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HAEMATOLOGICAL CHANGES iN AFRICAN CATFISH (*Clarias gariepinus*) JUVENILES EXPOSED to PHOSTOXIN TOXICITY

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Abstract
The present study was designed to determine the toxic effects of phostoxin on juveniles of African catfish (Clarias gariepinus). The acute toxicity bioassay was conducted to determine the 96h LC_{50} values following the probit analysis method. One hundred and twenty (120) juveniles of Clarias gariepinus(mean weight 25 ± 0.25 g) were exposed to varying concentrations of Phostoxin (0.01, 0.02, 0.03, 0.04 and 0.05 mg/l) including a control (0.0 mg/l) in duplicates in a static bioassay procedure for 96hours. The pH, dissolved oxygen, temperature and conductivity of the test water were monitored and recorded. The results obtained indicated significant (P<0.05) reductions with increased concentrations of phostoxin in haemoglobin (Hb), Red blood Cell (RBC), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). The white blood cell (WBC) and mean corpuscular haemoglobin concentrations (MCHC) in fish exposed to the pesticides were significantly (P<0.05) higher than that of the control. The data obtained from this work will contribute to the baseline haematological effects caused by phostoxin for monitoring the health status of Clarias gariepinus juveniles in the culture medium.

Keywords: Toxicity, Phostoxin, Haemoglobin, Bioassay, Pesticide and Probit

Introduction
Appreciation of fisheries and aquatic systems has been accompanied by increasing concern about the effects of growing human populations and human activities on aquatic life and water quality. Pesticides are widely used all over the world to control the harmful effects of pests on agricultural production. However, despite the good results of using pesticides in agriculture, their use in the environment is usually accompanied by deleterious environmental and public health effects (Nwani *et al*., 2010). Although agricultural use of chemicals is restricted to a limited number of compounds, agriculture is one of the few activities where chemicals are intentionally released into the environment to eliminate agricultural pests (Ojutiku *et al.,* 2014). Conversely, there is overwhelming evidence that agricultural use of pesticides has a major impact on water quality and leads to serious environmental consequences (Ojutiku *et al.,* 2014). Essentially, run-off of pesticides from agricultural farmlands into fish ponds could be toxic and could destroy cultured fish tissues and cause histopathological and haematological degradations on the organs and blood of the fish (Ubong *et al.*, 2015; Ogundiran *et al.*, 2010). Pesticides cause disease conditions, behavioral abnormalities, physiological malformation, histological, hematological and biochemical changes, cancer and gene mutations in fishes especially in their early stages (Napit, 2013).
The African catfish *Clarias gariepinus* is an ecologically and commercially important fish for the Nigerian aquaculture industry (Ita, 1980). These mud fish are frequently and widely cultured in ponds and they also occur freely in Nigerian's natural freshwater. Musa and Omoregie, (1999) reported that fish are intimately associated with the aqueous environment, such that physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish.

Phostoxin tablets and pellets contain aluminum phosphide (ALP) as the active ingredient and will liberate phosphine. Mild inhalation exposure causes malaise (indefinite feeling of sickness), fatigue, nausea, and respiratory distress in man. Moderate poisoning causes weakness, vomiting, and pain just above the stomach, chest pain, diarrhoea and dyspnoea (difficulty in breathing) (Agency for Toxic Substances and Disease Registry, 2000). Phostoxin which is either in form of tablets or pellets contains 55% aluminum phosphide as its active ingredient and 45 % inert agents which is used as the carrier of the active ingredient. Phostoxin has also been documented to liberate Phosphine gas when exposed to water (Gordon, 1972) and since phosphine gas is highly toxic to aerobic organisms, it could therefore be of considerable health risk to aquatic life. The phosphine gas is colourless and odourless in its pure form, but due to the presence of substituted phosphines and diphosphines, it has a foul odour resembling that of decaying fish (Chugh, 1992). Aluminum phosphide will rapidly react to form phosphine gas which is somewhat

soluble in water. The noxious gas which is not known to be absorbed dermally have their main routes of exposure to the body systems through ingestion and inhalation and have been shown to be highly toxic via both routes (Degesch, 1988). The contact of the gas with the gills readily affects the secondary lamellar, a tender projection from the primary lamellar having delicate epithelial lining for easy gaseous exchange. Ayoola and Ajani (2007) reported that, the effect of the gas on the delicate cellular membrane results in mucus accumulation that cause hypoxia which could lead to death in fish.

Haematological variables of fish under stress are of great significance in assessing the impacts of pollutants in the biota of a particular ecosystem. Therefore, haematology has been widely used as potent bio-indicator in aquatic toxicology (Sancho *et al.,* 2000). The study of the fish blood parameters are important for determining factors related to its physiological capacity (Affonso, 2007). Blood analysis is a valuable means of evaluating the physiological condition of cultured fish with respect to determining the effect of diets and other stressors on fish health changes in producing useful information to curb any unfavourable condition that may affect the fish health (Bello-Olusoji *et al.,* 2006). Adeparusi and Ajayi (2004) reported that analysis of blood is an important factor that could be considered in fish assessment. Therefore, this study was conducted to investigate:

a. effect of phostoxin on the physiochemical parameters of water in the test medium
b. LC_{50} values of phostoxin on *C. gariepinus* juveniles; and
c. effects of phostoxin on some haematological properties of *C. gariepinus* juveniles.

Materials and Methods

Collection of Experimental Fish

Two hundred and fifty (250) apparently healthy *C. gariepinus* juveniles of 11weeks old (mean weight: 25 ± 0.25g) were purchased from the Teaching and Research Farm of Fisheries and Aquaculture Department, The Federal University of Technology, Akure and transported to the Limnology laboratory of the Department of Fisheries and Aquaculture Technology for the experiment. The *C. gariepinus* juveniles were weighed with an electronic scale (Mettler Tolebo PB8001) and were distributed uniformly into the experimental tanks (10 L) containing 10 juveniles per tank. The fish were fed to satiation during the acclimatization period (5 days) with commercial feed (2mm of Rannan fish feed) and feeding stopped 24 hours prior to the commencement of the study to minimize the production of waste materials and reduce ammonia build up in the container. The water in the tank was renewed every 24 hours during the feeding period as recommended by Oyelese and Faturoti (1995).

Determination of Water Quality Parameters

The physico-chemical parameters (pH, dissolved oxygen (DO), temperature and conductivity) were measured in the experimental tanks using standard methods APHA (2005).

Preparation of Toxicant

1g of phostoxin was dissolved in 1000 ml of distilled water to form stock solution of 1000 mg/l. The pre-determined amounts of phostoxin solution were measured out using 5ml pipette into aquaria tanks containing 10 litres of water. This was prepared 24 hours before the experiment so that the toxicant will be thoroughly dissolved in water.

Determination of Median Lethal Concentration (LC_{50})

The median lethal concentration (LC_{50}) was determined using probit analysis and graphical method (Finney, 1971).

Haematological Analysis

After the 96 h some samples of fish were removed from each experimental tank for blood analysis. About 2-5ml of blood sample was collected in each duplicates of the treatment by the severance of the caudal peduncle as recommended by Blaxhall (1972) into the sample bottle containing 10ml ethylene diamine tetracetic (EDTA) as anticoagulant using different 5ml disposable syringes. The following blood parameters Red blood cell, Haemoglobin estimation, White blood cell and Packed cell volume were analyzed using the method prescribed by Svobodova *et al.,* (1991), while the Mean corpuscular volume, Mean cell haemoglobin concentration and Mean corpuscular haemoglobin were derived using their respective formulas according to Stockham and Scott (2008).

Statistical Analysis

All data obtained in both tests were analyzed using probit method and the graphical method (Finney, 1971) and multiple range test using Statistical Package for Social Sciences (SPSS version 20.0).

Results and Discussion

The result of this study indicated that there was a gradual decrease in the dissolved oxygen levels of the water as the concentration of the toxicant increased. This may be due to the fact that water was contaminated by the toxicant thereby reducing the dissolved oxygen level of

the water. The relatively low dissolved oxygen recorded in the present study agrees with the findings of Essien-Ibok *et al.,* (2014) who exposed *Heterobranchus bidorsalis* to urea fertilizer. The highest dissolved oxygen level was recorded at concentration 0mg/l while the least value was observed at concentration 0.05mg/l of Phostoxin. The pH and conductivity values in this study showed that there were significant differences ($P<0.05$) across the concentrations and they fall within the range which is ideal for biological productivity for fish as reported by Ekubo and Abowei, (2011). The temperature value in this study also showed significant differences ($P<0.05$) across the concentration of toxicants and was within the optimal range recommended by APHA (2005) for good growth of fish. The 96 h LC_{50} was value was observed to be was 0.025 mg/L (Figure 1).

Table 1: Water Quality Parameter Results obtained in Control and Test Tanks Containing *C. gariepinus* Juveniles Exposed to Phostoxin

Parameters	0.0mg/l	0.01mg/l	0.02mg/l	0.03mg/l	0.04mg/l	0.05mg/l
pH	7.57±0.01^{b}	7.55±0.03^{ab}	7.49±0.04^{a}	7.58±0.01^{b}	7.51±0.02^{ab}	7.47±0.02^{a}
Temp (^{o}C)	25.45±0.05^{b}	25.25±0.05^{a}	25.45±0.05^{b}	25.65±0.05^{c}	25.65±0.05^{c}	25.65±0.05^{c}
Conductivity	203.00±8.00^{a}	219.00±4.00^{ab}	218.00±4.00^{ab}	210.50±6.50^{a}	235.00±2.00^{b}	231.00±3.00^{b}
Dissolved Oxygen	4.70±0.10^{d}	4.65±0.05^{d}	4.50±0.00^{cd}	4.35±0.05^{c}	3.90±0.10^{b}	3.60±0.10^{a}

Means with different superscript across rows are significantly different ($P<0.05$)

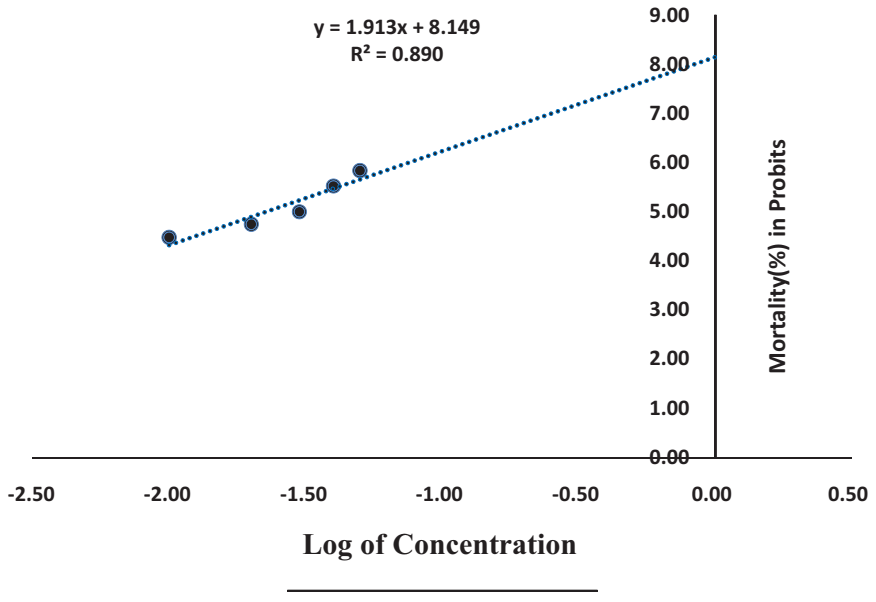

Figure 1: Median Lethal Concentration (LC50) of phostoxin on C. gariepinus juveniles.

The results of the haematological parameters of C. gariepinus exposed to varying concentrations of phostoxin are presented in Table 2. The results indicated that the Packed Cell Volume (PCV) of the fish in the control were significantly (P<0.05) different from those of the fish exposed to varying concentrations of phostoxin. PCV decreased with increase in the toxicant concentration across the treatments with the highest value (29.00±1.00) recorded in 0.0mg/l test solution while the lowest value (16.50±0.50) was recorded in 0.05mg/l of phostoxin. The values of the haemoglobin (HB) of the fish exposed to varying concentrations of phostoxin were significantly different (P<0.05) and decreased with increase in the concentrations (0.01, 0.02, 0.03, 0.04 and 0.05mg/l) of the toxicant across the treatments. The highest value (9.65±0.35) was observed in the control (0.0mg/l) while the lowest value (6.55±0.15) was recorded in the fish exposed to 0.05mg/l of phostoxin.

The red blood cell (RBC) values also decreased across the treatments with the highest value (3.28±0.13) recorded in the control (0.0mg/l) while the least value (2.20±0.10) was recorded in the fish exposed to 0.05mg/l of phostoxin.The results of the white blood cell (WBC) were significantly different (P<0.05) with the highest value (8650.00±50.00) recorded in 0.05mg/l test solution while the least value (6000.00±100.00) was observed in fish exposed to 0.0mg/l (control) of phostoxin (Table 2).Highest Mean Corpuscular Volume (MCV) value (88.57±0.33) was recorded in fish exposed to 0.0mg/l concentration while the lowest value (65.60±2.27) was recorded in 0.03mg/l concentration and it indicated significant differences across the treatments (P<0.05). The result for the Mean Corpuscular Haemoglobin Concentration (MCHC) showed significant differences (P<0.05) across the treatments with the highest value (41.05±0.53) recorded in 0.03mg/l and the least value (33.27±0.06) in the control treatment (0.0mg/l) of Phostoxin.Fish exposed to 0.05mg/l of Phostoxin had the highest Mean Corpuscular Haemoglobin (MCH) value of 29.81±0.68 and the test fish exposed to 0.02mg/l had the lowest MCH value of 26.32±0.69.

Table 2 : Haematological parameters of *Clarias gariepinus* juveniles exposed to different concentrations of phostoxin

	Parameters	**Concentrations**				
	0.0mg/l	0.01mg/l	0.02mg/l	0.03mg/l	0.04mg/l	0.05mg/l
HB (g/dl)	9.65±0.35^{d}	9.00±**0.10**^{c}	8.15±**0.05**^{b}	7.80±0.10^{b}	7.00±0.00^{a}	6.55±**0.15**^{a}
PCV **(%)**	29.00±**1.00**^{e}	26.50±**0.50**^{d}	23.00±**1.00**^{c}	19.00±0.00^{b}	17.50±0.50^{ab}	16.50±0.50^{a}
RBC **(10^6/mm^3)**	3.28±0.13^{c}	3.28±0.03^{c}	3.10±0.10^{c}	2.90±0.10^{bc}	2.55±0.15^{ab}	2.20±0.10^{a}
WBC **(10^3/mm^3)**	6000.**00** ±100.00^{a}	6600.00±**100.00**^{b}	7350.00±50.00^{c}	7600.00±100.00 ^{c}	8250.00±50.00^{d}	8650.00±50.00^{e}
MCV **(fl)**	88.57±0.33^{e}	80.91±0.91^{d}	74.17±0.84^{c}	65.60±2.27^{a}	68.70±2.08^{a}	75.05±1.14^{c}
MCHC **(%)**	33.27±0.06^{a}	33.98±1.02^{a}	35.50±1.33^{a}	41.05±0.53^{b}	40.04±1.15^{b}	39.71±0.30^{b}
MCH **(pg)**	29.47±0.06^{b}	27.49±0.52^{ab}	26.32±0.69^{a}	26.92±0.59^{ab}	27.55±1.62^{ab}	29.81±0.68^{b}

Means with different superscript across rows are significantly different (P<0.05)

The decrease or increase in certain blood parameters can be associated with the nature of fish species and the toxicants used in different studies (Ololade and Ogini, 2010).

The results obtained in the exposure of *Clarias gariepinus* to different concentrations of Phostoxin indicated that there was increase in WBC, MCH and MCHC as phostoxin concentration increased. This finding agreed with the reports of Akinrotimi *et al*., (2011) who studied acute haematological study of *Sarotherodon melanotheron* exposed to atrazine and reported that the fish response was attributed to stress, due to the ability of the white blood cells to act as defence mechanisms of the body. The increase in WBC showed an immune response to the toxicants. The result is in agreement with the reports of Akinrotimi and Gabriel (2012) whose study on submission of remarkable richness of toxicants on fish blood, showed that more of these white blood cells and their components were recruited to combat the stressor in the blood stream of the fish. Findings from this research showed that the RBC and Packed Cell Volume of *C. gariepinus blood* were higher in lower concentrations of phostoxin during the 96h exposure, which was in agreement with the studies of Oriakpono *et al.,*(2012) who exposed *Sarotherodon melanotheron* to crude oil and reported that the observed reduction in different blood parameters might have been as a result of malfunctioning of the fish haematopoietic system caused by toxicant exposure. Similar findings were reported for *C. gariepinus* exposed to malathion (Zubair, 2012).

The decrease in the PCV indicates the worsening of the condition of the organism and developing of anaemia. Haemoglobin concentration reveals the status of an organism oxygen level and the organism itself tries to maintain them as much as possible in the face of any stressor. This study shows that the mean haemoglobin reduced considerably when compared to the control values. Reduction in the values of haemoglobin in the blood of exposed fish, is usually caused by the effect of chemicals on the blood, as well as decrease in its oxygen carrying capacity, which also imply anaemia or validate the toxic effect of phostoxin on *Clarias gariepinus juveniles* (Gabriel *et al*., 2011).

Conclusion

The result obtained in this study revealed that phostoxin is highly toxic to *C. gariepinus*. Toxicity of phostoxin on *C. gariepinus juveniles* increased with increasing concentration of the pesticides. The LC_{50} value (0.025mg/l) of this study shows that phostoxin has a great toxic effect on the health status of *C. gariepinus*. However, the pesticide should be prudently used in controlling pests to avoid ecotoxicological hazards. More studies on the toxicity of phostoxin on other freshwater fish species are necessary to understand the mechanisms of actions of the pesticide.

References

Adeparusi, E. O. and Ajayi, A. D. (2004). Haematological characteristics of Nile Tilapia (*Oreochromisniloticus*) fed differently processed lima bean (*Phaseoluslunatus*L.) diets. *Journal of Research in Science and Management.***2**: 73 – 80.

Affonso, E. G., Tavares-Dias, M., Barcellos, J. F. M., Marcon, J. L., Menezes, G. C. and Ono, E. A. (2007). Hematological and biochemical parameters for the pirarucu Arapaima gigasSchinz, 1822 (Osteoglossiformes, Arapaimatidae) in net cage culture. *Electronic Journal of Ichthyology*.***2****:* 61-68.

Agency for Toxic Substances and Disease Registry (ATSDR). **(2000)**. Toxicological Profile for Aldrin/Dieldrin. TP-92/01. U.S. Department of Health and Human Services, Public Health Service. pp. 186-191.

Akinrotimi, O.A. and Gabriel, U.U. (2012).Haematological profiles of *Clariasgariepinus* brood fish raised in water recirculating aquaculture system. *Advances Agricultural Science and Engineering Research* **2:**97-103.

Akinrotimi, O.A., Aranyo, A.A. and Ibemere, I.F. (2011). Acute haematological study of *Sarotherodonmelanotheron*exposed to atrazine. *Advances Agricultural Science Engineering Res.***9:** 34-39.

American Public Health Association (APHA) (2005). Standard methods for the examination of water and waste water. *American Journal of Public Health and the Nations Health*.***56***(3): 387-388.

Ayoola, S.O. and Ajani, E.K. (2007). Histopathological Effects of Cypermethrin on Juvenile Nile Tilapia (*Oreochromisniloticus*). *African Journal of Livestock and Extension*.**6**:1-13.

Bello-Olusoji, O. A.,Omoare, V. Y. and Nwanna,C. (2006). Comparative studies on haematological characteristics of pond-cultured and wild tilapia (*Oreochromisniloticus*). *Nigerian Journal of Forestry.* **36**: 134 – 141.

Blaxhall, P. C. (1972). The haematological assessment of the health of freshwater fish: a review of selected literature. *Journal of fish biology*.***4***(4): 593-604.

Chugh S.N. (1992).Aluminium phosphide poisoning: Present status and management. *Journal of Associated Physicians India.***40**:401-405.

Degesch America, Inc. (1988). Material Safety Data Sheet: Aluminum Phosphide, Phostoxin, Degesch America, Weyers Cave, VA24486 USA.

Ekubo, A.A. and Abowei, J.F.N. (2011). Review of some water quality management principles in culture fisheries. *Research Journal of Applied Sciences, Engineering and Technology.***3**: 1342-1357.

Essien-ibok, M. A., Asuquo, I. E. and Ekpo, I. E. (2014). The assessment of acute toxicity of urea fertilizer against *Heterobranchusbidorsalis* fingerlings. *Global Journal of Fisheries and Aquaculture*, 2 (5):169-176.

Finney, D.J. (1971).ProbitAnalyis, 3rd ed. Cambridge University Press, Cambridge, .p. 20.

Gabriel, U. U., Uedeme-Naa, B. andAkinrotimi, O. A. (2011). Pollutant induced altered behaviours in fish: A review of selected literature. *Journal of Technology and Education in Nigeria*. ***16***(1): 9-26.

Gordon K. (1972). Training Manual for the Structural Pesticide Applicator. Rosemead, California, pp.123-140.

Ita, E.O. (1980). A review recent advance in warm water aquaculture resource and a proposed experimental design for maximizing fish production in Nigeria. K.L.R.I. Technical Rep Series.**51:**30-31.

Musa, S. O. andOmoregie, E. (1999). Haematological changes in the mudfish, Clariasgariepinus (Burchell) exposed to malachite green. *Journal of Aquatic Sciences*.***14***(1): 37-42.

Napit, M. K. (2013). The effect of pesticides on fish fauna of Bhopal lower lake (M.P.). *African Journal of Environmental Science and Technology.***7**(7): 725-729.

Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B.and Srivastava, S. K. (2010). Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish Channa punctatus (Bloch). *International journal of environmental research and public health*.*7*(8): 3298-3312.

Ogundiran, M. A., Fawole, O. O., Adewoye, S. O. and Ayandiran, T. A. (2010).Toxicological impact of detergent effluent on juvenile of African Catfish *(Clariasgariepinus)* (Buchell 1822). *Agriculture and Biology Journal of North America.***1**(3): 330-342.

Ojutiku, R. O.,Asuwaju, F. P., Kolo, R. J.,Obande, R. A. and Agbelege, O. O. (2014). Toxicity and Histopathological Effect of Cypermethrin on Juveniles of *Clariasgariepinus. British Journal of Applied Science and Technology.4(15): 2251-2264.*

Ololade, I. A. and Oginni, O. (2010). Toxic stress and haematological effects of nickel on African catfish, *Clariasgariepinus,* fingerlings. *Journal of Environmental Chemistry and Ecotoxicology*.**2**(2): 14-19.

Oriakpono, O., Hart, A.and Ekanem, W. (2012). Acute Haematological Response of a Cichlid fish *Sarotherodonmelanotheron* exposed to crude oil. *Journal of Toxicology and Environmental Health Science*.**4:**151-156.

Oyelese, O. A. andFaturoti, E. O. (1995). Growth and mortality estimates in Clariasgariepinus fed graded levels of processed cassava peels. *Journal of Tropical Forest Resources*.***11****:* 71-81.

Sancho, E., Cerón, J. J.andFerrando, M. D. (2000). Cholinesterase activity and hematological parameters as biomarkers of sublethalmolinate exposure in *Anguilla anguilla*. *Ecotoxicology and environmental safety*.***46***(1): 81-86.

Stockham, S. L., and Scott, M. A. (2008). Hemostasis, *Fundamentals of veterinary clinical pathology, 2nd ed. Ames (IA): Blackwell*.p 271–286.

Svobodova, Z., Pravda, D., Ravds, J. and Palackova, L. (1991). Unified method of haematological examination of fish. *Research Institute of Fish Culture and Hydrobiology, Vodnany, Czechoslovakia*.p.31.

Ubong, G., Nabuk, I. I., Ekanim, M. P. and Ubong, O. (2015). Acute toxicity of ariel detergent on the survival of fingerlings of Nile tilapia (*Oreochromisniloticus). International Journal of Current Research,* **7**(4): 14825-14828.

Zubair, A. (2012). Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of *Clariasgariepinus*. *African Journal of Biotechnology.*11(34); 8578-8585.