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Blood Chemistry, Antioxidants, Cytochrome P_{450} Activty and Heamatological Properties of *Clariagariepinus* (African Catfish) Exposed to Heavy Metals (Copper and Lead Salts)

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Abstract*— Fishes are one of the most important food resources and are considered as sources of the primary protein worldwide. Ongoing freshwater pollution increases the concentration of toxic metals in water and negatively affects fish health. These pollutants, which have a negative effect on fish, are released by agriculture, industrial wastewater discharge, raw sewage extraction, chemical waste, and oil spills due to fishing vessels. Waterborne metal exposure affects the physiological and biochemical factors in fish blood and tissues.The study investigated the changes in growth performance, hematological parameters, plasma components, and stress indicators of juvenile (African catfish) Clarias agriepinus, depending on varying exposures to copper and lead salts. In this study, 30 fish juveniles were engaged in each group of the five groups.C. agriepinus was exposed to the heavy metals at 20 and40 µg/l for first 15 and 30 days. Majority of the biomarkers such as GSH, catalase, cytP_{450}, protein, blood glucose were measured spectrophotometrically. The results showed that Cu and Pb exposures resulted in decreased daily length gain (DLG), and daily weight gain (DWG). In terms of hematological parameters, white blood cells and red blood cell (RBC) count significantly decreased after 2 weeks and four weeks. Plasma enzyme components including transaminases and Cyt P_{450} significantly increased. This work evidently demonstrated that at ecologically relevant concentrations, copper and lead can inhibit cellular functions of aquatic organisms. Most of the results obtained in this work were in line with some previous works.

Keynotes*— juvenile, pollution, concentration, plasma, waterborne, freshwater, biochemical

I. INTRODUCTION

Water as an essential of life is utilized for domestic, agricultural, industrial activities, and among others. Despite being an indispensable to humanity on the earth, it is poorly managed in many parts on the globe (Corcoran, 2010). Water is a universal solvent and it always dissolves other substances that get in contact with it. The contamination of water in particular ways makes it hard to find pure water (Desai*et al*., 2014). Even rainwater collects impurities from the atmosphere during precipitation. The contaminants increase when it runs off the land surface and gathers or mixes with sewage and industrial effluents (Ho *et al.*, 2012). Rivers, oceans and streams are the popular sources of water (Alrumman*et al*., 2016).

Anthropogenic activities have been documented as the main cause of water pollution in many parts of the world (Alrumman *et al*., 2016). Rivers, lakes, underground waters and oceans sre exposed to a great deal of contamination from various human activities that alter water quality by changing both its physiochemical and biological parameters; it makes it unsuitable for domestic use and several other purposes. However, pollution affects ecosystems and makes lives unfit for species, migration and extermination of species (as a result of damages to reproductive system) (Briggs, 2003).

Water pollution involves the release of toxic substances, pathogens, substances radioactives into water bodies (Wong *et al*., 2001). Lack of oxygen in a water body (eutrophication) is caused by excessive plant growths on water surface. According to the water cycle, naturally, Water around is absorbed into the land (soil) and rivers or stream Organic pollutants are biodegradable by microbes and can be converted to a form that brings benefits to the aquatic life (Makokha *et al*., 2008).

A Few of the metal pollutants include lead (Pb), arsenic (As), mercury (Hg), chromium (Cr), nickel (Ni), barium (Ba), cadmium (Cd), cobalt (Co), selenium (Se), vanadium (V), Some minerals like

Zinc (Zn), copper (Cu), iron (Fe) manganese (Mn), sulphur (S), boron (B) are useful to lives in small and prescribed doses above which they are toxic. Cyanides, thiocyanides, phenolic compounds, fluorides, radioactive substances, are harmful to man and animals (Kinraide *et al*., 2007).

All the categories of reactive species (RS) are oxidative stressors when the balance between reactive species (ROS) formation and detoxification is compromised RS causes damage to cellular macromolecules causing lipid, nucleic acid, and protein alterations. These paths of biochemical mechanism are for the initiation, progression phases of diseases such as atherosclerosis, ischemic heart diseases, diabetes, and cancers, liver diseases. There must be a number of radical scavenging enzymes to maintain a threshold level of RS inside the cell In order to maintain proper cell signaling, (Imlay *et al*., 2003).

The antioxidant enzymes in body cells consist fall in two primary and secondary antioxidants enzymes (catalases, superoxide dismutases (SOD), and glutathione peroxidases (GPX); glutathione reductase, glutathione-S-tranxferase, glucode-6-phosphate dehydrogenase). All play crucial roles in maintaining homeostasis of radical species in the cells. The induction of these enzymes reflects a Specific response of these enzymes to oxidative stress is demonstrative of their activation (Tappel*et al*., 1982). SOD converts superoxide ions to H_2O_2 and oxygen molecule (Hayes *et al*.,1995). Glutathione-S-transferases (GST) detoxify cytotoxic compouds to protect tissues against oxidative damage (Hayes *et al*., 1995). Besides certain roles in the endogenous metabolism, Glutathione-S-transferases are also associated with the detoxification of drugs, carcinogens, and environmental pollutants in man and animals (Shen *et al*., 1991).Studies once revealed that GPx and GST are inactivated by hydroperoxides which exert toxicity either directly by oxidation of SH groups of proteins or indirectly by hydroxyl radicals' formation (Pigeolet*et al*., 1990). The enzymatic action of catalase in decomposing H_2O_2 is shown below (Hoshino and Mishima, 2008)

$$2H_2O_2 + O_2 \rightarrow 2H_2O$$
$$H_2O_2 + AH_2A \rightarrow 2H_2O$$

The turnover rate of catalase is the highest among all antioxidant enzymes.

Aminotransferases are special enzymes that are connected with the reversible transfer of the amino group from alpha loci of α-amino-acids to an oxoacid. Over 60 such enzymes have been characterized. Examples are aspartate aminotransferase (AST, EC 2.6.1.1, formerly known as glutamate-oxalacetate transaminase or GOT) and alanine aminotransferase (ALT, EC 2.6.1.2, formerly known as glutamate-pyruvate transaminase or GPT) (Mijovic *et al.,* 1987). These enzymes are widely distributed in human organs; a contributory factor of their both broad application to a variety of pathologic conditions and reputation for lacking specificity.

The reactions catalyzed by AST and ALT are:
L-aspartate + 2-oxoglutarate ↔ oxalacetate + L-glutamate, catalyzed by AST
L-alanine + 2-oxoglutarate ↔ pyruvate + L-glutamate, catalyzed by ALT
The three oxo acids are components of the tricarboxylic acid cycle or carbohydrate metabolism. The two aminotransferases are keys to shunting these substrates into several metabolic pathways. AST is important in the transport of reducing equivalents across the mitochondrial membrane through the malate aspartate shuttle (Ioannou *et al*.,2006).

The heme iron in the CYP enzymes requires regular supply of electron for catalytic roles. This electron supply for CYP enzymes majorly comes from NAD(P)H. (Denison*et al*., 2003).
It is a usual practice to correlate physical and chemical parameters with biomarkers in pollution monitoring studies in order to evaluate the effects of xenobiotics on aquatic organisms (Abdel-Moneim and Abdel-Mohsen, 2012). The reactions of organisms towards chemical contaminants are employed as biomarkers or lookouts of environmental conditions. Biomarkers are measurable biological scenarios that arise in animals due to acute or chronic exposures to contaminants. These morphological deviations are clearly employed in biomonitoring studies. Moreover, histological investigations are economical techniques to determine the health status of the resident populations (Devi *et al*., 2013). When the concentration of a toxicant has been raised to a level sufficient to elicit cellular injury, sublethal changes develop in the target tissue and they could be very well evaluated (Al-balawi *et al.,* 2013). The effects of pollutants on cell's structure and function are diverse due to the fact that some of them alter the

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permeability property of membrane systems. A few others are inducers of enzymes causing cellular dysfunctions culminating irreversible necrotic changes (Al-Yousuf and El-Shahawi,1999).

Liver is prone to damages from varios types of toxicantsm, else considered an ideal indicator of aquatic pollution (Sniderman, 2004)). In fish, liver is a detoxifying site which shows alterations in histological architecture and deviation from normal biochemical and physiological responses on being contaminated (Angulo, 2002).

II. RESEARCH ELABORATION

Significance of the study

The environmental pollution and pollutants by heavy metals are popularly global threats in aquatic domain because of their toxicity. Domestic, industrial and anthropogenic activities have become the source of contamination to natural aquatic systems (Velez, D. and Montoro, 1998).

The heavy metal discharges have harmful effects on the environmental equilibrium and aquatic properties (Akinmoladun *et al.,* 2007). In animal species, bad effects of these pollutants can not be negligible for fishes (Olaifa *et al.,* 1998). The pollutants such as heavy metals bioaccumulate in food chain and cause the antagonistic effects and death to fish (Farkas *et al.,*2002).The toxic effects of heavy metals can affect the individual growth rates, physiological functions, mortality and reproduction in fish (Amundsen *et al.,* 1997). Heavy metals get into fish through gills, digestive tract and body surface. The gills are the significant routes of direct metal uptake (Romeo *et al.,* 1999)

In view of this, this study had gained importance in order to establish some of the aforementioned facts and to settle some research issues. This study would differentiate between the resulting disorders of acute and chronic poisoning of freshwater catfish upon the assessment of heamatological, biochemical parameters and blood chemistry.

Aim of the study

The aim of this study is to evaluate the effect of subchronic toxicity of copper sulfate and lead nitrate in the juvenile *Claria gariepinus* African catfish.

Objectives of this study

The main objective of this study is to

i. To determine the growth performance and nutritional parameters of the*Clarias agariepins* (African catfish) exposed to heavy metals.

ii. To determine the antioxidant parameters in the liversand kidneys of the *Clarias agariepins* (African catfish) exposed to heavy metals.

iii. To assess the blood chemistry of *Clarias agariepins* (African catfish).

iv. To evaluate the heamatological parameters in the *Clarias agariepins*(African catfish) exposed to heavy metals of varied concentrations.

III. METHODS

Samplng Procedure150 African black catfish were purchased from Oyo state fishery research laboratory. The fish were authenticated at the Zoology department, University of Ibadan, Ibadan. They were divided into 5 groups inside plastic aquariums and acclimatized for two weeks namely; control (30), CU-20 (30), CU-40 (30) and Pb-20 (30), Pb-40 (30).

The fishes were sacrificed by cervical dislocation after which the blood was collected: some into the anticoagulant tubes for blood glucose test and other into the ordinary tubes for heamatological examinations. The livers and kidneys were collected into the sucrose buffer solution to be homogenized for biochemical assays.

Heamatological analysis

The process followed was based on the instruction manual of hematology analyzer The total red blood (RBC) count, total number of white blood cell (WBC), neutrophil, and platelet (PLT), Mean corpuscles volume (MCV),Mean corpuscles hemoglobin (MCH), Mean corpuscles and Hemoglobin concentration (MCHC) were assessed.

Biochemical Analysis

Determination of Protein Concentration.

The protein concentration of the liver homogenate was determined by means of spectrometry. The biuret method as described by Gornallet *et al*.,(1949) with some modifications: the addition of potassium iodide to prevent precipitation of cu^{2+}ions as cuprous oxide. Principle: In alkaline solution the amino group of proteins reduced cuprous ions to the Cupric form which then form a blue-coloured complex with the protein that absorbs maximally at 540nm.Sodium

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potassium tartrate is added to stabilize the complex (Gornallet *et al*., 1949).

Superoxide Dismutase (SOD) Activity

The activity of SOD was determined by the method of Misraand Fridovich (1972).

Principle

The ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase Superoxide (O_2*) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced by O_2*introduced increases with increasing pH (Valerino and McCormack, 1971) and also increases with increasing concentration of epinephrine.These results led to the proposal that autooxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide radical and enhance inhabitable superoxide dismutase.

Estimation of Reduced Glutathione (GSH) level

The method of Beutler *et al.* (1963) was followed in estimating the level of reduced

Glutathione (GSH).

Principle

The reduced form of glutathione comprises in most instances the bulk of cellular nonproteins sulfydryl groups. As such, deproteinization of samples with sulphosalicylic acid is necessary to ensure that noprotein cysteine thiol groups can react with colour reagent. This method is therefore based upon the development of a relatively stable yellow coloured product when 5,5′–dithiobis-2-nitrobenzoic acid (DTNB; Ellman'sreagent) is added to sulfhydryl compounds of which glutathione comprises the bulk in tissues.The resulting chromophoric product possesses maximum absorbance at 412nm and the amount of reduced glutathione in the sample is proportional to the absorbance at this wavelength.

Assessment of markers of liver and kidney function (Blood chemistry)

Estimation of aspartate aminotransferase (AST) activity.

AST activity in the serum was measured using RANDOX standard assay kits, and the Procedure was according to the manufacturer's instruction.

Principle

α-oxoglutarate+L-aspartate → GOT L-glutamate+Oxaloacetate

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Determination of Alanine aminotransferase (ALT) activity.

ALT activity in serum was measured using RANDOX standard assay kits, and the procedure according to the manufacturer's instruction.

Principle

α-oxoglutarate + L-alanine $\xrightarrow{\text{GPT}}$ L-Glutamate + pyruvate

Alannine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

IV. RESULTS

Table 1: Growth performance and nutritional parameters of the African catfish exposed to heavy metals For 15 and 30 days.

15 Days	Protein (mg)	Glucose (mg/dl)	Growth Index
Control	8.5405±0.1	47.0000±5.6	83.3200±23.5
Cu-20	7.3805±0.1	61.0000±23.3	29.2500±7.4**
Cu-40	8.6130±0.2	87.0000±3.5	33.8500±8.6**
Pb-20	10.1210±3.4	99.5000±4.9*	41.1000±8.4**
Pb-40	7.2065±0.4	93.0000±18.3	26.3750±5.5**
30 Days	Protein (mg)	Glucose (mg/dl)	Growth Index
Control	8.5405±0.2	69.9004±10.6	354.0000±173.6
Cu-20	7.3805±0.2	67.4545±2.8*	80.0000±62.4**

Cu-4069.8990±0.7	8.6130±0.6*	71.3880±2.9*	73.5666±23.7**
Pb-20	10.1210±0.3	60.6540±5.8	71.9333±10.5**
Pb-40	7.2065±0.1	69.8990±0.7	50.3000±9.7**

Data are presented as mean±SEM (n=5), P-value<0.05 were considered significant.

Table 2: Antioxidant parameters and blood chemistry of the liver of African catfish exposed to heavy metals For 15 and 30 days.

15 Days	SOD (U/mg protein)	CAT (U/mg protein)	GSH (U/mg protein)	ALT (U/L)	AST (U/L)	CYP-450 (ρg/ml)
Control	489.5927±735	454.1667±35.3	133.0334±0.	87.59399±9.9	78.84616±19	69.90042±10.6
Cu-20	507.3819±103	129.4433±2.5	129.4433±2.5	89.47369±74	158.6539±63.9	67.4545±2.8
Cu-40	436.1854±6.3	150.6167±52.0*	119.6170±10.7	70.3007±1.3	373.0769±32.6**	71.388±2.9
Pb-20	319.6819±10.8	354.1667±29.4	128.6505±0.7	72.55639±0.6	117.3077±5.4	60.654±5.8
Pb-40	533.2146±54.7	133.6700±157.4*	124.4717±0.7	66.16542±9.8	164.3846±12.1	69.89±0.7
30Days	SOD (U/mg protein)	CAT (U/ng) (U/mg protein)	GSH (U/mg protein)	ALT (U/L)	AST (U/L)	CYP-450 (ρg/ml)
Control	429.2711±10.1	454.1667±35.3	133.4588±0.7	65.78948±24.9	113.4616±5.4	78.12048±5.1
Cu-20	279.7615±9.4**	166.6665±5.8***	127.9433±0.4	92.85716±11.1	149.0385±93.8	71.53614±0.7
Cu-40	73.74022±9.7**	71.33333±0.7***	120.772±0.6	92.48121±8.5	360.5769±28.5*	98.51206±5.1**
Pb-20	112.9457±45.2**	71.33333±0.7***	126.1505±1.3	115.0376±26.5	275.9616±178.1	66.9006±0.2
Pb-40	21.25238±0.4**	145.8334±41.2***	128.4323±0.6	103.7594±1.06	391.3462±53.0*	73.51205±0.2

Data are presented as mean±SEM (n=5), P-value<0.05 were considered significant.

Table 3: Antioxidant parameters and blood chemistry of the kidney of African catfish exposed to heavy metals For 15 and 30 days.

15 days	SOD (U/mgprotein)	CAT (U/mg protein)	GSH (U/mg protein)	ALT (U/L)	AST (U/L)
Control	54.42±69.141	56.232±24.31	3.5106±0.1	109.123±10.16	68.132±7.51
Cu-20	43.1123±4.18	111.671±66.24*	3.4213±0.1	115.01324±5.664	90.4211±12.20
Cu-40	38.818±7.213	80.749±8.618*	3.2234±2.4	117.2347±21.41	126.245±3.06
Pb-20	49.2411±4.79	161.763±4.58*	3.557±0.5	118.2132±6.10	110.747±4.0
Pb-40	32.128±6.70	154.721±6.461*	3.328±0.2	121.5211±3.51	128.567±8.57
30 days	SOD (U/mgprotein)	CAT (U/mg protein)	GSH (U/mg protein)	ALT (U/L)	AST (U/L)
Control	24.24±9.741	44.982±39.23	3.4206±0.263	139.605±12.06	102.105±5.513
Cu-20	21.1212±5.28	101.771±7.264*	2.4708±0.123	144.0789±1.674	113.4211±16.27
Cu-40	18.1818±8.07	60.749±15.648	3.5874±2.621	149.4737±11.477	114.605±2.046
Pb-20	24.2424±8.59	156.884±3.48****	3.6559±0.946	150.2632±6.620	128.947±5.0
Pb-40	18.1818±8.50	152.924±7.516****	3.1339±0.364	158.552±2.591	137.76±7.257

Data are presented as mean±SEM (n=5),P-value<0.05 were considered significant.

Table 4: Heamatological parameters in the African catfish exposed to heavy metals For 15 days.

	WBC (Cell/µL)	LYM %	MID %	GRA %	RBC (×10^6/µL)	HGB (g/dl)	HCT %	PLT (×106/L)
Control	181.0000±28.2	52.9500±0.6	26.7932±36.9	31.89225±7.1	19.5517±17.4	10.5500±0.7	31.0000±1.4	23.2050±27.0

CU-20	171.5000±2.1	99.20000±0.8	0.7500±0.9	0.0000±0.0	2.7150±1.8	10.3000±0.4	30.5000±0.7	70.7500±26.7**
Cu-40	143.0000±63.6	97.1000±3.3	2.9000±3.3	2.2500±2.4	2.6500±3.3	10.5000±0.7	32.6500±3.7*	87.2500±5.7**
Pb-20	160.0000±14.1	45.8000±1.0	31.4000±0.8	21.6500±2.32.8	0.8000±0.1	14.0000±0.5	40.0000±2.8*	84.7000±0.5**
Pb-40	131.0000±74.9	12.7000±0.8	36.7500±2.0	79.4000±	2.5000±0.1	12.7000±0.8	36.7500±2.0*	79.4000±2.8**

Data are presented as mean±SEM (n-5), P-value<0.05 were considered significant.

Table 5: Heamatological parameters in the African catfish exposed to heavy metals for 30 days.

	WBC (Cell/µL)	LYM %	MID %	GRA %	RBC (×106/µL)	HGB (g/dl)	HCT %	PLT (×106/L)	MCH %
Control	119.30±11.7	53.15 ± 6.1	19.40±3.6	27.45±9.8	2.475±0.9	11.9± 2.4	32.55 ± 17.6	51.0± 0.1	50.0± 9.4
Cu-20	124.10±0.1	56.20 ± 0.1	20.30±0.1	23.5± 0.1	2.77± 0.1	10.6± 0.1	37.4± 0.1*	50.5± 0.7	38.1± 0.1
Cu-40	110.50± 0.1*	51.5± 24.6	19.45± 0.6	29.00± 24	3.84± 1.4	16.55 ± 6..0	40.4± 2.6*	30.± 9.1	43.25 ± 0.3
Pb-20	123.15±1.3	38.2± 25.3	20.55± 0.3	20.25± 4.5	3.01± 0.3	12.5± 2.6	41.9± 6.4*	50.± 16.9	39.4± 1.9
Pb-40	79.55± 32.5*	41.± 29.2	18.70± 2.9	12.75± 6.0	2.87± 0.5	12.4± 2.7	40.± 8.4*	33.± 6.3	43.3± 1.4

Data are presented as mean±SEM (n=5),P-value<0.05 were considered significant.

V. CONCLUSION

The result in the growth index obtained from the fish exposed to copper and lead had shown that the metabolic processes of the fish were adversely impaired.One critical point in understanding at the molecular level of the toxicity of thiol-binding mineral elements such as Cu and Pb is that in *in vivo* assays, toxicity occurs at micromolar concentration. The results obtained showed that metal concentrations that are high enough to be toxic may still be too low to deplete cytosolic glutathione pools to a significant extent on a stoichiometric basis. Moreover, experiments showed that in isolated organisms, such as in aquarium fish exposure to elements such as copper and lead causes a decrease in the activity of several involved enzymes.On the contrary, acute and chronic exposure of fish to thiol-binding elements, such as copper, caused a decrease in the circulating concentration of glutathione and in a shift of the balance of disulphide, “oxidized”, to thiol, “reduced”, glutathione towards more oxidized status (Hall, 2014). Indirect evidence of the efficacy of exposure to thiol-binding metals at environmental and occupational levels to induce oxidative stress and to decrease the glutathione pool emerges also from the study of biomarkers such as antioxidant enzymes and soluble oxidized metabolites (Garcon *et al*.,2004). The effect exerted by “one atom” of the toxic metal must thus be able to yield a multiplicative effect, such as that corresponding to the inhibition or inactivation of key enzymes that contain thiol groups as part of functional or regulatory mechanism.

Severe histopathological lesions and cellular alterations were observed in major target organs (liver and kidney) of fish exposed to heavy metals at different concentrations which could be attributed to the significant accumulation of the heavy metal in these organs beyond the prescribed limits of WHO/FAO. Hence, there is a need of stringent check on the industrial and domestic effluents.

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