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Response of *Clarias gariepinus* juveniles to varying concentrations of copper in water containing *Pteridium aquilinium* (Bracken Fern) and Poultry manure

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Abstract: *Pteridium aquilinum* (four fully formed fronds and 3-4 young shoots) were acclimatized in tanks containing 20 litres of water and 10 mg/l poultry manure for one week before the addition of different concentrations of copper (0, 1.8, 3.2, 5.6 and 10.0 mg/l) as hydrated copper chloride for twenty four hours. At the end of this period, juvenile *Clarias gariepinus* (mean weight 40g, length 22cm) which had been acclimatized for two weeks were introduced into the tanks containing *P. aquilinum*, poultry manure and different concentrations of copper as copper chloride for 96 hours. Each concentration of copper and 10mg/l poultry manure served as a treatment. This study aimed at evaluating the response of *C. gariepinus* juveniles when grown in water containing *P. aquilinum,* poultry manure and varying concentrations of copper. Alkalinity, phosphate and nitrate increased in water while copper content decreased at the end of the experiment. Concentrations of copper in water and fish flesh were significantly lower ($p< 0.05$) than in *P. aquilinum* with the highest concentration of 2776 mg/kg copper in the ferns exposed to 10mg/l copper and poultry manure. No significant differences were observed in the haematogical indices of fish. Histopathology showed changes in the gills, liver and kidneys. The muscle tissues showed no visible lesion even at 10mg/l copper and poultry manure. It was concluded that the water, *C.gariepinus* juveniles contained less copper than *P. aquilinum* in the presence of poultry litter.

Keywords: Uptake, copper, *Clarias gariepinus*, Haematology, histopathology

Introduction

Fish in polluted waters accumulate heavy metals in their tissues with higher concentrations in water producing greater levels in tissues (Jezierska and Witeska, 2006). Natural hyper accumulators are plants that can tolerate and incorporate high levels of toxic metals in their tissues with no signs of toxicity (Bennett *et al.,* 2003; Mokhtar *et al.,* 2011). For phytoremediation to succeed, the plants must extract large concentrations of heavy metals from the soil or water into their roots, translocate the heavy metal into the surface biomass, and produce a large quantity of plant biomass. The accumulation of some heavy metals and trace elements by some plants has been demonstrated (Ma *et al.*, 2001; Choo *et al.,* 2006, Olaifa and Omekam, 2014). Copper is essential as a trace element for several metabolic activities of fish and plays important roles in many enzymatic activities. It also serves as an herbicide, fungicide and an algaecide (Wani *et al.*, 2011).

Clarias gariepinus is a commonly cultured fish species which is hardy and can withstand many adverse environmental conditions. Manure is regarded as a complete fertilizer with organic and inorganic components which can be used without other chemicals (FAO, 2003). The compositions of different poultry manures including heavy metal contents from different locations have been reported (Nnaji *et al.*, 2011).

Pteridium aquilinum (bracken fern) is a vascular wetland plant found in Nigeria which was employed in this study to extract copper present in water at varying concentrations and to test the efficacy of the remediation using *C.gariepinus* juveniles in a 96-hour bioassay. Poultry manure was used to stimulate the growth of *P. aquilinum* in water.

Materials and Methods

Pteridium aquilinum plants (whole rhizomes) were obtained from within the University of Ibadan Campus and acclimatized for one week. Four fully formed fronds and 3-4 young shoots were later taken out and grown in experimental tanks containing 20 L water and varying concentrations of copper (1.8mg/l, 3.2mg/l, 5.6mg/l and 10.0mg/l/) as hydrated copper chloride (Reish and Oshida, 1987) and 10 mg/L poultry manure (Ndimele, 2009) for one more week. The compositions of poultry manure used were given as calcium (10.39), magnesium (1.46), potassium

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0.45), sodium (0.45), manganese (0.32), iron (0.58), copper (0.04) and zinc (0.22) mg/kg respectively (Olaifa and Omekam, 2014). Each concentration of copper and 10mg/l poultry manure served as a treatment. The control experiment contained no copper chloride or poultry manure.

The concentration of copper was calculated (Tab. 1) from hydrated copper chloride according to Odiete (1999) as 1g of copper equals molecular weight of copper chloride divided by the atomic weight of copper chloride. The required concentration of copper in water was calculated as the weight of copper measured multiplied by the molecular weight of hydrated copper divided by the atomic weight of copper. This gave the required weight of copper in copper chloride per litre of water. The weights of copper in the different concentrations were multiplied by 20 L (the volume of water per tank). The different concentrations of copper were introduced into the experimental tanks at the end of the week and left to stand for 24 hours before introduction of *C. gariepinus*.

Tab. 1: Concentrations of copper from copper chloride Used for the Experiment.

Requirement of Copper (mg/l)	Concentration of copper chloride (mg/l)	Copper chloride in 20L of water
1.8	4.833	96.66
3.2	8.591	171.82
5.6	15.034	300.68
10.0	26.847	536.94

Juvenile of *Clarias gariepinus* (mean weight 40g and length 22cm) were kept in bowls containing 20L of water and acclimatized for two weeks before introduction to experimental tanks. During acclimatization, water was replaced every other day and the fish fed twice daily with multipurpose compounded feed at 3% of their body weight. Feeding of fish was stopped 24 hours before the introduction in to the tanks.

The initial and final physicochemical parameters of the water used for the experiment were measured and recorded. A mercury-in-glass thermometer (Paragon Scientific Ltd, Birkenhead, Wirral, UK) was used to measure water temperature with the bulb of the thermometer fully immersed in each tank for two minutes before the reading. Nitrate, alkalinity and phosphate were determined (Murphy and Riley, 1962). A pH meter (Jenway 3015 pH, 0.0 accuracy; Genway, Staffordshire, UK) was used to obtain the pH of each water sample. Dissolved oxygen was determined by titration as: D.O in mg/l = (ml of titrant) (N) (8) (1000)/Sample volume in ml. Where N =1 and represents the normality of the solution used to titrate the sample (Montgomery, 1990).

Blood was drawn from the posterior caudal vein (Schmitt *et al.,* 1999) and analyzed for packed cell volume, red blood cells (Blaxhall and Daisley, 1973; Jain, 1986), white blood cells, mean cell

volume (PCVx1000/RBC), Mean Cell haemoglobin concentration (Hb content/PCV), mean cell haemoglobin (Hb/RBC pictograms), Haemoglobin (Optical density of the test x concentration of standard x dilution /optical density standard = X gm % haemoglobin) (Dacie and Lewis, 1975).

All water samples were filtered before analysis while whole fish and pooled samples of *P. aquilinum* were digested using perchloric and hydrochloric acid digestion (Pratt, 1965; Isaac and Korbor, 1971). Milled sample (0.5g) was weighed out in a 25ml volumetric flask. 5mls of the mixture of perchloric and hydrochloric acid solution were added. The flask and its content were heated on a hot plate for 45minutes-1hour at 150°C and 200°C until a clear solution was obtained. The flask was cooled and made up to the 25 ml mark with deionised water. Copper contents in fish and fern samples were determined using a Buck Scientific VGP 210 atomic absorption spectrophotometer.

The organs of fish-gills, kidneys, intestines, muscles and livers were obtained after dissection of fish samples. Small portions of each organ was fixed and passed through a series of dehydration using graded concentrations of xylene, embedded in wax and sectioned unto glass slides before staining with haemotoxylin and eosin (H&E) dyes. The sections were examined under the light microscope (Ministry of Agriculture, Fisheries and Food, 1984). The data obtained were analysed using ANOVA and Duncan's multiple range test (Duncan, 1955).

Results

The response of *Clarias gariepinus* juveniles in water containing varying concentrations of copper and 10 mg/L poultry manure and are presented in Tables 2 -

7, figure 1 and plates 1-5.

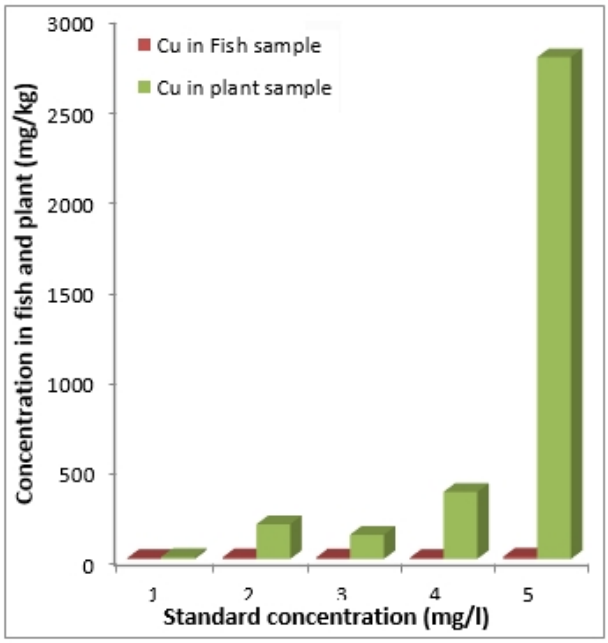

Fig. 1: Chart showing the concentrations of copper in *C. gariepinus* and *P. aquilinum* and plant samples in varying concentrations of copper (Key: 1= 0.0mg/l, 2= 1.8 mg/l, 3= 3.2 mg/l, 4= 5.6 mg/l, and 5= 10.0 mg/l)

Tab. 2: Physico-chemical qualities of water containing varying concentrations of copper and 10mg/l poultry manure at the onset and end of study.

Parameters /Period		Concentration (mg/l)				
		0.0	1.8	3.2	5.6	10.0
Dissolved	Onset	5.5	6.1	4.9	5.2	3.8
Oxygen (mg/l)	After	4.9	5.0	4.9	5.2	5.1
Temperature	Onset	27	27.5	26.5	26.5	26
(^{0} C)	After	26	26	27.5	27	27.5
Alkalinity	Onset	122	240	236	224	224
(mg/l)	After	224	468	360	122	352
Phosphate	Onset	1.85	6.79	1.85	4.63	11.11
(mg/l)	After	1.76	3.56	80.27	15.12	27.94
Nitrate	Onset	0.26	0.26	2.85	3.38	2.85
(mg/l)	After	0.35	0.43	2.60	3.56	4.98
Potassium	Onset	0.61	0.88	0.78	1.00	1.05
(mg/l)	After	1.97	1.37	1.03	1.31	0.74
Copper	Onset	0.0	0.04	0.08	0.18	0.12
(mg/l)	After	0.01	0.13	0.31	0.07	0.61

Tab. 3: Number and time of mortality of *C. gariepinus* in water with varying concentrations of copper and 10 mg/L poultry litter in 96 hours

Concentration (mg/l)	Number of test fish	Mortality (Replicate1)	Time of death (hours-R1)	Mortality (Replicate 2)	Time of death (hours-R2)
0.0	10	-	-	-	-
1.8	10	-	-	-	
3.2	10	-	-	1	32
5.6	10	-	-	1	26
10.0	10	1	26	1	28

Tab. 4: Percentage mortality of fish exposed to varying concentrations of copper and 10 mg/l poultry manure in 96 hours

Concentration of copper (mg/l)	Log. Concentration	Number of test fish	Percentage Mortality (Replicate1)	Percentage Mortality (Replicate2)
0.0	0.0	10	-	-
1.8	0.26	10	-	-
3.2	0.51	10	-	10
5.6	0.75	10	-	10
10.0	1.0	10	10	10

Table 5 shows the haematological indices recorded after exposing *Clarias gariepinus* to varying concentration of copper (1.8mg/l, 3.2mg/l, 5.6mg/l and 10mg/l) after 96 hours. There were no significant differences in all the haematological parameters in all fish samples exposed to varying copper concentrations of copper.

Plates 1-5 and Table 6 and show the histopathology of *C. gariepinus* in water containing varying concentrations of Copper and 10 mg/l poultry manure.

Discussion

Juveniles of *Clarias gariepinus* were exposed to water containing varying concentrations of copper, 10mg/l poultry manure and *Pteridium aquilinum*. Foaming was observed on the surface of the water in all treatments except the control after the first 24 hours. This could have been due to changes in dissolved oxygen concentrations with the least D.O. level at the onset of the study in the treatment with 10mg/l copper. Alkalinity, phosphate and nitrate increased in water while copper content decreased at the end of the experiment.

Fish live in close and prolonged contact with their environment; therefore, a change in water parameters also affects the physiology of fish (Wani *et al*., 2011). All fish exposed to copper had skin erosion which increased with increasing copper concentrations similar to previous reports (Tawari-Fufeyin, 2008). The highest mortality of fish (20 percent) occurred in 10 mg/l Copper and 10mg/l poultry manure. Skin haemorrhage was observed around the mouth of dead fish from the water containing 10mg/l of copper though mortality was lower than in previous report (Olaifa *et al*., 2004) and was assumed to be due to some uptake of copper by *P. aquilinum* and possible formation of complexes with organic matter from poultry manure. In the complexed and adsorbed

Tab. 5: Haematological indices of *Clarias gariepinus* juveniles in water containing varying concentrations of copper and 10mg/l poultry manure.

Blood Parameters	Concentrations of copper (mg/g)				
	0.0	1.8	3.2	5.6	10.0
PCV %	23.50	21.50	24.00	23.50	22.50
WBC (X $10^{9/L}$)	13825.0	14550.0	15100.0	13200.0	12100.0
TP (g/100ml)	3.05	2.90	3.80	2.90	3.25
Hb	7.45	7.10	7.90	7.60	7.10
PLT (X $10^{9/L}$)	125000.0	155000.0	143000.0	159500.0	144000.0
RBC (X $10^{12/L}$)	1.81	1.43	2.59	1.95	1.46
Lymphocytes (x $10^{9/L}$)	59.0	66.0	64.0	60.0	62.0
Neutrophil	37.00	30.00	32.00	36.50	32.50
Eosinophil x$10^{9/L}$	2.00	2.50	2.50	2.50	3.50
Monocyte x$10^{9/L}$	2.0	1.50	1.50	1.00	2.00
Albumin (g/dl)	1.10	1.55	1.70	1.20	1.25

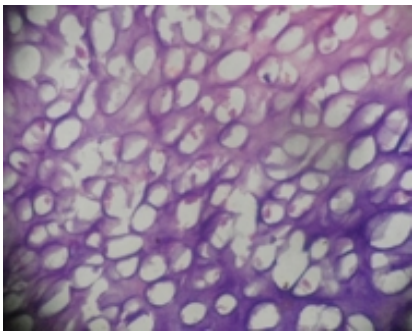

Plate 1a:0.0mg/lCu,gill

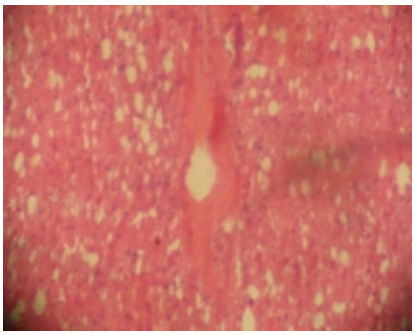

Plate 1b: 0.0mg/l Cu, liver

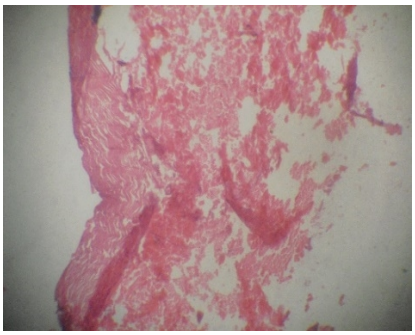

Plate 1c: 0.0 mg/l Cu, intestine

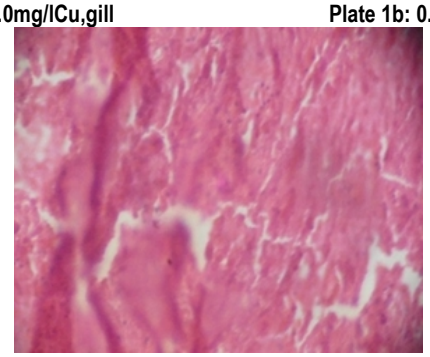

Plate 1e: 0.0mg/l Cu, muscle

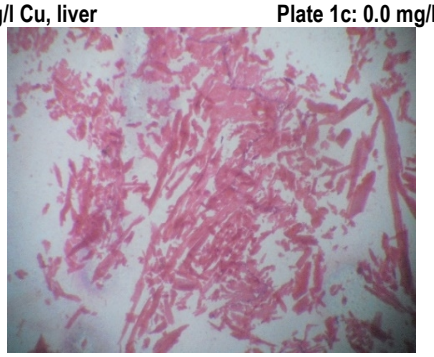

Plate 1d: 0.0 mg/l Cu, kidney

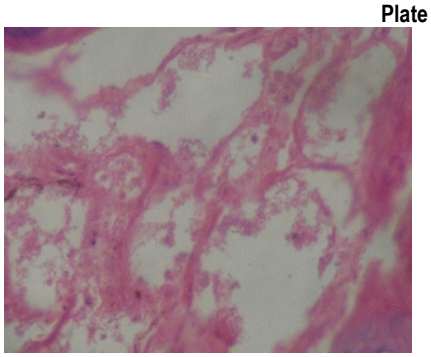

Plate 2a: 1.8 mg/l Cu, gill

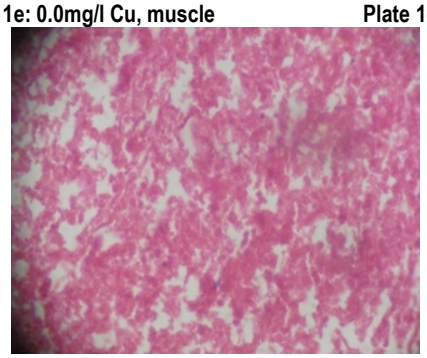

Plate 2b:1.8 mg/l Cu, liver

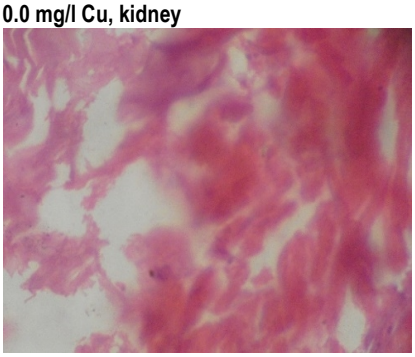

Plate 2c:1.8 mg/l Cu, muscle

Plates1-5: Photomicrographs (H& E) of gills, liver, intestine, kidney and muscles of *C. gariepinus* exposed to varying concentrations of copper in water containing 10 mg/l poultry litter.

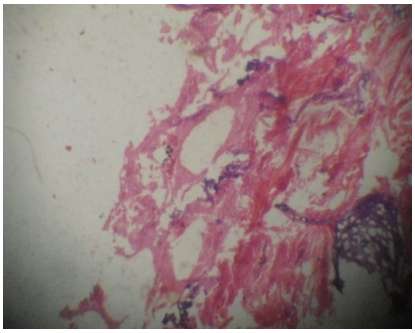

Plate 3a: 3.2 mg/L Cu, gill

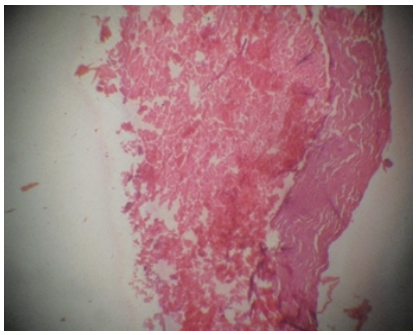

Plate 3b: 3.2 mg/L intestine

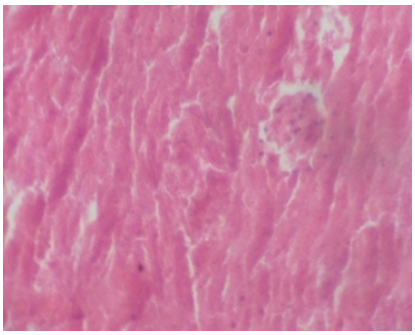

Plate 3c:3.2 mg/l Cu, kidney

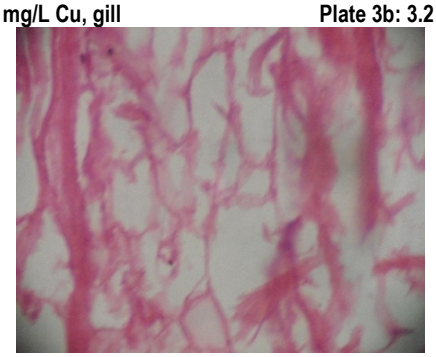

Plate 4a:5.6 mg/l Cu, gill

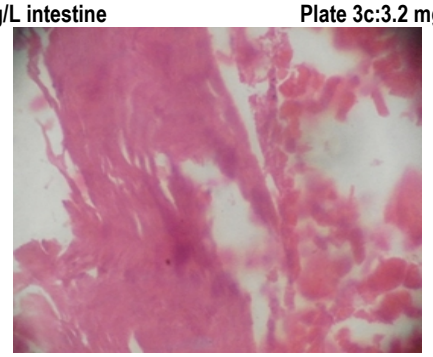

Plate 4b:5.6 mg/l Cu, intestine

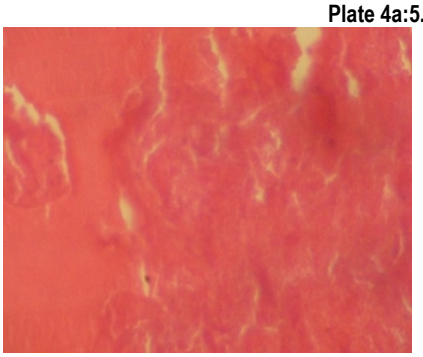

Plate 4c: 5.6 mg/l Cu, kidney

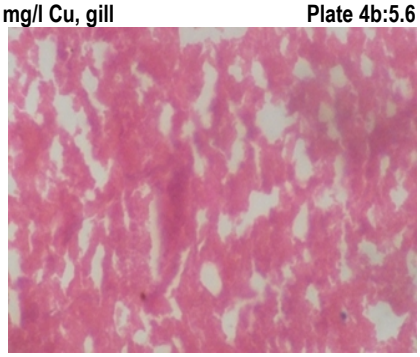

Plate 4d: 5.6 mg/l Cu, liver

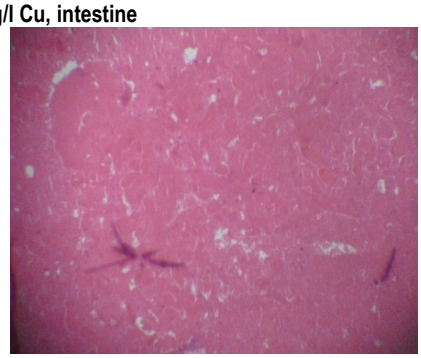

Plate 5a: 10mg/l Cu, kidney

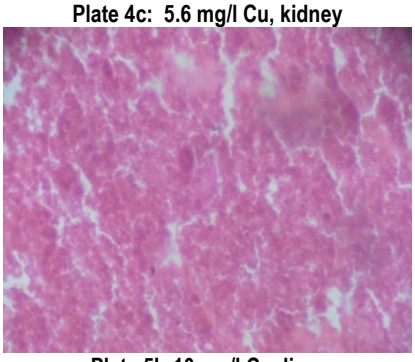

Plate 5b:10 mg/l Cu, liver

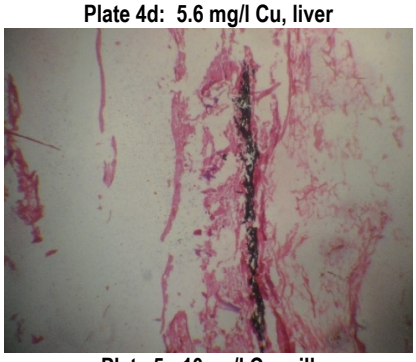

Plate 5c:10mg/l Cu, gill

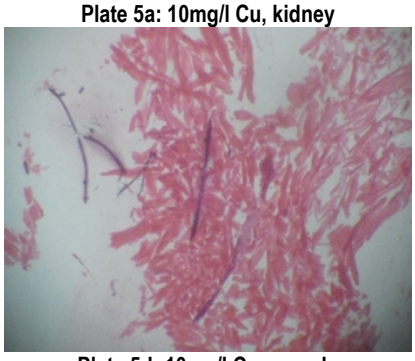

Plate 5d: 10mg/l Cu, muscle

Plates1-5: continued.

states, the toxic effect of copper is reduced (Oronsaye and Ogunbor, 1998). Generally, the concentrations of copper in water and fish flesh were significantly lower ($p<0.05$) than in *P. aquilinum* with the highest concentration in the ferns exposed to 10mg/l copper and poultry manure (2776 mg/kg copper; Tab. 2 and Fig. 1). This result was similar to earlier report (Olaifa and Omekam, 2014).

There were no significant differences in the haematological parameters (Tab. 5) of fish exposed to copper. The count of red blood cells is a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation (Adeyemo *et al.,* 2008).

The Histopathology showed changes in the morphology of gills, liver and kidneys of *C. gariepinus* used in the study at higher concentrations of copper in the water (Tab. 6 and plates 1-5) but the muscle showed no visible lesion even at 10mg/l copper and

Tab. 6: Summary of Histopathology of *C. gariepinus* in water containing varying concentrations of copper, 10mg/l poultry litter and *P. aquilinim*.

Concentration	Tissue	Effects
0.0mg/l	liver	Diffuse congestion (central venous and portal), There was diffuse hepatic vacuolation
0.0mg/l	Gill	No visible lesion seen
0.0 mg/l	Muscle	(Scattered) No visible lesion seen
1.8mg/l	Gill	Severe mucosal erosion and marked disrupted tissues
1.8mg/l	Liver	Fatty infiltration, diffuse, marked
1.8mg/l	Kidney	No visible lesion seen
3.2mg/l	Intestine	The villi were severely stunted, There was mucosal necrosis
3.2mg/l	Gill	Mild mucosal erosion (diffuse).
3.2mg/l	Kidney	No visible lesion seen
5.6mg/l	Gill	Severe mucosal erosion with disintegration of the secondary lamellae
5.6mg/l	Intestine	Severe erosion
5.6mg/	Kidney	Marked interstitial oedema
5.6 mg/l	Liver	Severe Fatty infiltration
10 mg/l	Gill	Severe necrosis of the lamellae.
10 mg/l	Liver	Diffuse vacuolation.
10 mg/l	Kidney	No visible lesion seen
10 mg/l	Muscle	Scattered. No visible lesion seen

poultry manure. Accumulation of metals such as iron, copper, manganese or cobalt is organ-specific; time-related. Copper shows an affinity for liver but under conditions of contamination, metals deposit in the same organ exerting toxic effects. The accumulation of metals in organs of fish depends on both uptake and depuration rates (Jezierska and Witeska, 2006; Wani *et al.*, 2011).

The gills were affected in all the treatments with necrosis at 10mg/l. The gills are in close contact with water and can accumulate heavy metals like copper easily while the liver is a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment (Mohamed, 2009; Camargo and Martinez, 2007). Metals in the kidney increase at a slower rate but this organ is also a good indicator of pollution (Jezierska and Witeska, 2006; Wani *et al.*, 2011). Kidneys of *C. gariepinus* were not greatly affected except at 5.6mg/l copper which showed marked changes. The accumulation of metals in various organs of fish causes structural lesions and functional disturbances (Jezierska and Witeska, 2001).

Conclusion

During this study, the *P. aquilinum* extracted copper from water depending on the initial concentrations in the water. However, there was a short period of 24 hours between the introduction of copper and the fish into the experimental tanks. This could have been responsible for the changes observed in the organs of fish. Further studies will be required to know the effect of a longer time interval on the response of *C. gariepinus.*

References

- ✓ Adeyemo O.K., Ajani F., Adedeji O.B. and Ajiboye O.O. (2008) Acute Toxicity and Blood Profile of adult *Clarias gariepinus* Exposed to Lead Nitrate. The Internet Journal of Hematology; 4: 1-8.
- ✓ Bennett L.E., Burkhead J.L., Hale K.L., Terry N., Pilon M and Pilon-smits E.A.H. (2003) Analysis of transgenic Indian mustard plants for phytoremediation of metals-contaminated mine tailings. Journal of Environmental Quality, 32: 432-440.
- ✓ laxhall P.C. and Daisley K.W. (1973) Routine haematol-ogical methods for use with fish blood. Journal of fish biology, 5: 771-781.
- ✓ Camargo M.M. and Martinez C.B. (2007) Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyology, 5: 327-336.
- ✓ hoo T.P., Lee C.K., Low K.S. and Hishamuddin O. (2006) Accumulation of chromium (VI) from aqueous solutions using water lilies (*Nymphaea spontanea*). Chemosphere, 62: 961-967.
- ✓ Dacie J.V and Lewis S.M. (1975) Practical haematology, 5th edition. Churchill Livingstone, 285pp.
- ✓ Duncan R. M. (1955) Multiple range and multiple f-tests. Biometrics, 11: 1- 42.
- ✓ FAO (2003) Recycling of animal wastes as a source of nutrients for freshwater fish culture within an integrated livestock system. www.fao.org/docrep/field/003/AC5 26E0 1.htm
- ✓ Isaac A.R. and Korbor J.D. (1971) Atomic absorption and flame photometry. Technique and uses in soil, plant and water analysis. In: Walsh (ed) Instrumental methods for analysis of soils and plant tissues. Soil Science Society, American Madison, Wisconsin. USA: 17-38.
- ✓ Jain N.C. (1986) Schalm's Veterinary Haematology. 4th edition, Lea and Febiger.
- ✓ Jezierska B. and Witeska M. (2001) Metal Toxicity to Fish, University of Podlasie, Siedlce, Poland.

- ✓ Jezierska B and Witeska M. (2006) The Metal uptake and accumulation in fish living in polluted waters. In: Twardowska, Allen, Haggblom and Stefaniak (eds) Soil and Water Pollution Monitoring, Protection and Remediation, A Dordrecht, The Netherlands: 107-114.
- ✓ Ma L.Q., Komar K.M., Tu C., Zhang W., Cai Y. and Kennelley E.D. (2001) A fern that hyperaccumulates arsenic. Nature, 409: 579-579.
- ✓ Ministry of Agriculture, Fisheries and Foods (1984) Manual of veterinary investigation: Laboratory techniques, Volume 2. Reference Book 390. Third Edition. Ministry of Agriculture, Fisheries and Food. Her Majesty's Stationery Office, London, United Kingdom.
- ✓ Mohamed F.A. (2009) Histopathological studies on *Tilapia zilli* and *Solea vulgaris* from Lake Qarun, Egypt. World Journal of Fisheries and Marine Sciences, 1: 29-39.
- ✓ Mokhtar H., Morad N. and Fizri, F.F.A. (2011) Hyperaccumulation of Copper by Two Species of Aquatic Plants. International Conference on Environment Science and Engineering IPCBEE vol.8, IACSIT Press, Singapore: 115-118.
- ✓ Montgomery H.A.C, Thom N.S and Cockburn A. (1990) Determination of dissolved oxygen by winkler method and solubility of oxygen in pure and sea water. Journal of Applied Chemistry, 14: 280-296.
- ✓ Murphy J. and Riley J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 2: 31-36.
- ✓ Ndimele P.E. (2009) Evaluation of phyto-remediative properties of water hyacinth *Eichhornia crassipes* (Mart.) Solms) and Biostimulants in Restoration of oil –polluted wetlands in the Niger Delta. A PhD thesis submitted to the Faculty of Agriculture and Forestry, University of Ibadan.
- ✓ Nnaji J.C., Uzairu A., Gimba C. and Kagbu J.A. (2011) Heavy metal risks in integrated chicken–fish farming. Journal of Applied Sciences, 11: 2092-2099.
- ✓ Odiete W.O. (1999) Environmental Physiology of Animals and Pollution. 1st Edition. Diversified Resources Ltd., Lagos.
- ✓ Olaifa F.E., Olaifa A.K. and Onwude T.E. (2004) Lethal and sub lethal effects of copper to the African catfish (*Clarias*
- ✓ *gariepinus*) juveniles. African Journal of Biomedical Research, 7: 65-70.
- ✓ Olaifa F.E., Omekam A.J. (2014) Studies on Phytoremediation of copper using *Pteridium aquilinum* (bracken fern) in the presence of biostimulants and bioassay using *Clarias gariepinus* juveniles. International Journal of Phytoremediation, 16: 219-234.
- ✓ Oronsaye J.A.O. and Ogunbor E.O (1998) Toxicity of copper to *Oreochromis niloticus* fingerling in Ikpoba River. Indian Journal of Animal Science, 68: 1001-1003.
- ✓ Pratt P.F. (1965) Digestion with perchloric and hydrochloric acid for total element. In methods of soil and plant analysis, Agronomy, pp 1010-1021. American Society of Agronomy. Inc. Madison, Wisconsin. USA.
- ✓ Reish D.L. and Oshida O.S. (1987) Manual of Methods in aquatic environment research. Part 10: Short-term static bioassays. FAO Fisheries Technical Paper, No 247. Rome.
- ✓ Schmitt C.J., Blazer V.S., Dethloff G.M., Tillitt D.E., Gross T.S., Bryant Jr. W.L., De Weese L.R., Smith S.B., Goede R.W., Bartish T.M. and Kubiak T.J. (1999). Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants. Information and Technology Report USGS/BRD-1999-0007. U.S. Geological Survey, Biological Resources Division, Columbia.
- ✓ Tawari-Fufeyin P., Igetei J. and Okoidigun M.E. (2008) Changes in the catfish exposed to acute cadmium and lead poisoning. Bioscience Research Communication, 20: 271-276.
- ✓ Wani A.A., Sikdar-Bar M., Borana K., Khan H.A., Andrabi S.S.M and Pervaiz P.A. (2011) Histopathological Alterations Induced in Gill Epithelium of African Catfish, *Clarias gariepinus*, Exposed to Copper sulphate. Asian Journal of Experimental Biological Sciences, 2: 278-22.