HAEMATOLOGICAL AND SERUM ENZYMES BIOMARKERS OF HEAVY METALS IN *Chrysichthys nigrodigitatus* AND *Cynoglossus senegalensis*

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ABSTRACT

Heavy metals are constituents of marine environment that occur as a result of pollution principally due to the discharge of untreated wastes into water bodies by many industries. Haematological, serum enzymes activity and heavy metal concentrations in feral populations of *Chrysichthys nigrodigitatus* (Lacepede:1803) and *Cynoglossus senegalensis* (Kaup: 1858) from Ebute-Oba section of the Lagos Lagoon, were evaluated from July-September, 2012 in order to determine and establish the relationship between heavy metal levels, serum enzymes activity and haematological parameters in *Chrysichthys nigrodigitatus* and *Cynoglossus senegalensis*. Levels of metals in *C. nigrodigitatus* and *C. senegalensis* in mg/l were as follows: (Cr: 0.07 ± 0.001, Cu: 0.027 ± 0.01, Fe: 0.481 ± 0.214, Pb: 0.001 ± 0.001, Zn: 0.316 ± 0.575) and (Cr: 0.010 ± 0.07, Cu: 0.029 ± 0.01, Fe: 0.467 ± 0.217, Pb: 0.021 ± 0.044, Zn: 0.433 ± 0.499) respectively. Serum alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkalinephosphase (ALP) activities in *C. nigrodigitatus* and *C. senegalensis* measured in IU/l were 64.995±44.123, 16.345±166, 157.787±34.88 and 73.758±48.768, 29.532±20.100, 45.707±285 respectively. ALP activity between the two species showed a significance difference ($P<0.05$). Haematological parameters recorded for *C. nigrodigitatus* were Hb with 13.325 ± 8.293g/dl, PCV (25.53±11.79%), RBC ($2.95±0.73.10^6mm^3$), WBC($124.19±56.41x10^4mm^3$), MCHC(32.46±2.18g/l), Neutrophils (41.00±27.91%) and Lymphocyte (64.33±31.94%). While values for *C. senegalensis* were: Hb (11.33±8.04 g/dl), PCV (34.00±22.83%), RBC ($2.70±1.98x10^6mm^3$), WBC ($275.20±167.27\ 10^4mm^{-3}$), MCV (178.94±126fl), MCH (59.63±44.50 Pg), MCHC (33.33±33.28g/l), Neutrophils (39.00±30.33%) and Lymphocytes (80.00±71.77%). Accumulation of heavy metals in both fish species leads to an increased ALT, AST and decreased ALP activities. Conversely, accumulation resulted in reducing Haemoglobin and an increased Red Blood Cells and Lymphocyte counts. Haematological and serum enzymes activities are predilective biomarkers for the detection and monitoring of aquatic ecosystems pollution. Therefore, there is high level of pollution in the area and is of environmental concern for aquatic organism.

Keywords: Biomarkers, Haematological, Serum Enzymes, Heavy Metals, Fish

INTRODUCTION

Aquatic environment are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants, and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life (Karbassi *et al.,* 2006). The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment (Yang and Rose, 2003).

A large part of these elements exert their toxic effects by generating reactive oxygen species (ROS), causing oxidative stress. Most of the heavy metal ions are toxic or carcinogenic in nature and pose a threat to human health and the environment. According to Olatunji and Abimbola (2010), the city of Lagos is the most populous in Nigeria and is projected to be the fifth most populous in the world by 2015. The city harbours over 75% of the industrial outfits within the country which include textile industries, chemical and paint industries, breweries and bottling companies, metal industries, ship yards, plastic and petro-chemical factories, paper mill and sawmills. These industries discharge untreated wastes into the several drainages and canals that find their way into the Lagos lagoon (Okoye, 1989).

The Lagos Lagoon which lies within longitudes $6^0 25'$ and $6^0 43'$E and latitudes $3^0 22'$ and $3^0 40'$N is the largest of the three lagoon systems occurring in the Lagos area receiving over 80% of the land-derived run-offs laden with various types of wastes. An estimated $10{,}000m^3$ of industrial effluents are discharged into the Lagos Lagoon per day (Singh *et al.,* 1995; Don-Pedro *et al;* 2004).

However, there has been growing interests in the determination of the levels of heavy metals in fish (Kalay *et al.,* 1999). Fishes are good bioindicators of the degree of pollution in the aquatic environment due to their propensity to bio-accumulate toxic metals and other contaminants in their internal organs, most especially their edible muscle tissues which could reach toxic concentrations leading to serious health hazards.

Fish has attracted much attention in biomonitoring water pollution due to its special biological characters such as relatively big body size, long life cycle, easy to raise etc.
More importantly fish species are at the top position in the aquatic food chain and may directly affect the health of humans, which make it much significant for biomonitoring. Fish which is at high trophic level of the aquatic food chain accumulate substantial amount of metals in their soft and hard tissues (Mansour and Sidky, 2002), Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles. Once absorbed, pollutants are transported in blood stream to either a storage point i.e. (bone) or to the liver for transformations and/or storage (Obasohan, 2008). Heavy metals are high priority pollutants because of their relative high toxicity and persistent nature in the environment. A sound understanding of dynamics of the concentration and distribution of heavy metals and their compounds in various compartments of the environment is a priority for good environmental management programmes all over the world (Don-pedro *et al.,* 2004). Hence it is important to regularly monitor the levels of metals and other contaminants that are being discharged into the aquatic ecosystems which can in turn be accumulated by fisheries resources. This study was undertaken to examine the relationship between heavy metal levels with alanineanino transferase (ALT), Aspartateamino tansferase (AST), Alkalinephosphatase (ALP) activities and haematological parameters in *Chrysicthys nigrodigiatus* and *Cynoglosnus senegalensis*.

MATERIALS AND METHODS

Description of study area

Ebute-Oba is a fish landing site located along the shores of the Lagos Lagoon, situated between latitudes $6^{0}27^{1}58.3^{11}$ N and $6^{0}27^{1}55.9^{11}$N and longitudes $3^{0}29^{1}32.2^{11}$ E and $3^{0}23^{1}49.0^{11}$ E in the vicinity of the Third Mainland Bridge in Isale-Eko Lagos Island (Fig.1). The landing site experienced similar environmental conditions characteristic of the Lagos Lagoon, notably semi-diurnal tidal regime and seasonal salinity variations due to rainfall. Both finfish and shell fish commonly found in the market around Lagos Island come from landings from this site. Fisher- folk which comprise of Eguns make their catch from a wide expanse of the Lagos Lagoon making use of cast and seine nets with motorized canoes. Amongst the notable species caught and landed are; *Chrysichthys nigrodigitatus*, *Ilisha africana*, *Cynoglossus senegalensis*, *Penaeus notialis*, *Machrobrachium machrobrachium* and *M. vollenhovenii*.

A common scene around this landing site is a constant vehicular movement which is predominant throughout the day.

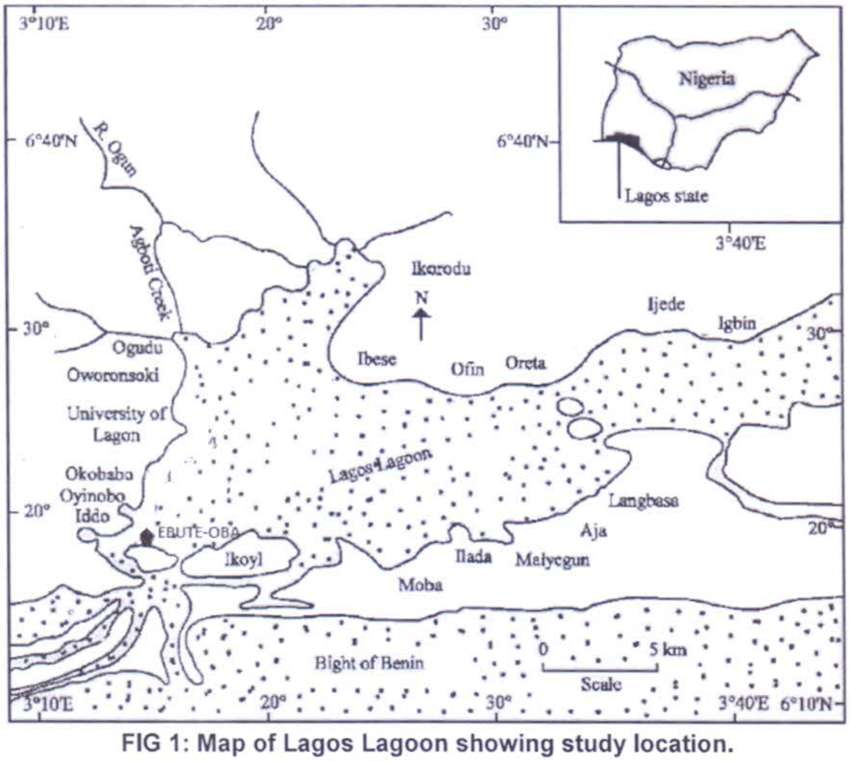

FIG 1: Map of Lagos Lagoon showing study location.

Field sampling and measurement of mophometric features

Samples of *Chrysichthys nigrodigitatus* and *Cynoglossus senegalensis* were collected twice a month between July and September, 2012 between 09.00 – 12.00 hours on each sampling day. Total length (cm) and body weight (g) was measured using a measuring board and an electric weighing balance respectively. A total of 30 samples of *C. nigrodigitatus* and 30 samples of *C. senegalensis* were collected.

Blood collection

Blood was collected from the caudal blood vessels on the field to avoid stress and this lasted for about 5minutes. After blood collection, fish samples were transported to the ecotoxicology laboratory of the Marine Sciences Department, University of Lagos in ice-chest for heavy metal analysis of the tissues.

Surface water samples were collected using 1litre water sampling bottles. The bottles were preserved in a cool dry place and transported to the laboratory for further analysis of water quality parameters not measured in-situ. Parameters measured in-situ include; surface water temperature, hydrogen ion concentration, electrical conductivity, salinity, turbidity and dissolved oxygen. Chemical oxygen demand was measured ex-situ in the laboratory. The surface water temperature of the water sample was determinate in-situ by using mercury-in-glass thermometer calibrated in degree centigrade (^{0}C).The thermometer was immersed in the water for about five (5) minutes and the height of the mercury was read on the graduated glass tube. The pH measures hydrogen ion activity in water. The pH of the water was determined with the aid of a pH meter. This was done by dipping the probe in the water and reading off the values from the meter. The conductivity of the water samples was measured using the Horiba U-10 water quality checker and values were read off from the meter. This was done in-situ with the use of a hand held refractometer. A drop of the water sample was placed on the prime of the meter of the refractometer and the meter was adjusted to ‰$_0$ mark and viewed through the eye piece. The daylight plate was closed and the salinity of the water sample read on the scale. Turbidity is a measure of the amount of light. This was measured at the study site using the Horiba water checker, model U-10 probe. The dissolved oxygen level was measures in-situ using a DO meter. The probe was placed in the water and the reading was recorded from the meter. The measurement of COD is based on the principle that almost all organic compounds in water can be oxidized to CO_2 and water by the action of strong oxidizing agents under acid condition. This was done in the laboratory.

Laboratory analyses of heavy metals

Determination of Heavy Metals in Fish Samples were carried out using Atomic Absorption Spectrophotometer (AAS).

Metal concentrations for all extracts were determined using Perkin Elmer Model 200 Atomic Absorption Spectrophotometer (air-acetylene flame). Accuracy was assessed by analyzing three replicates of selected samples yielding standard deviations lower than 10% for all the metals (Cd, Cr, Cu, Fe, Pb and Zn).

Determination of heavy metals in water samples

Water sample is first aspirated into the flame or electrothermal device where it is vaporized and atomized. Ration of the proper wavelength is then passed through the vapour containing the ground state atoms of the metal where absorption occurs. The magnitude of the AAS absorption signal is directly proportional to the concentration of the analyze metal in the sample solution.

Statistical analysis

Variations in heavy metal levels in *C. nigrodigitatus*, C. senegalensis and water were tested by one-way Analysis of Variance (ANOVA) at 5% level of significance. Post-hoc comparison of means between fish species were carried out using Least Square Difference (LSD) test to determine which values differed significantly. Comparison of mean heavy metal levels between *C. nigrodigitatus* and *C. senegalensis* was carried out by independent T-test. Pearson correlation analysis was carried out to determine the relationship between heavy metals in fish, serum enzymes activity and haematological parameters. The analysis was performed using the SPSS 17.0 package.

RESULTS

Heavy metals in fish species and water

Heavy metals contents in the flesh of Chrysichthys *nigrodigitatus*, *Cynoglossus senegalensis* and water during the sampling periods is presented in tables 1. The result on the table reveals that heavy metals concentration in the flesh of both fish species and water is generally low and are below WHO/FAO/FEPA allowable limits. No Cadmium was detected in all matrices measured

Analysis of Variance (ANOVA) of the mean values of the heavy metals concentration of *C. nigrodigitatus*, *C. senegalensis* and water showed a significant difference. There was a significant difference ($P<0.05$) in Cr and Fe between *C. nigrodigitatus* and water for the sampling periods. There was a significant difference ($P<0.05$) between Fe concentration in *C. senegalensis* and water all through the sampling period. The result of serum enzymes activity in fish species is presented in table 2. T-test analysis showed no statistical significant difference (P>0.05) between mean serum AST activity value in *C.nigrodigitatus* (65.00U/l±44.12) and *C.senegalensis* (73.76U/L±48.77). No statistical difference (P>0.05) was recorded between mean serum ALT activity value in *C. nigrodigitatus* (16.35 IU/l±12.16) and *C. senegalensis* (29.53U/l±20.10). A statistical significant difference was recorded (P< 0.05) between mean serum ALP activity value in *C.nigrodigitatus* (157.79 IU/l±34.89) and *C.senegalensis* (45.71U/l±35.29) for the sampling periods. Haematological parameters in fish species are presented in table 3. T-test showed no statistically significant difference (P>0.05) for all haematological parameters recorded between *C. nigrodigitatus* and *C. senegalensis*. The Morphometric parameters are presented in table 4. Water Quality Parameters of the study area are presented in table 5. Pearson Rank Correlation (P=0.05) showed no statistical significance (P>0.05) for all water quality parameters recorded for the sampling periods.

Table 1: Heavy metals concentration in flesh of *C. nigrodigitatus, C.senegalensis* and water

Species	Mean±SD (mg/l)					
	Cd	Cr	Cu	Fe	Pb	Zn
C.nigrodigitatus	-	0.007±0.00	0.027±0.01	0.481±0.21	0.001±0.00	0.316±0.57
C.senegalensis	-	0.010±0.00	0.029±0.01	0.467± 0.21	0.021±0.04	0.433±0.49
WHO Guidelines for fish	2.0	0.15	0.15-1.0	-	2.0	1.5
Water	-	0.014±0.00	0.020± 0.04	1.098± 0.44	0.001±0.00	0.458±0.68
WHO Guidelines for Water	0.005	0.05	-	-	0.01	3.0

Table 2: Serum enzymes activity in *C. nigrodigitatus* and *C. Senegalensis*

Species	Mean IU/L ±SD		
	AST	ALT	ALP
C.nigrodigitatus	64.995±44.123	16.345±12.166	157.787±34.88
C.nigrodigitatus	73..758±48.768	29.532±20.100	45.707±35.285

Table 3: Haematological Parameters in *C. nigrodigitatus* and *C.senegalensis*

Species	Mean±SD											
	Hb g/dl	PCV %	RBC 10^6mm^{-3}	WBC 10^4mm^{-3}	MCV Fl	MCH Pg	MCHC g/l	Neutrophil %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
C.nirodigitatus	13.32 ±8.29	25.53 ± 11.79	2.95 ± 0.73	124.19 ± 56.41	97.89 ± 50.56	36.46 ± 12.95	32.46 ± 2.18	41.00 ± 27.91	64.33±31.94	-	-	-
C.senegalensis	11.33 ± 8.04	34.00 ± 22.83	2.70 ± 1.98	275.20 ± 167.27	178.94 ± 126	59.63 ± 44.50	33.33 ± 33.28	39.00 ± 30.33	80.00±71.17	-	-	-

Table 4: Length and weight of *C. nigrodigitatus* and *C. senegalensis*

Species	Morphometric Parameters	Range	Minimum	Maximum	Mean of Standard Deviation
C.nigrodigitatus	Length (cm)	27.000	26.000	53.000	37.41±9.52
	Weight (g)	1133.000	522.000	1655.000	859.50±40.9
C.senegalensis	Length (cm)	14.000	29.000	43.000	36.93±5.48
	Weight (g)	942.000	473.000	1415.000	748.71±35.15

Table 5: Water quality parameters

Parameters	Range	Minimum	Maximum	Mean Standard Deviation
Water Temperature (°C)	1.00	24.00	25.00	24.17±0.41
Dissolved Oxygen (mg/l)	0.57	1.40	1.97	1.71±0.22
PH	1.50	6.30	7.80	7.08±0.59
Conductivity (µScm)	1100.00	6900.00	8000.00	7650.00±33.70
Turbidity(FTU)	2.00	1.00	3.00	2.00±0.89
Salinity (‰)	2.00	3.00	5.00	4.17±0.98
COD (mg/l)	16.00	24.00	24.00	32.33±5.43

DISCUSSION

Bioaccumulation of heavy metals in tissues of marine organisms has been identified as an indirect measure of the abundance and availability of metals in the marine environments (Kucuksegin *et al.,* 2006). For this reason, monitoring fish tissue contamination serves an important function as an early warning indicator of sediments contamination or related water quality problems (Mansour and Sidky, 2002; Babatunde, 2012) and enables appropriate action to protect public health and the environment. Several studies (Ademoroti 1996; Allen 1995; Karthikeyan *et al.,* 2007) have indicated that fish are able to accumulate and retain heavy metals from their environment. Adeyeye et *al.,* (1996) also showed that the concentration of metals was a function of fish species as it accumulates more in some fish species than others. Apparently, *C. nigrodigitatus* and *C. senegalensis* accumulated heavy metals in the flesh with essential metals in higher levels than non-essential metals. The average concentrations of Cd, Cr, Cu, Fe, Pb and Zn in flesh of the two species showed the following trend (*C. nigrodigitatus*); Fe>Zn>Cu>Cr>Pb>Cd and (*C.senegalensis*); Fe>Zn>Cu>Pb>Cr>Cd. Except for Fe, mean concentration of heavy metals were higher in *C. senegalensis* when compared to *C. nigrodigitatus*. From the study, the concentration of Cr, Pb, and Zn in *C. nigrodigitatus* and *C. senegalensis* showed lower levels compared to WHO maximum permissible limits. An important observation was the elevated levels of metals especially Cr, Fe and Zn in both species which could be directly attributable to bioaccumulation as evidently depicted by the accumulation factors. Metals (Copper and Zinc) play an important role in cellular metabolism acting as co-factor in a number of important enzymes. However, they can become toxic when elevated concentrations are introduced into the environment (Younis *et al.,* 2012). The concentrations of the metals (Cd, Cr, Cu, Fe, Pb, and Zn) in flesh of the two fishes were lower than WHO limit and pose no potential threats to public health.

Pollution monitoring using enzyme inducement or enzyme dispersion in fish or other aquatic organisms has been proposed for studying polluted environments. Various organic and inorganic wastes in industrial and domestic effluents are responsible for water pollution. Conventional laboratory toxicity studies cannot be extrapolated to the natural environment because they lack natural ecological realism (Abdelmeguid *et al.*, 2002). Since fish often respond to toxicants in a similar way to higher vertebrates they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. Plasma enzymes depends on the rate of release of enzymes from damaged cells, which in turn depend on the rate at which damage is occurring and at the extent of cell damage. The varied levels of aminotransferase observed in the two species investigated in the present study when compared to the mean levels recorded in other species (Younis *et al.,* 2012; Aliakbar *et al., 2010)* suggest high activity levels in these species. It has been reported that the variation in enzyme activities in heavy metals exposed fish is due to increased permeability of the cells as well as the direct effect of heavy metal on the tissues (Roy, 2002).

The pattern of AST and ALT activities recorded in this study showed that cellular damage could arise due to chronic exposure to heavy metals in the environment of the test species. No significant difference between plasma enzymes activity between the fish species occurred in the study, the results of this investigation showed a relatively high level of AST and ALT activities which is a consequent of tissue impairment due to heavy metal exposures.

Alkalinephosphatase (ALP) activity varied significantly between the two species with the activity higher in *C. nigrodigitatus* and lower in *C. senegalensis*. This may imply that *C. senegalensis* is less sensitive to heavy metals exposure compared to *C. nigrodigitatus*, since reduction in ALP activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis (Onikienko, 1963; Kori-Siiakpere *et al.,* 2010). Reduced ALP activity by stressors probably indicates an altered transport of phosphate and an inhibitory effect on the cell growth and proliferation. Conversely, elevated plasma ALP activity as observed in *C. nigrodigitatus* may be as a consequence of osteoblastic activity increase or due to intra and extra hepatic obstruction of bilary passage (Jyotti and Narayanan 1999).

Blood parameters in fish have been studied to elucidate physiological adaptation and to assess the health of fishes (Vazquez and Guerrero 2007; Yildz, 2009). Blaxhall and Diasley (1973) reported the use of haematocrit (PCV) and haemoglobin in checking anaemic conditions and reported values for fish usually vary from 20% to 35% (Pietse *et al.,* 1981) and do not exceed 50% (Clarke *et al.,* 1979).

The mean PCV value of 34.00% for *C. senegalensis* and 25.52% for *C.nigrodigitatus* recorded in the present study is within this range but this value for *C. nigrodigitatus* is lower than *C. nigrodigitatus* (35.52%) and *C.furcatus* (35.5%) from kwa River (Etim *et al.,* 1999). The difference in mean haemoglobin, PCV and RBC values between *C.. senegalensis* and *C. nigrodigitatus* could be attributed to differential responses caused by heavy metal exposures as it is well known that some metals cause early mortality of mature red blood cells and inhibition of haemoglobin formation through inhibition of erythrocyte alpha-amino levulinic acid dehydrogenase (ALA-D). Red blood cell (RBC) counts of 2.71×10^6 mm^3 for *C. senegalensis* and 2.95×10^6 mm^3 for *C. nigrodigitatus* was significantly high, according to Lenfant and Johnson (1972) erythrocyte counts greater than 1×10^6 mm^3 is considered high and indicative of high oxygen carrying capacity of the blood which is characteristic of fishes capable of aerial respiration and high metabolic rate. A strong inverse correlation (r=-0.94) which occurred between haemoglobin and Iron in *C. nigrodigitatus* compared to a weak positive correlation (r=0.28) recorded between haemoglobin and Iron in *C. senegalensis* could indicate that haemoglobin increased with a fall in Iron and vice- versa in *C. nigrodigitatus* while there was a very slight increase in Iron with a corresponding slight increase in haemoglobin concentration, this observed relationship could imply that *C. nigrodigitatus* is more sensitive to Iron contamination compared to *C. senegalensis*. This is reflected in the slight difference between mean RBC count of *C. nigrodigitatus* (2.95×10^{6} mm^3) and *C. senegalensis* (2.7×10^{6} mm^3). The mean haemoglobin value of *C. senegalensis* (11.33 ±8.04g/dl) and *C. nigrodigitatus* (13.32±8.29g/dl) was high and fell within the range (5.6-15.8g/dl) reported for *C. gariepinus* (8.70g/dl) (Sowunmi, 2003). These values are also higher than 4g/dl recorded for *Heterotis niloticus* (Fagbenro et *al.,* 2000). This observed haemoglobin level may be connected to an adaptive response to heavy metal contamination or low dissolved oxygen recorded. The observed lymphocytes that were numerous than any other differential cells for C. nigrodigitatus (64.33±31.94%) and *C. senegalensis* (80.00±71.17%) and basophils that were seldomly seen are typical of most fishes (Ezzat *et al.,* 1974; Kori-Siakpere and Ake 2005). Abundance of neutrophils in both species is similar to the observations of Breazile et *al.,* (1982) and Kori-Siakpere and Ake (2005) and thought to be normal picture for fish blood. The major findings of this study are that heavy metals were accumulated by both species at concentration below WHO/FAO/FEPA permissible limits for human consumption. Heavy metals are toxic substances, with changes in enzymes activities in serum of fish exposed to various concentrations. Results of the study indicated that sub-lethal and chronic concentrations observed may cause changes in the haematological parameters and enzymztic activities of the studied fishes, hence could be used as biomarkers of metal contamination and detection in aquatic systems such as the Lagos Lagoon.

CONCLUSION

Although results of heavy metal concentrations studied showed that consumption of the two species would not have any potential health implications; Similar studies may be performed to check contamination with other toxic metals such as mercury, cobalt, nickel and arsenic in commercial fish. Periodical monitoring of contaminants levels in fish by using enzymatic activities and haematological parameters in both commercial and feral fish is needful to checkmate any possible future accumulation and/ or increase as a result of waste and effluents discharge into the Lagoon. The use of feral fish species in local water bodies should be utilized as sentinel organisms for monitoring and management of the contamination status of these aquatic systems and should be complementary with conventional physico-chemical water monitoring regimes.

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