HISTOPATHOLOGY OF THE MUSCLE AND HEAVY METAL CONCENTRATIONS OF *PERIOPHTHALMUS PAPILLO* FROM ELECHI CREEK, PORT HARCOURT, NIGERIA

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Abstract: In polluted coastal areas mudskippers are the potential bioindicators and bio-accumulators of pollutants and may directly or indirectly be related with human health issues, especially as they are consumed as a local delicacy. The histopathology of the muscle and concentration of selected heavy metals (Pb, Cr, Cd, Ni and V) of *Periophthalmus papillo*, from Elechi Creek, Port Harcourt, Nigeria, were investigated for 12 months from May 2016 – April 2017. Samples of *P. papillo* were collected quarterly from five stations along the Elechi Creek. The stations were selected based on high industrial activity and effluent discharge. Five samples of fish were collected per station, quarterly and 2g of their muscle was used for histology and analysed to determine the heavy metal concentrations. Histological examination of the muscle samples was done and images captured with a Lecia DFC280 Digital Camera attached to a light microscope (Lecia 6000B). Data were analysed statistically to determine means, analysis of variance and mean separation using SPSS 2007. The following ranges and mean concentrations, respectively, were recorded for the metals under consideration: Cr 15.79-19.52mg/kg, 17.67±0.68mg/kg; Pb 4.37-16.48mg/kg, 9.73±2.45mg/kg; Cd 0.44-1.19mg/kg, 0.74±0.13mg/kg; Ni 2.18-7.77mg/kg, 4.68±1.06mg/kg, and V 0.11-0.37mg/kg, 0.30±0.12mg/kg. Concentrations higher than the permissible values were obtained for all the metals in all the stations. Histopathological micrographs showed severe organ degeneration in all samples. The concentrations of these metals were high and some exceeded international standards, especially for Cr, Pb and Cd. These concentrations may pose a health risk to consumers on one hand and to the mudskippers as their muscles are showing signs of degeneration. It is recommended that bio-monitoring of the sample stations should be conducted regularly and the public should be educated on the dangers of consuming fish harvested from the examined locations. Effluent discharge should also be regulated by the appropriate regulatory agencies.

Key words: Heavy metals, *Periophthalmus*, mudskipper, Elechi creek, Nigeria

INTRODUCTION

Water bodies act as reservoir of heavy metals (Kargin *et al.*, 2001) putting the organisms at risk. These heavy metals get into the water bodies directly from point sources (such as, effluents) and non-point sources (such as, runoff) (Yilmaz and Tilmaz, 2004). Metals deposited in the aquatic environment may accumulate in organisms and get transmitted along the food chain where they cause ecological damage and pose a threat to human health due to biomagnification over time (Yilmaz and Yilmaz, 2004). Some aquatic organisms can concentrate heavy metals up to 105 times the concentration present in the water (Nwoka *et al.*, 2014) and these may have physiological effects that are not easily noticed in ecological studies.

The mudskippers, *Periophthalmus papillo*, are amphibious fishes that use their pectoral fins to walk on land. They absorb and accumulate many different pollutants released into the coastal environment by industrial, agricultural, domestic and transportation activities both by ingestion and diffusion (Polgar *et al.*, 2010). In polluted coastal areas mudskippers are the potential bioindicators and bio-accumulators of pollutants and directly or indirectly related with human health issues, as they are consumed in different regions (Aligaen and Mangao, 2011). The physiological, histological, and embryological changes in mudskippers are of interest as strong indicators of water quality parameters because they can accumulate very high concentrations of toxic compounds in their tissues (Polgar *et al.*, 2010) without noticeable signs.

Heavy metals get into the water bodies directly from point sources as effulent and non-point sources as runoff and more insidiously as atmospheric deposit that are transported from long distances (Yilmaz and Tilmaz, 2004). These metals affect every level of the food web from producers in the lowest trophic levels to the highest order of carnivore by residing in the system and magnifying with every trophic state (Benson *et al.*, 2009). Changes in oxidation state of the heavy metal can have profound effect on their toxicity and bio-availability (Donat and Briland, 2005). Discharges of inorganic and organic micro-pollutants from various industrial, agricultural and municipal sources have resulted in permanently contaminated water, sediments and the accumulation of chemicals in the aquatic food-chain. The metals discussed in this article were among the priority list of pollutants compiled by the Environmental Protection Agency of United States and they include - Chromium (Cr), Lead (Pb), Cadmium (Cd), Nickel (Ni) and Vanadium (V). Cr has biological importance in trace concentration, in excess or deficiency it produces a toxic effect. Cr VI has very toxic and carcinogenic properties (Tuzen, 2003). Anthropogenic sources include paints, cements, motar, anti-corrosives and oil industry activities (Agbozu and Ekweozor, 2001). The health implications of Cr toxicity include oxidative damage of blood cells, hemolysis and subsequent kidney and liver failure. Pb has long biological half life, so its elimination or excretion from the body is difficult (ATSDR, 2007). It has been reported to be a multi system toxicant; it affects every organ and its key organelles, it competes with calcium for uptake site in the bone and displaces it. Anthropogenic sources are oil and gas, lead pigment paints, batteries, fishing sinkers and explosives. Cd is the most toxic heavy metal; it affects reproductive rate of biota in the aquatic environment and there have been claims of such reproductive disruption in laboratory rats (Afshan *et al.*, 2014). Chronic toxicity includes impaired kidney function, tumors, hypertension and hepatic dysfunction (Mansour *et al.*, 2002). Cd is widely distributed in the aquatic environment and is stored within the lipid component of the fish, so it is easily absorbed by the human body (Osiow, 2005). Ni in small quantities is essential but high quantities constitute damage to human health, both allergic and carcinogenic effects of Ni have been well documented (Campbell and Nikel, 2006). The International Agency for Research on Cancer (IARC) (2014) listed some Ni compounds as Group 1 human carcinogen. Nickel in correlation with Cd has been associated with increased incidence of cancer in humans (IARC, 2014). Both acute and chronic effect of Ni causes skin dermatitis. Anthropogenic sources of Ni include metallurgical process, combustion and incineration of fossil fuels. Vanadium is a constituent of crude oil. In fact, the most abundant trace metal found in petroleum samples (Amorim *et al.*, 2007). The IRIS and IARC (2014) classified Vanadium pentaoxide as a human carcinogen at a level of $9.0x10^{-3}$ mg/kg/day.

Elechi Creek lies between longitude 6°58'10.83" to 00'23.64" E and latitude 4°4'11.34" to 4°4'43.33" N. It is a tidal, brackish water system. It is seen to receive waste water discharges from a number of facilities associated with industries located along the water body (Obire *et al.*, 2003). Irrespective of the volume of waste discharged into this creek, artisanal fishermen still fish edible organisms which are sold to the public. This study was carried out to determine the histopathological effects of the heavy metals Cr, Pb, Cd, Ni, and V, in the edible parts (muscle) of *P. papillo* from Elechi creek. The hypothesis is that metal concentrations will result into histopathological damage to the fish muscle.

MATERIALS AND METHODS

Study area

Samples were collected from the following locations along the creek (Fig. 1):

Station 1 – Eagle Cement Water Front

This station is situated at latitude 4° 47'41.85" and longitude 6° 56'29.43". Eagle Cement is a cement manufacturing company surrounded on the right by Pathfinder (NNS Okemini) and on the left by an oil tank farm. Illegal crudes recovered by NNS Okemini are often destroyed by regular burning, Accidental spills from discharging vessels increase pollution load in this station.

Station 2 – Saipem Water Front

This station is situated at latitude 4° 46'24.55" and longitude 6° 58'50.49". Saipem, a multinational company involved in Engineering, Construction and Fabrication, is surrounded on the left by Neptune Maritime Company and an oil tank farm. Accidental spills from discharging refined product carriers affect pollution load in this station.

Station 3 – Master Energy Water Front

This station is situated at latitude 4° 46'30.27" and longitude 6° 58'45.80". Master Energy, a refined product marketing company, receives petroleum products from oil vessels. It is surrounded on both sides by vessels loading or discharging petroleum products, adjacent to it is NEPAL oil tank farm. The surrounding and adjoining river always have oil film coats on the surface due to the activities of the companies. Accidental spills, washout from oil vessels and other effluents increase the pollution load of the estuary.

Station 4- Nigerian Agip Oil Company Water Front

This station is situated at latitude 4° 47'55.68'' and longitude 6° 58'15.76''. NAOC is a land and offshore oil producing company, surrounded on the right by an abattoir, and toileting facilities of the adjoining communities. The Agip jetty has some vessels around it.

Station 5 - Ogbakiri Water Front

This station was situated at latitude 4° 47'37.69" and longitude 6° 54'37.32". This station was chosen as the control for this study, as there appeared to be no factories or vessels loading or offloading products.

Fish Sampling

Samples (*Periopthalmus papillo*) were collected from the creek at each of the sampling stations during the study period on quarterly basis that is, four times in the twelve months sampling period covering the wet and dry seasons from May, 2016 to April, 2017. *Periopthalmus papillo* was caught with the aid of a locally made trap known as the jik trap. Samples were washed and transported in properly labelled ice chests to the laboratory.

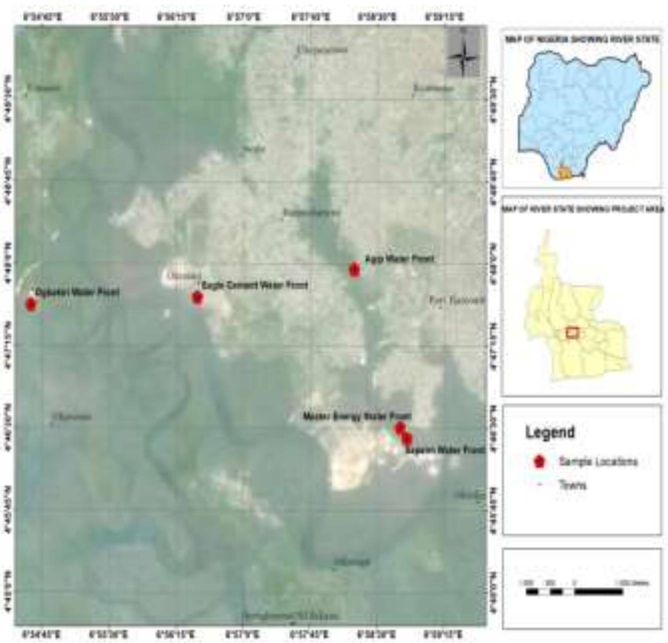

Figure 1: Map of Elechi Creek Showing Sample Locations

Analysis of Fish Samples

Two grams of fish muscle, were cut and oven dried at 500°C to ash. The ashes were digested with 5mls of 10% HNO_3 and warmed on a water bath to dissolve. The mixture was filtered and made up to 50ml using distilled water. The filtrate was stored in plastic bottles for metal analysis.

The digests were analysed for Ni, V, Pb, Cr and Cd using the GBC Avanta Atomic Absorption Spectrophotometer (Davies *et al.*, 2006; Adebowale *et al.*, 2009).

Histological Examination/Toxicity Test

For histological examination, fish samples were dissected under sterile conditions, weighed and immersed into Alcoholic Bouin's fixative. Then they were fixed in 10% neutral buffered formalin at room temperature for 24hrs. After serial dehydration steps in alcohol, samples were embedded in paraffin. The blocks of embedded tissue were sectioned at 5µm, and sections were routinely stained with haematoxyline and eosin (HeE) and mounted on DPX. Images were acquired with a Leica DFC280 digital camera attached to a light microscope (Leica 6000B).

Data Analysis

The heavy metal concentrations in the muscle from the study locations were subjected to SPSS statistical software using one-way analysis of variance (ANOVA) to test for significant differences. Post hoc comparison was done with Tukey HSD to determine the stations that were statistically significant.

RESULTS

Concentrations of heavy metals in the muscle of *P. papillo* from the study locations

The concentrations of the metals in *P. papillo* from all locations are presented in Table 1. Chromium (Cr) had a mean concentration of 17.67±0.68mg/kg but the highest and lowest mean values were recorded in Stations 2 (19.52±2.49mg/kg) and 4 (15.73±1.72mg/kg). The differences were not significant at p=0.05. The mean concentration of Pb was 9.73±2.45mg/kg. The highest mean value of Pb was recorded in station 4 (16.48±1.58mg/kg) while the lowest (4.14±0.96mg/kg) was recorded in station 5. Statistical tests showed that Pb concentrations were similar in stations 1 and 5 but different from station 4.

Cadmium concentrations averaged 0.74±0.13mg/kg from all locations. Fish samples from station 1 had significantly higher concentrations than those from station 2. Nickel had an overall mean concentration of 4.68±1.06mg/kg. The highest mean concentration (7.77±0.54mg/kg) of Ni was recorded in station 1 and the lowest (2.18±0.16mg/kg) was recorded in station 4. Statistical tests showed significant differences in Ni concentrations between the stations: stations 4 and 5 were similar but different from station 1.

The mean concentration of Vanadium was 0.3±0.12mg/kg and Station 1 was higher than those from stations 4 and 5 at p=0.05.

Table 1: Mean concentration (mg/kg) of heavy metals in the muscle of *P. papillo* from Elechi Creek, Port Harcourt, Nigeria

Station	Cr	Pb	Cd	Ni	V
1	17.09 ±1.03	4.37[b] ±0.38	1.19[a]±0.08	7.77[a]±0.54	0.37[a] ±0.04
2	18.84 ±1.83	10.10[ab]±2.10	0.44[b]±0.03	6.13[ab]±0.68	0.37[a] ±0.04
3	19.52 ±2.49	13.54[ab]±2.54	0.74[ab]±0.14	4.24[ab]±0.22	0.23[ab]±0.02
4	15.73 ±1.72	16.48[a] ±1.58	0.71[ab]±0.08	2.18[b]±0.16	0.11[b] ±0.02
5	17.18 ±1.01	4.14[b] ±0.96	0.60[ab]±0.10	2.57[b]±0.17	0.12[b] ±0.02
	*0.05	**0.2	**0.05	***0.6	

*WHO, 2003; **FAO, 1983; ***WHO, 2002 (permissible limits for fish)

Histology

Histopathological damages were observed in the fish muscle cells from all stations. These included hypertrophy of myotomes, infiltration of inflammatory cells, loss of muscular bundle and necrosis. The plates below are photo micrographs of normal muscles (from Patnaik *et al.*, 2011) and those of *P. papillo* of Elechi creek from the five stations.

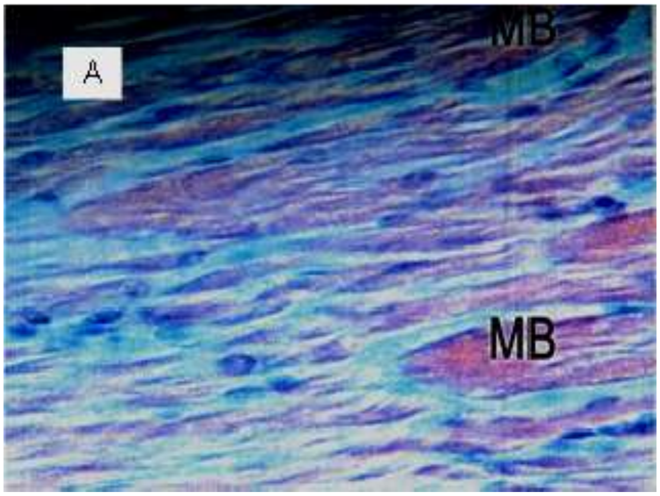

Plate 1: Photomicrograph of Fish Muscle from a Laboratory Control Study (H&E x40) (Patnaik *et al*., 2011) MB – *Normal Muscular Bundle*

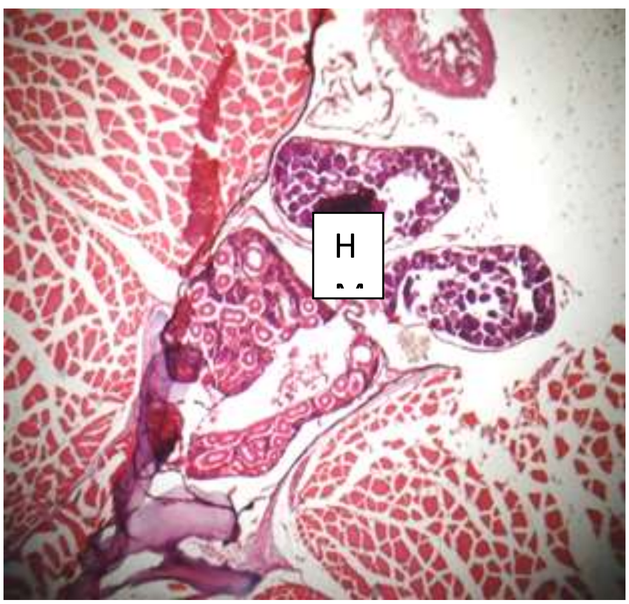

Plate 2: Micrograph of *P. papillo* Muscle from Station 1 (H&EX40) *HM-Hypertrophy of Myotomes*

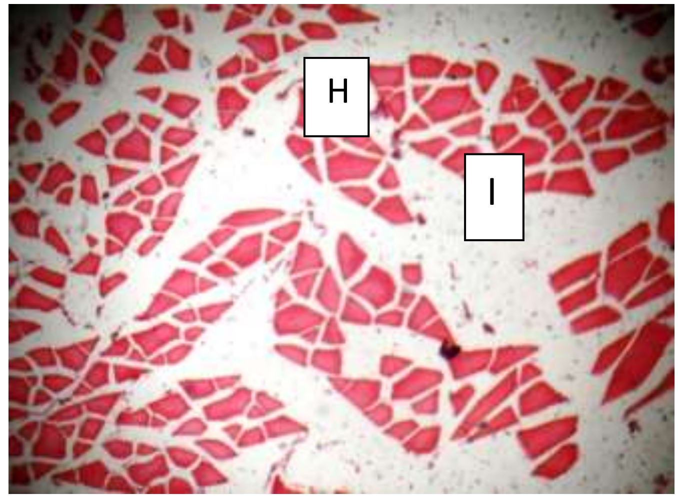

Plate 3: Micrograph of *P. papillo* Muscle from Station 2 (H&EX40) *II-Infiltration of Inflamatory cells*

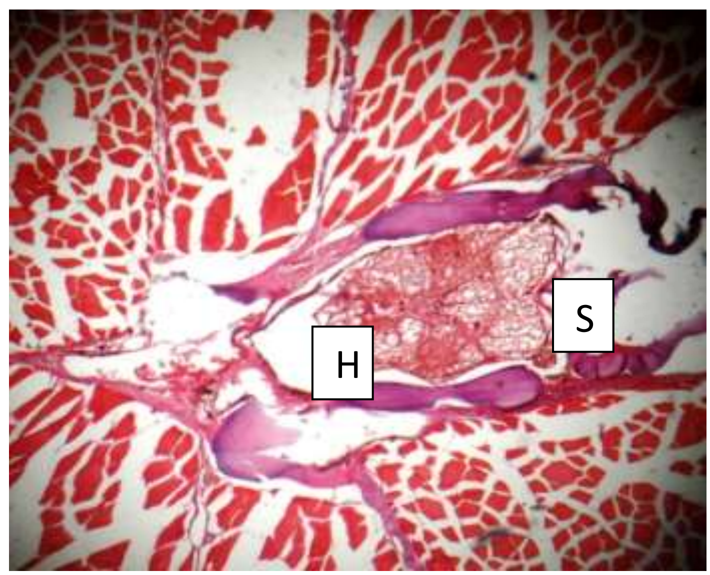

Plate 4: Micrograph of *P. papillo* Muscle from Station 3 (H&E.X40) *SE-Stromal Edema, HM-Hypertrophic Myotomes*

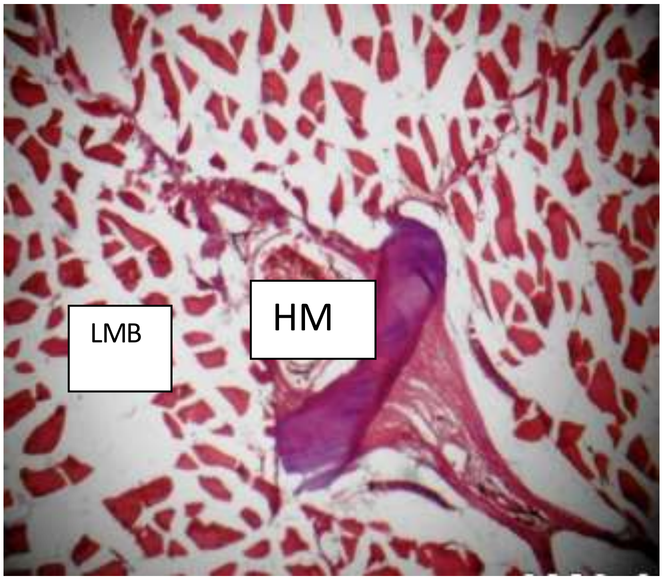

Plate 5: Micrograph of *P. papillo* Muscle from Station 4 (H&EX40) *LMB-Loss of Muscular Bundle, HM-Hypertrophy of Myotomes*

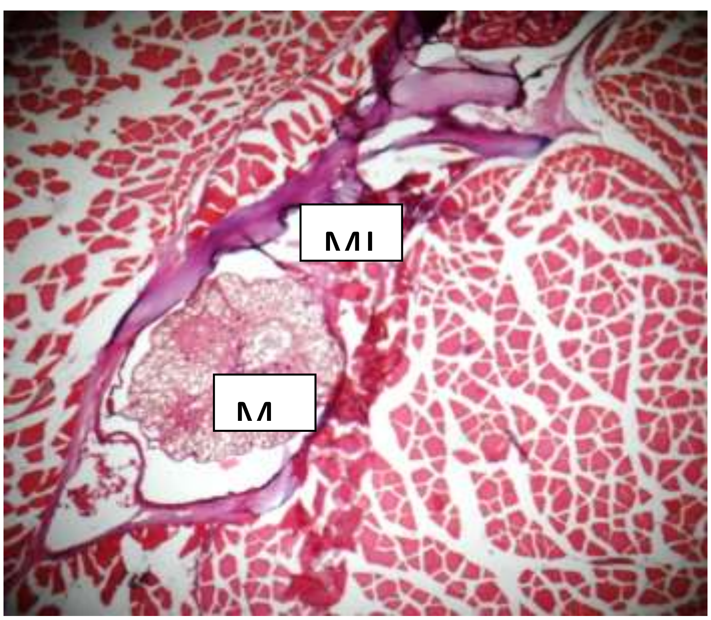

Plate 6: Micrograph of *P. papillo* Muscle from Station 5 (Mag.X40) *MN-Muscular Necrosis, MI-Muscle Inflammation*

DISCUSSION

Chromium concentration was very high in the muscle of the fish samples in all the stations as it far exceeded the WHO (2003) and FEPA (2003) and FAO (2002) permissible limits of 0.05mg/kg, 0.15mg/kg and 1.0mg/kg. The findings of this study were in agreement with the results of Javed and Nazura (2014) who reported high concentration of Cr in the gill, muscle and liver of *Channa punctatus* at Kasimpur, India, but disagrees with the findings of Ijeoma *et al*. (2015) who reported lower concentrations of Cr in the soft tissue of a crab from Delta State, Nigeria. Chromium has biological importance in trace concentration, but excess or deficient levels produce a toxic effect (Tuzen, 2003). The high concentration of Cr reported in this research implies that the consuming population may be at risk of Cr toxicity.

Pb concentrations were also high and exceeded the WHO (2002), permissible limit of 0.2mg/kg. Lead has long biological half-life, so its elimination or excretion from the body is difficult (ATSDR, 2007). High Cd concentrations, exceeding the FAO / WHO (2002) permissible limit of 0.05mg/kg, were also recorded in this study which were similar to the observations made by Edward *et al*. (2013) in *Clarias gariepinus* in Abuloma and Otitoju and Otitoju (2013) in the soft tissue of *Tympanotus fuscatus* in Oron rivers, both in Nigeria. Cadmium is a non-essential, highly toxic heavy metal and it reduces the reproductive rate of biota in the aquatic environment and there have been claims of such reproductive disruption in laboratory rats by Afshan *et al*., 2014.

Nickel concentrations in this study were also higher than the WHO (2002) and FEPA (2003) mean permissible limits of 0.6mg/kg and 0.5mg/kg. Nickel is essential in small quantities but constitute damage to human health in higher concentrations causing allergic and carcinogenic effects (Campbell and Nikel, 2006). The International Agency for Research on Cancer (2014) IARC listed some Ni compounds as Group One human carcinogen. Nickel in correlation with Cd has been associated with increased incidence of cancer in humans (IARC, 2014) and both acute and chronic effects of Ni causes skin dermatitis.

Vanadium concentration was the least heavy metal in the muscles examined. Vanadium is a constituent of crude oil and is the most abundant trace metal found in petroleum samples (Amorim *et al*., 2007). There is paucity of information on V as previous workers did not find V in their studies but its presence in this study could be as a result of the presence of illegal refineries around Elechi Creek in recent times. The IRIS and IARC (2014) classified vanadium pentaoxide as a human carcinogen at a level of $9.0x10^{-3}$ mg/kg/day. The V concentration in the fish muscle far exceeds this value.

Das and Mukherya (2000) observed that as muscle tissues come in contact with pollutants dissolved in water or sediment, reactions in the ultra structure of the muscles are spontaneous. They asserted that separation of muscular bundles could result as the initial response to such stimuli. Further response could induce hyperacidity and excitability in animals leading to release of lactic acid and subsequent muscular fatigue. This observation corresponds with our visual observation of some of the fishes with skin discoloration and slack muscle fibres such that the skin could be separated from the muscle with forceps. Patnaik *et al.* (2011) observed marked thickening and separation of muscle bundle, heamolysis, necrosis and lesions with reduced compactness in the muscle tissue expoused to 4.3ppm of $PbNO_3$ after 28 days. He also observed intra edema with some dystrophic changes on exposure of fish muscle to 1.6ppm of CdCl after 28 days. His observations are similar to the findings of the present study.

CONCLUSION

Results from this study show that concentrations of the heavy metals examined exceeded reference values. Fish muscles showed varying degrees of degeneration. This could adversely reduce the fish population and endanger the health of the public.

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