamination level in aquatic system because it occupies different food chain levels, (Karadede-Akin and Unlu, 2007). Heavy metal are important group of chemical pollutants, whereby food is the main route for entry into our body, some heavy metals irreversibly are bound to human body tissues. E.g Calcium to kidneys and Lead to bones.

Heavy metal occurs in the environment both as a result of natural processes as well as contaminants from human beings activities (Franc *et al.*, 2015). Some heavy metals are known as essential toxic substances. These include, nickel, zinc, Lead, Cadmium, copper and chromium. Heavy metal has positive and negative effect on human health and surroundings (Alduljaleel & shuhaimi-othman, 2011).

Furthermore, the contaminants also concentrate in some of the organs of fish and can cause lethal and a range of sub lethal effects (Ozmen *et al.*, 2008) it has been reported that active metabolite body parts such as liver, gill and kidney concentrate more amount of heavy metal than other parts like muscle (Obasohan, 2007). Fishes are often seen at the top of the aquatic food chain and may accumulate large amount of heavy metals from the environment (Mansour and Sidky, 2012) In addition, fishes are one of the most indicative aspects in freshwater ambience for the evaluation of heavy metals pollution and health risk potential of human upon consumption (Papagiannis *et al*., 2004).

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Tilapia fishes have a great capacity for metal bioaccumulation (Mokhtar *et al.*, 2009). Heavy metals are accumulated through different organs of the fish because of the affinity between them, in this process all heavy metals are concentrating at different levels in different organs of the fish body parts. Most studies have been concerted only on the accumulation of heavy metals in the edible part (muscles), in view of the fact that it is the main fish part that is consumed by human beings (Rao and Padmaja, 2000).Muscle is not always a good indicator of the entire body fish contaminations and therefore it is vital to analyse other body parts as well, such as the gills and liver (Obasohan, 2007).Due to the existence of metal-binding proteins in some tissues, for an example metallothioneins in the liver can accumulate significantly higher heavy metal concentration than the muscles (Uysal e*t al*., 2008a).

The main aim and objectives of this study was to determine some heavy metals (Cr, Cd, Zn, Pb, Fe, Ni) concentration in samples of Tilapia fishes (*Oreochromis niloticus*) obtained from three different markets (Choba market, Creek Road market, and Mile 1 market in Port Harcourt), Compare the metals profiles in the fish from the three markets and Compare the concentration with acceptable limits from standard organisation (WHO, FEPA, 2000).

Heavy metals concentration in Tilapia fishes

Due to feeding and living in the aquatic environments fishes are particularly vulnerable and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants. Fishes, in comparison with invertebrates are more sensitive to many toxicants and are a convenient test subjects for f indication of ecosystem health.

Heavy metals are produced from a variety of natural and anthropogenic sources. In aquatic environment, heavy metal pollution results from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products, and through Wastewater Treatment Plants (WWTPs). Coal combustion is one the most important anthropogenic emission sources of trace elements and an important source of a number of metals. The contamination of heavy metals and metalloids in water and sediment, when occurring in higher concentrations is a serious threat because of their toxicity, long persistence and bioaccumulation and bio magnification in the food chain. Fishes are considered to be most significant bio monitors in aquatic systems for the estimation of metal pollution level, they offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats. In addition, fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases. Studies from the field and laboratory works showed that accumulation of heavy metals in a tissue is mainly dependent on water concentrations of metals and exposure period, although some other environmental factors such as water temperature, oxygen concentration, pH, hardness, salinity, alkalinity, and dissolved organic carbon may affect and play significant roles in metals accumulation and toxicity to fish. Ecological needs, size and age of individuals, their life cycle, feeding habits and the season of capture were also reported to affect experimental results from the tissues. Fishes has the ability to uptake and concentrate metals directly from the surrounding water or indirectly from other organisms such as small fish, invertebrates and aquatic vegetation.

Fishes accumulate pollutants preferentially in their fatty tissues like liver and the effects become apparent when concentrations in such tissues attain a threshold level. However, this accumulation depends upon their intake, storage, elimination from the body, this means that metals which have high uptake and low elimination rates in tissues of fishes are expected to be accumulated to higher levels. Heavy metals can be taken in the fish from ingestion of contaminated food through the alimentary tract or through the gills and skin.

The heavy metals concentration in fish tissues reflects past exposure through water and or food and it can demonstrate the content situation of the animals before toxicity affects the ecological balance of populations in the aquatic environment (Oguzie, 2013).

Obasohan, *2007* reported that the fish health status in some polluted systems (estimated by the condition factor) indicated that the fish have a lower condition. Very low levels of pollution may have no apparent impact on the fish itself, which would show on obvious signs of illness, but it may decrease the fecundity of fish populations, leading to a long term decline and eventual extinction of this important natural resource.

Heavy metals can be categorized biologically as essential and non-essential.The non-essential metals (e.g. foreign elements)and their toxicity rise with increasing concentra tions.Essential metals(e.g.,Copper,Zinc, Chromium, Nickel, Cobalt, Molybdenum, Iron on the other hand have a known importance biological roles(Oguzie, 2003) and toxicity occurs either at metabolic deficiencies or at high concentrations. The deficiency of an essential metal can therefore cause an adverse health effect, whereas its high concentration can also result in negative impacts which are equivalent to or worse than those caused by non-essential metals

The toxicity of metal to fishes is significantly affected by the form in which they occur in water.The ionic forms of metals or simple inorganic compounds are toxic than complex inorganic or organic compounds. The toxic action of metals is particularly pronounced in the early stages of fish develop pment and adversely affects various metabolic processes in developing fish (embryos in particular), resulting in develop mental retardation, morphologically and functional deform ities or death of the most sensitive individuals. Heavy metals are cable to disturb the integrity of the physiological and biochemical mechanism in fish that are not only an important ecosystem component but also used as a food source.

Materials And Methods

Study Area

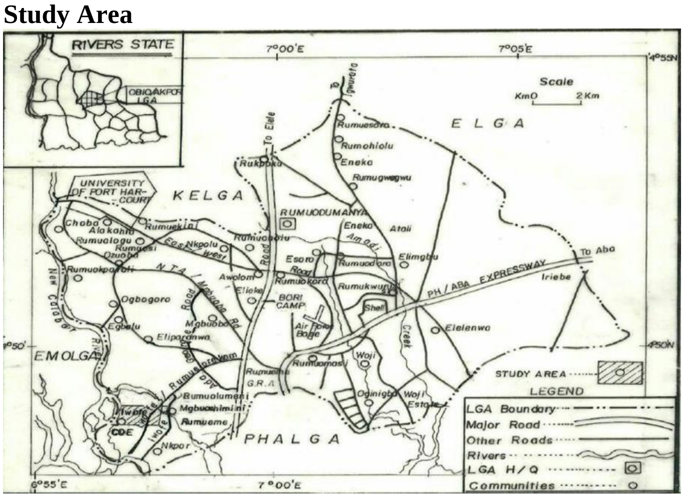

Fig.1. Map of the study area.

Three waterbodies were selected in Port Harcourt, Nigeria for the study

These include; New Calabar river, Elechi and Woji creeks.

Collection of Samples, Preparation and Analysis

Fresh tilapia fishes (*Oreochromis niloticus)* were obtained directly from the fish sellers in each station. The fishes are commonly consumed by the Local population of rivers state. The Tilapia fishes were carefully preserved with Ice in clean polyethylene bags, ice was used to minimize the tissue decay and to maintain moist conditions during transportation. The fishes were placed in an isolated container during transportation and immediately taken to Plant science and Biotechnology Laboratory at Faculty of Science of the University of Port Harcourt.

The fresh Tilapia fishes (*Oreochromis niloticus*) samples were thawed at room temperature (250^{o}C), then were ashed holistically in a muffle furnace at a temperature of 630^{0}C for 3hrs. The ashed samples were dissolved in 10ml concentrated Hydrochloric Acid and was heated on an electro-thermal heater hotplate. The solution of the ash was diluted to 50ml with distilled water and was analyzed for metal ion by Atomic Absorption Spectrophotometer.

Concentration of chromium (Cr) cadmium (Cd), zinc (Zn), lead (Pb), nickel (Ni), and cobalt (Co) were measured from the Tilapia fish (*Oreochromis niloticus*).

Lead Ion Procedure

Lead ion was analyzed by an Atomic Absorption Spectrophotometer at 283.3nm wave length. The wave length was selected with a narrow slit with air and acetylene gas flow was adjusted. Other setting as recommended for the instrument employed was attended to and regulated. Hallow Cathode lamp was given adequate time to stabilize before aspirating standards for equipment with standard lead concentrations, the aspiration tubing and system were flushing with distilled water severally before aspirating the test sample solution on the sample experimental condition used for the standard.

The concentration of Lead ion in the sample was extrapolated from the standard graph of lead ion plotted. The concentration was expressed in mg/l or ppm from the equipment corrections were made necessary in units of choice.

Iron Ion Procedure

248.3nm wavelength was selected. Air and gas flow was adjusted slit width and the other vital setting as was recommended for the instrument was adjusted.

Hallow cathode lamp was stabilized and hollow adequate time to energize. The instrument was calibrated with standard Iron ion concentration to obtain a standard plot. The sample was aspirated into the instrument and the concentration from the standard iron ion graph in ppm or mg/l.

Cadmium Ion Procedure

229nm wavelength was selected. Air and acetylene gas was adjusted. Other settings as recommended for instrument, Hollow cathode lamp gas allowed adequate time to stabilize. Sample standard and was aspirated respectively and the result were extrapolated from the standard graph.

Zinc Ion Procedure

Sample preparation sample as above 213.8nm wavelength was selected, air gas pressure flows was adjusted. Silt width and other settings as was recommended were adjusted. Hallow cathode lamp was allowed to stabilize.

Standard Zinc ion concentration was separated to calibrate the equipment and to obtain a standard graph of the ion tested. Aspiration system was flushed with de-ionized water occasionally before further use. The sample solution was aspirated and the concentration of zinc ion in the sample was extrapolated and recorded in mg/l or ppm.

Chromium Ion Procedure

The machine was first energized and kept for about 15minutes to stabilize. Wavelength of 357.9nm recommended for chromium ion absorption was selected; air and acetylene flow into the burner system was adjusted and regulated. Other essential settings as was recommended in the standard operational procedure (manual) were adjusted. The Hallow cathode lamp was allowed adequate time to stabilized standard chromium solutions were aspirated into the burner system and their equivalent absorbance were obtained. The burner chamber, the aspirator tubing were flushed thoroughly with de-ionized water, then the test sample solution was aspirated and the corresponding absorbance was obtained. The concentration of chromium in the sample was extrapolated from chromium standard graph plotted by the recording system.

Nickel Ion Procedure

The machine was stabilized for 15minutes. Wavelength of 232.0nm was selected. Air and acetylene flows were adjusted, other necessary setting as recommended for the instrument were adjusted. Hollow cathode lamp was allowed adequate times to stabilize standard Nickel solutions of known concentration were aspirated and their corresponding absorbance was recorded. The sample solution was aspirated after flushing the instrument system with de-ionized water. The absorbance displaced on the screen or measurement system was also recorded.

The concentration of Nickel in the sample was extrapolated from the standard graph.

Results and Discussion

The results of heavy metals from three (3) representative sampling sites are presented in table 1. Concentration of heavy metals detected in the three stations shows the variation among heavy metals accumulation in the sample analyzed. Among the metals studied from the stations (Ni, Fe, Zn, Pb, Cd, Cr) concentration of Iron (Fe) had the highest concentration while Cadmium and chromium were not detected. However, Iron (Fe) concentration in Station1 (New Calabar river) was higher than other heavy metals like Ni, Pb, Zn in that same station. Nickel (Ni) had the lowest concentration of all studied heavy metals. The concentration of heavy metals in the fish which were analyzed in these three different locations were lower than the maximum permissible levels recommended by WHO except Nickel (Ni) concentration in Station 1 that has a value of 0.55mg/kg higher than the permissible level recommended.

Table 1. Heavy metal concentrations in *O.niloticus* against Acceptable limits.

Parameters (Mg/kg)	Stations			
	1	2	3	Acceptable or permissible limits by WHO
Fe	54.17 ± 0.25^{a}	50.10 ± 0.20^{b}	35.30 ± 0.45^{c}	100.0
Ni	0.55 ± 0.02^{a}	3.36 ± 0.02^{b}	0.37 ± 0.01^{c}	0.5
Pb	3.62 ± 0.02^{b}	0.43 ± 0.02^{c}	3.06 ± 0.03^{c}	1.5
Zn	8.38 ± 0.02^{b}	7.31 ± 0.02^{c}	9.32 ± 0.03^{a}	30.0

Note: Values in each row with the same superscript are not significantly different at $P < 0.05$.

The significant differences for all the metals at P< 0.05 were observed between fishes and the Station studied.

The iron (Fe) concentration in Station 1(New Calabar river) was the highest with the mean value of 54.17 ± 0.25 which was near the market and Indomie company where all waste are disposed, followed by 50.10 ± 0.20 at Station 2 (Mile 1 Market) and the lowest was 35.30 ± 0.45 at Station 3 (Creek Road market). The levels of Iron (Fe) in these fish samples from the three locations were less than those recommended by World Health Organization (WHO) as reported by El-moselhy *et al.*, 2014, that the allowable level for Fe in these fishes are 100.0mg/kg.

Furthermore, there was significant difference ($P < 0.05$) in the concentration of Nickel present in the three different locations with the Nickel concentration in station1 being the highest with the mean value of 0.55 ± 0.02. The value obtain is higher than those recommended by WHO secondary standard that the allowable level for Ni in these fishes are 0.5mg/kg. The mean value of the station II & III are 0.43 and 0.37 respectively.

There was also a little significant ($p < 0.05$) in the concentration of Lead (Pb) in the different stations ranging from 3.62 ± 0.02, 3.36 ± 0.02 & 3.06 ± 0.63 respectively. The levels of Lead (Pb,) in these fish samples from the three different stations were greater than those recommended by WHO similar to that reported by Nwani *et al.*, 2009, that the allowable level of Ni in these fishes are 1.5mg/kg.

There are also a significant difference ($p < 0.05$) in the concentration of Zinc (Zn) in the three different stations ranging from 7.31 to 9.32 and the values are 8.38 ± 0.02, 7.31 ± 0.02 and 9.32 ± 0.03 respectively. The levels of Zn in these fish samples from the three stations were less than values recommended by WHO for these fishes are 30.0mg/kg.

Table 2. Measures of dispersion and variability of the heavy metal Concentrations.

Parameters	Mean	Median	SD	Mean Range
Fe	46.52	50.10	9.93	35.30-54.17
Ni	0.45	0.43	0.09	0.37-3.36
Pb	3.35	3.36	0.28	0.43-3.62
Zn	8.34	7.31	1.01	7.31-9.32

The metals can be arranged in descending order in their order of concentration from the pollution prone areas i.e stations I, II and III; Fe > Zn > Pb > Ni

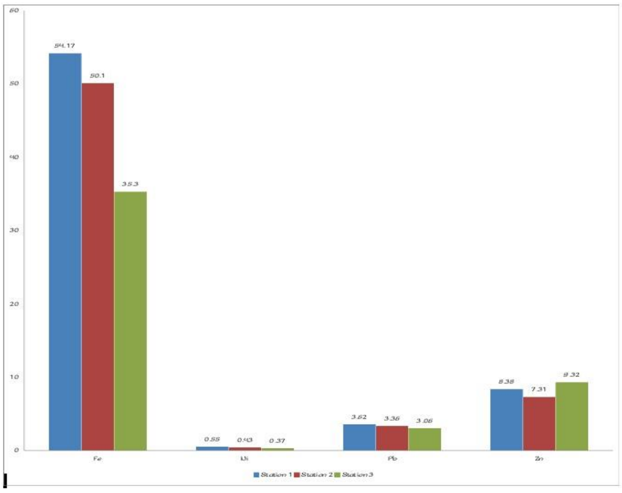

Fig. 2. Histogram of heavy metal concentrations in *Oreochromis niloticus* from three waterbodies from Port Harcourt.

The results (Fig.2) above shows that Fe was highest in Station 1 and lowest in station 3(Fe =54.17mg/kg, 50.1, 35.3 for stations 1, 2, 3 respectively).Ni also recorded the highest value in station1 and lowest in 3. (Ni=0.55mg/kg, 0.43, 0.37 in stations 1, 2, 3 respectively). Similarly, Pb recorded the highest value in station 1 and lowest in 3 (Pb=3.62mg/kg, 3.36, 3.06 for staions 1, 2, 3 respectively) whereas Zn recorded highest in stations 3 and lowest in 2 (Zn= 8.38mg/kg, 7.31 and 9.32 in stations 1, 2, 3 respectively).

Conclusion and Recommendation

The present work shows that different metals accumulate at different concentrations in various Stations. The differences in the level of accumulation in different station can be attributed to different anthropogenic activities in each station. The soluble form of heavy metals is thought to be more harmful because it is more readily available and easily transported to aquatic organism.

This study indicated the difference in the heavy metals concentration found in Tilapia fishes (*Oreochromis niloticus*) in the three water bodies in Port Harcourt, Niger Delta; Nigeria.

It can be concluded generally that Fe, Pb, Zn, Cd, Ni and Cr contents in the Tilapia fishes (*Oreochromis niloticus*) analysed in this study were well below the limit set by WHO for human consumption except for Nickel concentration in Station 1 which exceeded the regulatory limit. Therefore, Consumption of Tilapia fishes from these three stations may not pose any threat to human health and are saved for now. However, consumption of Tilapia fishes (*Oreochromis niloticus*) at Station 1 may pose a human health risk due to high concentration of Nickel. This study also indicates that fishes could be useful in estimating bioavailability of metal to fresh water biota.

Due to the toxic effects of these metals when present in high concentration, we recommend that factories and industries should recycle their waste which may possibly contain these heavy metals and put them into other useful products instead of dumping them directly to the water bodies. Polluted or contaminated environment should be cleaned up. More so, further studies on bioaccumulation of other chemicals to help elucidate the impact of contaminants on the human health is recommended.

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