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The Impact of Heavy Metals on Haematological Parameters and Enzymatic Activies in Chrysichthys Nigrodigitatus and Pythonichthys Macrurus

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Abstract: Human activities have led to the accumulation of toxic metals in the aquatic environment. Heavy metals are serious pollutant in the aquatic environment because of their environmental persistence and ability to be accumulated and cause deleterious impact on aquatic organism. This study was carried out to investigated the levels of some selected heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in two fish species: *Chrysicthys nigrodigitatus* and *Pythonichthys macrurus* obtained from Makoko area of the Lagos lagoon between the months of July-September, 2012 and also to determined the effects of these metals on haematological parameters and enzymes activity of the fish species. The mean concentration of heavy metals in mg/l obtained for *C. nigrodigitatus* were Cd (ND), Cr (0.010±0.001), Cu (0.036±0.022), Fe (0.580±0.137), Pb (0.001±0.001), Zn (0.218±0.346) and P. macrurus; Cd (0.001±0.002), Cr (0.005±0.005), Cu (0.046±0.066), Fe (0.359±0.270), Pb (ND), Zn (0.228±0.376) were below the standards of WHO/FAO/FEPA permissible limit. The mean level of the enzymes recorded in (IU/L) for *C. nigrodigitatus* were Aspartate aminotransferase AST (71.95 ± 26.76), Alanine Aminotransaminase ALT (22.367±12.83), Alkaline phosphatase ALP (164.61 ± 26.65) and for *P. macrurus* were AST (115.82±41.04), ALT (37.79± 25.16) and ALP (59.80±21.99) respectively. Statistical significance difference ($P<0.05$) occurred in serum ALP between the two fish species. There was no statistically significant difference ($P>0.05$) recorded in all haematological parameters, the values revealed a high Haemoglobin and PCV concentration for both fishes. Results for *C. nigrodigitatus* were Hb (12.34 ± 2.15g/dl), PCV (37.00±6.45%), RBC (3.57± 0.92$10^6$$mm^{-3}$), WBC (136.20±59.06$10^4$$mm^{-3}$), MCV (108.06±28.46FL), MCH (35.99 ±9.45Pg), MCHC (33.32 ± 0.03g/l), Neutrophils (35.33±8.76%), Lymphocytes (64.67±8.76 %) and *P.macrurus* values were Hb (13.84 ±2.65g/dl), PCV (41.50± 7.94%) RBC (4.18±1.04 $10^6$$mm^{-3}$), WBC (115.87 ± 28.04 $10^4$$mm^{-3}$), MCV (101.37± 16.92FL), MCH (33.86±5.62Pg), MCHC (38.87±13.59 g/l), Neutrophils (39.00± 13.87%) and Lymphocytes 60.67±13.60 (%). Deductions from this study revealed an alteration in the haematological parameters and enzymes activity of *C. nigrodigitatus* and P.macrurus. The result indicated that the concentrations of these heavy metals fall within their respective acceptable permissible limits recommended by the WHO/FAO/FEPA. Hence the consumption of these fish species may not pose any threat to human health. However periodic monitoring of these metals in the fish and water is highly essential to public health due to bioaccumulation.

Key words: Heavy Metals · Haematological Parameters · Enzymatic Activies · Fish

INTRODUCTION

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increase flux of metallic substances in the aquatic environment [1]. The metals are of special concern because of their diversified effect and the range of concentration which stimulated toxic ill effect to aquatic life forms. Industrial waste constitutes the major source of metal pollution in natural water [2]. Aquatic environment are exposed to a number of pollutants that are majorly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas, these pollutants pose a serious threat to aquatic life.

Heavy metals are high priority pollutants because of their relative high toxicity and persistent

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nature in the environment. Therefore knowledge of the changing concentration and distribution of heavy metals and their compounds in various compartments of the environment is a priority for good environmental management programme all over the world [3].

Fishes are good bio indicator of the degree of pollution in the aquatic environment due to their propensity to bio accumulate toxic metals and other contaminant in their internal organs, most especially their edible tissues. They could reach toxic concentration which could lead to serious health hazard [4]. They accumulate metals to concentration many times higher than that present in water or sediment [5]. Among the aquatic fauna, fish is the most susceptible to heavy metal contamination than any other aquatic fauna [6]. Pollution enters fish through five main routes: via food or non-food particles, gills, oral consumption of water and the skin. The absorbed pollutants are then carried in the blood stream to either a storage point or to the liver for transformation and/or storage [7, 8].

Enzyme activity are considered as sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water for the presence of toxicants[9]. A major site of toxic action for metal is interaction with enzymes, resulting in either enzyme inhibition or activation.

Human activities have led to the accumulation of toxic metals in the aquatic environment [1]. Heavy metals are serious pollutant in the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organism; metal ions at high toxicity are known to cause deleterious impact on organs and blood vessel in fish [10, 11]. By virtue of its position the Lagos lagoon is surrounded by the densely populated and industrialised Lagos metropolis making it a convenient dumping site for numerous industrial and domestic wastes. According to Singh *et al.,* [12] an estimated 10,000m^3 of industrial effluents are discharged into the Lagos lagoon per day.

In view of this, it is important to control the discharge of heavy metals and other contaminant into the aquatic environment because of their slow rate of degradation and the ability to accumulate up the food chain. This study is to investigate the level of some selected Heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in the flesh of *Chrysichthys nigrodigtatus and Phthonichthys macrurus,* to determine the enzyme activity (ALT, AST and ALP) and haematological parameters in *Chrysichthys nigrodigtatus and Phthonichthys macrurus.*

MATERIALS AND METHODS

Description of the Study Area: The Lagos lagoon is a large expanse of shallow water covering an area of about 208 km^2 (Figure 1). The Lagos lagoon lies between

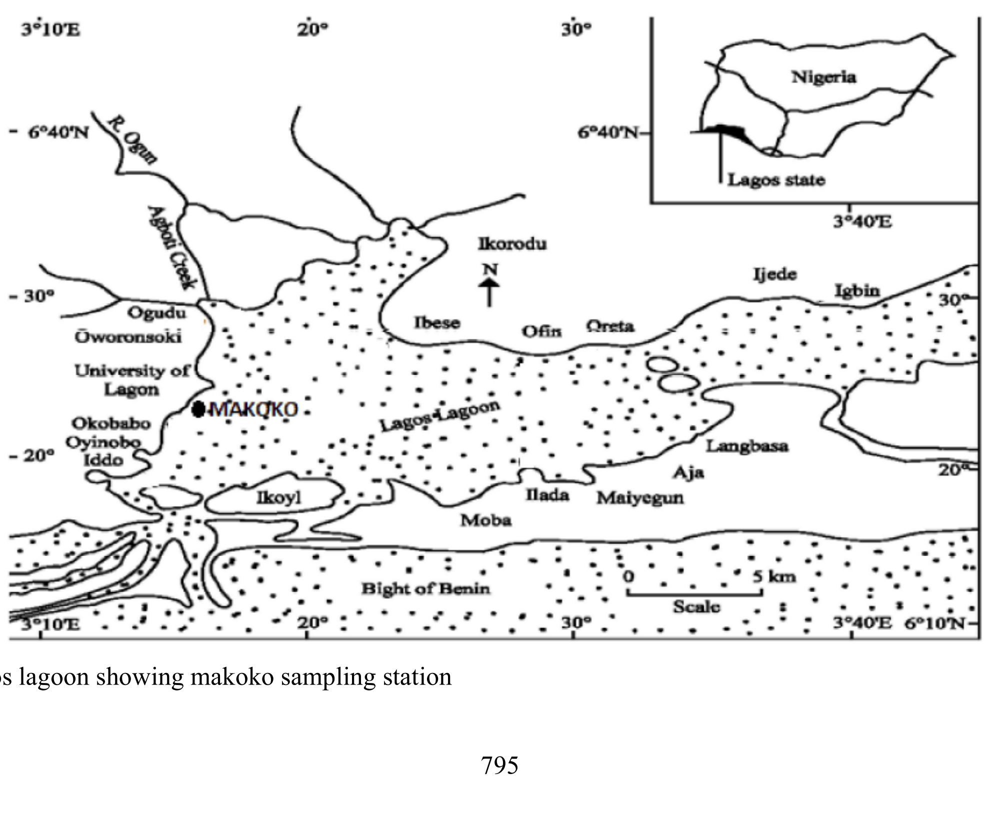

Fig. 1: Lagos lagoon showing makoko sampling station

longitudes 30° 333' E and 30° 35' E and latitudes 60°24' N and an area of 208km^2 and is the largest of the lagoon systems of the West Africa sub- region. It is the largest of the nine coastal lagoons of South-western Nigeria (Others are Yewa, Badagry, Iyagbe, Ologe, Kuramo, Epe, Lekki and Mahin Lagoons). It serves as an important seaport, nursery ground for fisheries and a source of food supply and for recreational purposes. As a result of lagoon's rich fauna, especially in fishes, fishing activities are usually witnessed on a daily basis.

Makoko market is mainly used by fishermen as a landing site for fisheries resources from the Lagos lagoon were a large number of fish species are landed alive. The geographical location is on longitude 6°32^{1}N and latitude 3°35^{1}E was taken using Global Position System (GPS).

Field Sampling: Samples of the experimental fish; *Chrysicthys nigrodigitatus (*badgrid catfish) and *Pythonichthys macrurus* was collected twice a month between the months of July to September at Makoko fishing site between the hours of 6:30am and 7:30 am. The mean standard length and weight of the *C. nigrodigitatus* was 31.50cm and 238.67g, for *P. macrurus* was 54.0cm and 286.67g respectively. Blood samples was collected in lithium heparin bottles and ethylene diamine tetra-acetic acid (EDTA) bottles for enzymes activities and haematological parameters respectively placed in iced chest to avoid possibilities of coagulation prior taking to Nigerian Institute for Medical Research (NIMR), Yaba Lagos for analysis. Heavy metals analysis both in flesh and water was carried out in the chemistry laboratory of the University of Lagos.

Water Sampling: Water samples were collected 15cm below the surface at the sampling site using 1litre plastic bottles and corked immediately while under the water. The samples were taken to the laboratory immediately for chemical oxygen demand and heavy metal analysis. Metals in water samples are determined using Atomic Absorption Spectrophotometer.

Surface Water Temperature (°C): Surface water temperature was determined in-situ using the mercury-in-glass thermometer calibrated in degree centigrade (°C). The thermometer was immersed in the water for three (3) minutes and the level of the mercury was read on the graduated glass tube.

Hydrogen Ion Concentration (pH): pH is a measure of the hydrogen ion concentration in water. It was determined using the pH meter on the field (in situ) by dipping the probe into the water and the value on the meter recorded.

Salinity (°/$_{00}$): The water salinity was determined in-situ using a hand held refractometer. This was done by placing a drop of the water sample on the prime of the meter of the refractometer and the meter was adjusted to 0% marks and viewed through the eye piece. The day light plate was closed and the salinity of the water sample was read on the scale.

Turbidity (FTU): Turbidity is a measure of the amount of light penetration into an aquatic system. The turbidity of the water samples at the study site was measured using the Horiba water checker, model U-10. The probe of the U-10 was placed in the water and the value was recorded from the meter.

Dissolved Oxygen (mg/l): The dissolved oxygen level was measured in-situ using a Dissolved oxygen meter, the probe of the meter was placed in the water and the reading was recorded from the meter.

Conductivity (µScm^{-1}): Conductivity is a measure of how well a solution conducts electricity. The conductivity of the water samples were measured using the Horiba water checker, model U-10. The probe was immersed in the water and the value on the meter was recorded.

Blood Analysis:

Collection of Blood and Haematological Examination: Blood from the caudal blood vessel of *C. nigrodigitatus* and *P. Macrurus* was collected on the field using 5ml sterile plastic syringe fitted with 0.8 x 40mm hypodermic needle. 2ml of the blood was collected and introduced into ethylene diamine tetra-acetic acid bottles (EDTA) for haematological examination and another 2ml of blood samples into Lithium heparin bottles for enzymatic assay. The bottles were directly placed in iced chest to avoid possibility of coagulation and were taken to the laboratories for further examination.

Estimation of Liver Enzyme in Serum: The blood samples in lithium heparin bottles were centrifuged at 3000 revolution per minute (rpm) for 10 minutes which led to the separation of the plasma and serum. The serum was collected for the analysis of the activities of amino transamine (AST), alanine amino transamine (ALT) and alkaline phosphatase (ALP) using Reitman and Frankel [13] method with Randox assay kits.

Statistical Analysis: One way analysis of variance (ANOVA) at 5% level of significance was used. When the ANOVA revealed significant differences, Post-hoc comparison of means between fish species was carried out using Least Square Differences (LSD). Independent T-test was used to analyse for the significance of metals between species. Pearson correlation analysis was used to ascertain the relationship between heavy metals in fish, haematological parameters and enzymes activity using SPSS 17.0 package.

RESULTS

Heavy Metals Content in the Flesh of *C. Nigrodigitatus* and *P. Macrurus* and Water: Results for the mean concentration of heavy metals in the flesh of *C. nigrodigitatus, P. Macrurus* and water are represented in table 1. Cadmium (Cd) had a maximum mean concentration of 0.001mg/l in *P.macrurus,* lead with 0.001mg/l in *C. nigrodigitatus* and 0.002mg/l in water, these two metals were not detected in others. Fe had the highest mean concentration of 0.58mg/l in *C. nigrodigitatus* and 0.359mg/l in *P. Macrurus*. Generally the heavy metal concentration in both fish species and water of Makoko was low and below the WHO/FAO/FEPA required limit.

Analysis of variance (ANOVA) in the mean values of the heavy metals concentration in *C. nigrodigitatus, P. Macrurus* and water for chromium indicate significant difference (P<0.05).There was significant difference (P<0.05) for Zn concentration in *C. nigrodigitatus* and in water and *P. macrurus* and water content showing, significant difference also in Pb. There was no significant different (P>0.05) recorded in Cd and Cu concentration throughout the sample period.

Serum Enzyme Activity in *C. Nigrodigitatus and P. Macrurus:* The T-test analysis showed significant difference (P<0.05) for mean serum ALP activity in *C. nigrodigitatus* (59.80±21.99IU/L) and *P. Macrurus* (164.61±26.65IU/L) for the sampling period. There was no significant difference (P>0.05) between mean serum ALT activity in *C. nigrodigitatus* (22.367±12.83IU/L) and *P. Macrurus* (37.79 ± 25.16IU/l) and also no significant difference (P>0.05) between mean serum AST in *C.nigrodigitatus* (71.95 ± 26.76IU/L) and *P.macrurus* (115.82 ± 41.04IU/L) during the sampling period (Table 2).
HAEMATOLOICAL PARAMETERS IN FISH.

Table 3 shows the T-test analysis indicate no significant difference (P>0.05) for all the haematological parameters in *C. nigrodigitatus* and *P. Macrurus* during the sampling period.

Table 1: Level for heavy metals in *C. Nigrodigitatus, P. Macrurus*, water and WHO standard

	Cd mg/L	Cr mg/L	Cu mg/L	Fe mg/L	Pb mg/L	Zn mg/L
C. nigroditatus	ND	0.010±0.001	0.036±0.022	0.580±0.137	0.001±0.001	0.218±0.346
P. macrurus	0.001±0.002	0.005±0.005	0.046±0.066	0.359±0.270	ND	0.228±0.376
WHO Guideline for fish	0.2	0.15	-	-	1.5	1.50
WATER	ND	0.019±0.005	0.029±0.011	1.67±0.978	0.002±0.02	0.844±0.392
WHO Guideline for water	0.005	0.05	-	-	0.01	3.0

Table 2: Levels for enzyme activities in serum of *C. nigrodigitatus* and *P. Macrurus*

	AST (IU/L)	ALT (IU/L)	ALP(IU/L)
C. nigroditatus	71.95 ± 26.76	22.367 ± 12.83	164.61 ± 26.65
P. macrurus	115.82 ± 41.04	37.79 ± 25.16	59.80 ± 21.99

Table 3: Haematological parameters in serum of *C. nigrodigitatus* and *P. Macrurus*

PARAMETRS	*C. nigroditatus*	*P. macrurus*
Haemoglobin (g/dL)	12.34 ± 2.15	13.84 ± 2.65
Packed cell volume (%)	37.00 ± 6.45	41.50 ± 7.94
Red blood cell (10^6 mm^{-3}	3.57 ± 0.92	4.18 ± 1.04
White blood cell (10^4 mm^{-3}	136.20 ± 59.06	115.87 ± 28.04
Mean corpuscular Volume *(FL)*	108.06 ± 28.46	101.37 ± 16.92
Mean Corpuscular Hb (Pg)	35.99 ± 9.45	33.86 ± 5.62
Mean Corpuscular Hb conc (g/L)	33.32 ± 0.03	38.87 ± 13.59
Neutrohyl (%)	35.33 ± 8.76	39.00 ± 13.87
Lymphocyte (%)	64.67 ± 8.76	60.67 ± 13.60
Monocyte	ND	0.33 ± 0.52
Eosinophile	ND	ND
Basophile	ND	ND

Table 4: Water Quality Parameters

	Minimum	Maximum	Mean	Standard deviation
Dissolved oxygen (mg/L)	0.10	1.80	1.02	0.610
pH	5.60	7.50	6.517	0.83
Conductivity (µScm)	1600.00	4800.00	3416.67	1348.21
Turbidity (FTU)	1.000	3.000	2.00	0.89
Temperature(°C)	24.00	25.00	24.33	0.52
Salinity	1.00	2.00	1.50	0.56
Chemical Oxygen demand	24.00	32.00	27.50	3.78

Table 5: The Length and weight relationship of *C. nigrodigitatus* and *P. Macrurus*

Specie	Minimum	Maximum	Mean	Standard deviation
C. nigroditatus				
Length (cm)	30.00	33.00	31.50	1.049
Weight (g)	190.00	307.00	238.67	51.97
P. macrurus				
Length (cm)	45.00	60.50	54.00	4.92
Weight (g)	210.00	320.00	286.67	38.94

Water Quality Parameters: There was a strong correlation (r =0.96) and a statistical significant difference (P <0.05) between conductivity and salinity. A strong relationship (r =0.82) and a statistical significant difference (P> 0.05) occurred between turbidity and salinity.

Other water quality parameters showed no statistical significance (P>0.05) (Table4).

DISCUSSION

Toxic pollutants including heavy metals are ubiquitous in polluted aquatic environment. Heavy metals are continually released into the aquatic environment from natural and industrial processes. Many of these metals occur naturally in the environment and are essential for normal metabolism of aquatic organisms. However, industrial and agriculture waste have elevated the levels of such metals in the aquatic environment. Among various water pollutants, heavy metals pose a great threat to fishes. From the results of the heavy metal concentration, it is observed that *P. macrurus* and *C. nigrodigitatus* accumulated low concentrations of metals. The pattern of concentration for the two species are almost similar; for *P. macrurus* (Fe > Zn> Cu> Cr> Cd> Pb) and *C. nigrodigitatus* (Fe> Zn> Cu> Cr> Pb> Cd). The level of accumulation have been found to be below WHO/FAO/FEPA permissible limits, hence fish landed from the Makoko area of the Lagos lagoon would not constitute human health hazards. Mean copper content found in the two species for this study i.e., *P. Macrurus* (0.046 ± 0.07mg/l) and *C. nigrodigitatus* (0.036±0.02) due to accumulation suggest the predisposition of these species to inhibition of Na^+ uptake in fish gills [14] which could result in significant loss of Na^+[15]. This may finally disrupt the enzyme sodium potassium ATP by membrane damage to the cells of both fish species.

The liver tissue in fish is more often utilized as an environmental indicator of pollution. Toxicants cause a disturbance in the physiological state of the fish which affect the enzymes activity, it then causes distortions in the cell organelles which may lead to the elevation in the activity of various enzymes. Transaminases play an important role in carbohydrate and amino acid metabolism in the tissue of fish and other organisms [16]. Alamine amino transferase is a key metabolic enzyme released on the damage of hepatocytes.

Both species showed a quite substantial quatum of Zinc content in the flesh of *P. Macrurus* (0.228±0.38mg/l) and *C. nigrodigitatus* (0.218±0.35mg/l). Short and long term exposure to zinc may indicate hepatic damage. Abdel- warith *et al.,* [16] have reported a significant increase in AST and ALT activity in Zinc exposed *Oreochromis niloticus*, hence it suffice to envisage similar trend in *P. Macrurus* and *C. nigrodigitatus* consequent to Zinc accumulation over time.

Alkaline phosphatase is a membrane bound enzyme found at bile pole of hepatocytes and also found in pinocyte vesicle and Golgi complex. It is present on all cell membranes where active transport occurs and hydrolase and transphosporylase in function. Decrease in ALP activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis [17]. The study of different biochemical and cellular constituents in blood is of fundamental importance in the physiological and physiopathological evaluation of animals, because quantitative variations in blood parameters can be induced by pollutants and other environmental factors [18]. According to Nussey *et al.,* [19], the study of the haematological picture is frequently utilized for the detection of physiopathological profile changes in different stress conditions such as exposure to heavy metals. Physiologically haemoglobin is crucial to the survival of fish, being directly related to the oxygen binding capacity of blood. However the Mean haemoglobin content of *C.nigrodigitatus* (12.34±2.15) is within the normal range recorded for African catfish [20, 21]. The mean PCV value for *C. nigrodigitatus* (37.00±6.45) and *P. macrurus* (41.50± 2.65) for both fishes agrees with [21], who reported that normal haemotocrit values usually fall within the range of 20-35% and rarely greater than 50% for fish, the higher mean PCV value for *P.macrurus* (41.50± 2.65) appears to be a characteristics of fish for the order Anguilliform. The variation between these parameters showed by this study may be connected to the level of heavy metal exposure recorded for the period of study. No significant difference in the heavy metals measured in the fish with Cd (0.001 ± 0.002mg/l); Cr (0.005 ± 0.005mg/l); Cu (0.046 ± 0.066mg/l); Fe (0.359 ± 0.270mg/l); Zn (0.228 ± 0.376mg/l) were below permissible limits for human consumption. This could be associated to an adaptive response to cope with stress caused by heavy metal presence in the fish species from the study location. It would be observed that the haematological parameters between *P. macrurus* and *A. anguila* revealed a possible distortion. The differences in these blood indices may be attributed to a defence reaction against toxicity through the simulation of erythropoiesis.

CONCLUSION

Contamination of aquatic environment by heavy metals either as a consequence of acute or chronic events constitutes additional source of stress for aquatic organism. Sub lethal concentration of toxicants in the aquatic environment, as recorded in the present study will not necessary result in outright mortality of organism but may result to effect in genotoxicity, teratogenicity, reproductive abnormalities and carcinogenicity in feral organism. Toxicants and pollutants can result in several physiological dysfunctions in fish which could induce changes in blood parameters and enzymes activity, which makes them predilective biomarkers for aquatic environmental pollution monitoring.An immediate attention from concerned authorities is required to protect the Lagos lagoon from potential pollution from a myriad of anthropogenic activities. The need arise for government to strictly monitor the source of domestic waste and industrial effluent discharge of heavy metals to prevent lethal concentrations which may be detrimental to humans who consume fishery resources from the lagoon. Ajani and Ayoola [22] observed that bioaccumulation and bio-concentration of various heavy metals in aquatic organisms also need effective monitoring.

It is therefore necessary that regulatory authority such as Federal Ministry of Environmental (FME) and other regulatory bodies ensure that existing laws are strictly obeyed and if there is a need for heavy fine on defaulters who fail to obey the laws governing the laws of discharge of industrial waste. Public awareness on the proper disposal of waste from the shanty community in Makoko should be implemented. However, more research needs to be carried out on the haematological parameters and enzymes activity on *C. nigrodigitatus* and especially *P. macrurus* as no reference or baseline values are available for comparison.

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