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Haematological and Serological Responses of Clarias Gariepinus to Sublethal Concentrations of Lead Nitrate

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ABSTRACT:

The acute toxicity tests lasting 96 hours were performed on Clarias gariepinus of mean weight 138 ± 12g and mean total length of 28.0 ± 1.5 cm. The LD50 of lead nitrate was 57.5mg/l. The experiment lasted for 70days after the control and the experimental groups were exposed to sub-lethal concentrations (20mg/l and 35mg/l) of lead nitrate. Haemoglobin concentrations were significantly ($p < 0.05$) decreased to values between 6.64 ± 0.23 g/dl to 5.42 ± 0.58 g/dl in all the sub-lethal concentrations compared to the control values of 8.58 ± 0.48 g/dl. Haematocrit values were similarly significantly ($p < 0.05$) lowered from the control value of 26.8 ± 0.68 iu/l to 20.8 ± 0.76 iu/l and 17.0 ± 1.67 iu/l for 20mg/l and 35mg/l lead nitrates respectively. The mean values of RBC count were significantly ($p < 0.05$) lowered from the control value of 4.38 × 1012 ± 0.26 to between 3.39 × 1012 ± 0.12 and 2.72 × 1012 ± 0.02 in 20mg/l and 35mg/l lead nitrate respectively. The WBC count significantly ($p < 0.05$) increased from 20720.00 ± 307.77 in the control to 20940 ± 875.72 and 23620.00 ± 402.01 in the two sub-lethal levels respectively. The serum enzymes of the control 40.00 ± 4.07 iu/l (ALP), 34.8 ± 1.18 iu/l (ALT) and 119.8 ± 13.24 iu/l (AST) were significantly ($p < 0.05$) higher than the experimental group at 20mg/l lead nitrate 38.2 ± 4.48 iu/l (ALP), 33.6 ± 4.43 iu/l(ALT) and 113.4 ± 14.77 iu/l(AST) while the values at 35mg/l lead nitrate were 31.0 ± 5.84 iu/l, 26.2 ± 2.67iu/l and 97.6 ± 14.15 iu/l respectively. There were no significant differences ($p < 0.05$) in MCHC, MCH and MCV values between the control and the experimental groups. This study has sufficiently contributed to the basic research needs of aquatic toxicology and fish pathology.

Key words: Clarias gariepinus, toxicological effects, haematological indices, lead nitrate.

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1.0 INTRODUCTION

The use of hematological techniques is gaining importance for toxicological research, environmental monitoring and assessment of fish health conditions [1]. Blood parameters are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants [2, 3].

Haematological analysis will enhance fish cultivation by facilitating early detection of situations of stress and or diseases that could affect production performance [4].

Enzymes are biochemical and physiological indicators used as biomarkers to identify possible environmental contaminations before the health of aquatic organisms is seriously affected and to develop water quality indices. Such a

biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish [4]. Lead has been found to have adverse effects on haematological parameters of fish [5, 6]. Sub-lethal concentrations of lead cause toxicity which results into oxidative damage in fish tissues through generation of free radicals in which reactive oxygen species (ROS) are most important in causing damage to cells and tissues[7, 8]. This will lead to death or low yield in terms of quality and quantity, resulting in the scarcity of the commodity and eventual high cost of the few available fish to the disadvantage of the populace [9, 10].

The aim of this study is therefore to determine the effects of sub-lethal concentrations of lead nitrate on some blood parameters and serum enzymes of the African catfish *Clarias gariepinus.*

2.0 MATERIALS AND METHODS

Acute toxicity tests were performed according to Adeyemo *et al*., [11]. Six plastic fish tanks of 52.8 litres capacity measuring 32cm x 50cm x 33cm were used for the study. Sixty *Clarias gariepinus* were selected after acclimatization and divided into 3 groups, A, B and C.

Group A consisted of 20 fish kept as control. Group B consisted of 20 fish treated with 20mg/l lead Nitrate in replicate. Group C consisted of 20 fish treated with 35mg/L Lead Nitrate. Experiment lasted for 70 days (10 weeks) after which blood samples were collected through cardiac puncture for haematology and serology. Haemoglobin (Hb), haematocrit (Ht), Red Blood cell (RBC) count, white blood cell (WBC) count, WBC differential count were carried out using the methods of Blaxhall and Daisely [12]. Red blood cell indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined according to Jain [13].

Serum enzyme analysis was done using the test kits [14] for Alanine Transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP).

Mean and standard error of the means of experimental fish were calculated for each treatment group and the control. Data were statistically analyzed using ANOVA [15].

3.0 RESULTS:

The LD_{50} of lead Nitrate was 57.5mg/l.

3.1 Haematology:

The results of the haematological responses of *Clarias gariepinus* to sublethal concentrations of lead nitrate after 70 days exposure are shown in Figures 1, 2, 3 and 4.

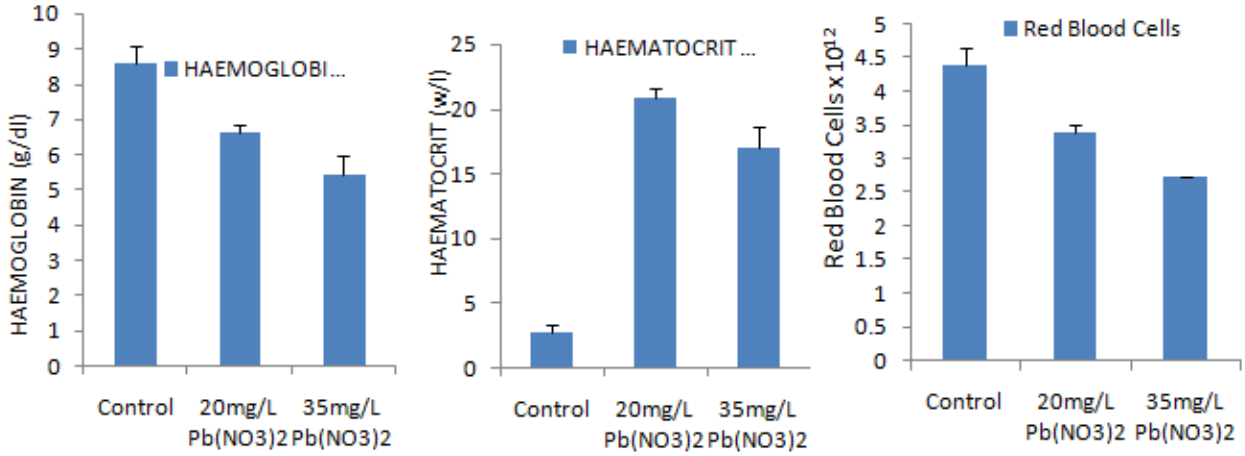

Figure 1: Haemoglobin, Haematocrit and Red blood cells (Mean ± SEM) of Clarias gariepinus after 70 days exposure to lead nitrate.

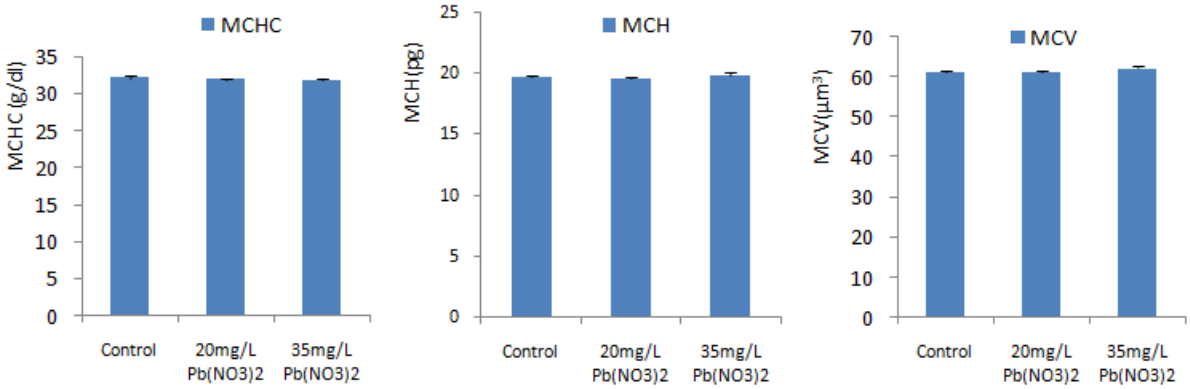

Figure 2: The Red blood indices (MCHC, MCH & MCV) of *Clarias gariepinus* after 70 days exposure to lead nitrate.

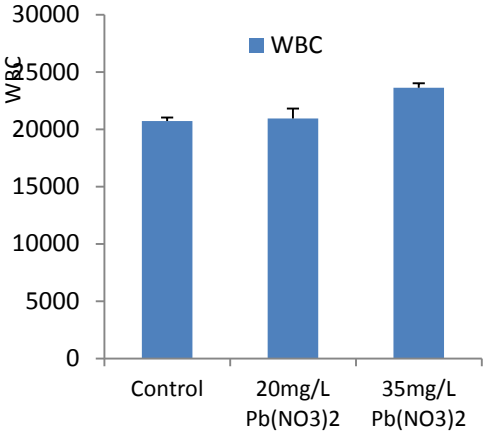

Figure 3: The White blood cells (Mean ± SEM) of *Clarias gariepinus* after 70 days exposure to lead nitrate.

Significant differences (P<0.05) between the haematological parameters of the control and treated groups occurred in Hb, Ht, RBC and WBC. No significant differences (P>0.05) in MCHC, MCH, MCV, lymphocytes and neutrophils were recorded between the control and treated groups.

3.2 Serology:

The results of the enzyme assay of Clarias gariepinus after 70 days exposure to lead nitrate are shown in Figure 5.

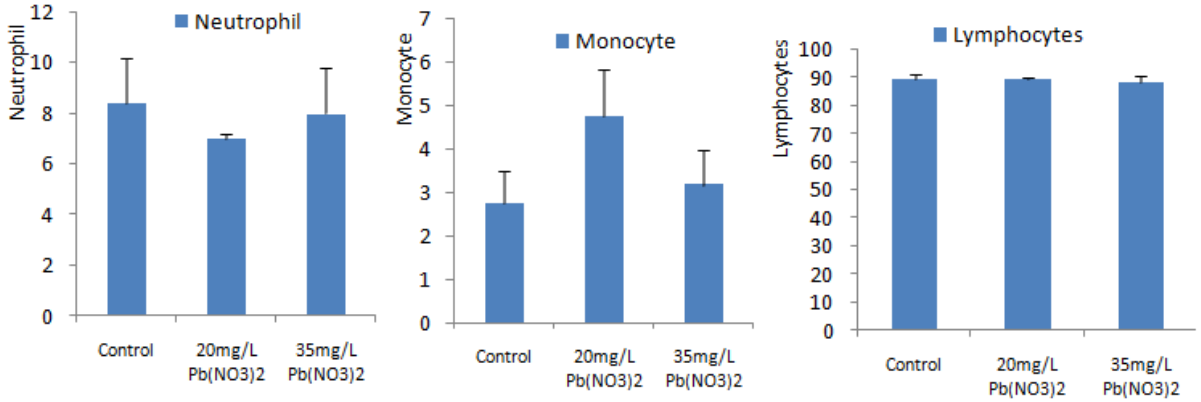

Figure 4: WBC Differential counts of *Clarias gariepinus* after 70 days exposure to lead nitrate.

Significant differences (P<0.05) occurred between the serum enzymes AST, ALT and ALP of the control and treated groups. The serum enzymes of the control were significantly higher than the treated groups.

4.0 DISCUSSION

Studies by Van Vuren[16] showed that when water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the

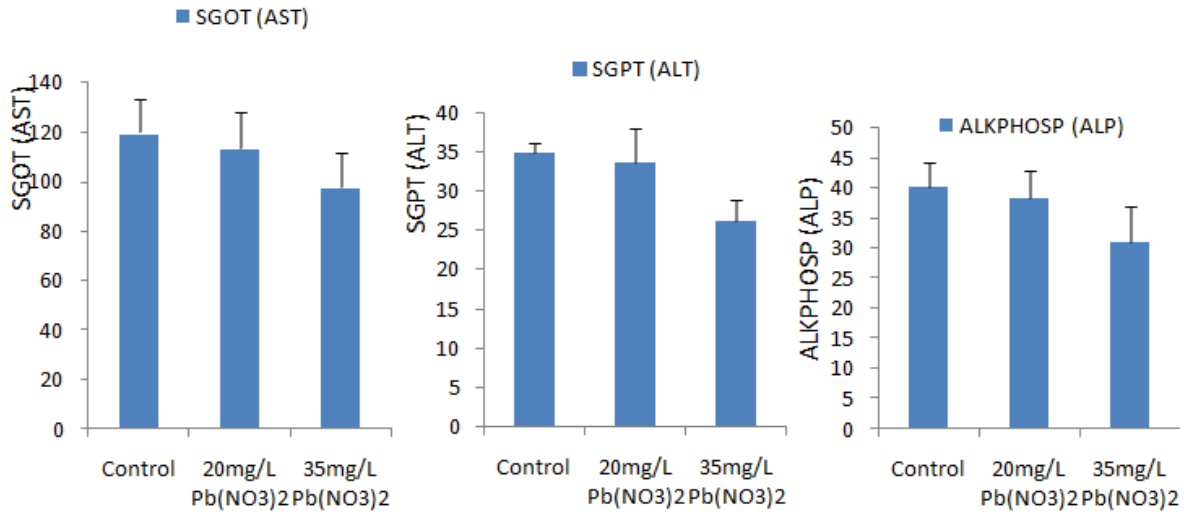

Figure 5: AST, ALT & ALP (Mean ± SEM) of *Clarias gariepinus* after 70 days exposure to lead nitrate

haematologic parameters. Thus water quality is one of the major factors responsible for individual variations in fish haematology since they are sensitive to slight fluctuations that may occur within their internal environment [17].

In this study, the main haematological response of the Catfish *Clarias gariepinus* to the exposure of lead nitrate was a significant (P<0.05) decrease of RBC count, Hb and Ht or packed cell volume (PCV) compared to the control group. Decreased RBC count, Hb and Ht in *Clarias gariepinus* after exposure to lead and cadmium was also reported by Tawari-Fufeyin *et al.,*[18] and Borane and Zambare[19]. This decrease in the values of the RBC, Hb and Ht could be attributed to haemolysis resulting in haemodilution, a mechanism for diluting the concentration of the toxicant in the circulatory system [20]. Larsen *et al.,* [21] reported consistent reduction of Hb in White fish *Coreganus* sp poisoned with lead nitrate. Christensen *et al.,* [22] reported that brook trout *Salvelinus fontinalis* exposed to lead for 6-8 weeks recorded decrease in Hb content. Tawari-Fufeyin *et al.,* [18] reported that *Clarias gariepinus* exposed to lead nitrate and cadmium sulphate showed increase in WBC production (Leucocytosis) which was also reported in *Clarias gariepinus* infected with bacteria *Pseudomonas fluorescens*[23], treated with malachite green[24], Copper and lead[5].

Enzyme assay showed significant decrease (P<0.05) in Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) levels in the experimental group when compared to the control. Adedeji[25] studied the acute effect of diazinon on blood plasma enzymes of *Clarias gariepinus.* In the study, significant decrease occurred in the serum enzymes of the exposed fish when compared to the control. This is because the major biochemical response to the effects of pollutants in fishes is the inhibition of enzymes [26].

5.0 CONCLUSION

This study revealed that exposure to lead at sub lethal levels can produce significant changes in the physiology of *Clarias gariepinus* as manifested by changes in the blood parameters and serum enzymes. Prolonged exposure through pollution by lead salts may lead to reduced productivity and or fish mortality due to disruption of the internal physiology.

REFERENCES

1. Shah, S.L., and Altinag, A. Haematologucal parameters of tench (*Tinca tinca L.)* after acute and chronic exposure to lethal and sublethal mercury treatments. Bull. Environ. Contam. Toxicol., 2004; 73:911-918.
2. Adhikari, S. and Sarkar Effects of cypermehrin and carbofuran on certain haematological parameters and prediction of their recovery in fresh water teleost, *Labeo rohita* (Ham) Ecotoxicol. Environ. Safety, 2004; 58:220-226.
3. Maheswaran, R, Devapaul A, Velmurugan B, Ignacimuthu S. Haematological studies of Freshwater fish, *Clarias batrachus* (L.) exposed to mercuric chloride. Inter. J. Integr. Biol., 2008; 2(1): 49-54.

4. Tavares-Dias M, Barcellos JFM. Peripheral blood cells of the armored catfish *Hoplosternum littorate* Hancock, 1828: a morphological and cytochemical study. Braz. J. Morphol. Sci., 2005; 22: 215-220.
5. Annune, P.A. and Ahuma, F.T.A. Haemotological changes in mudfish exposed to sublethal concentrations of copper and lead. J. Aquat. Sci., 1998; 13:33-36.
6. Adedeji, O.B. Effects of diazinon on blood parameters in the African catfish *(Clarias gariepinus).* African Journal of Biotechnology, 2009; 8(16): 3940-3946.
7. Verma K and SD Belsare. Superoxide Dismutase level in fish tissues as indicator of Lead Toxicity. In Fisheries and Fish Toxicology, APH Publishing Corporation, New Delhi. India , 2005:137
8. Ikeogu CF, CI Nsofor and IO Igwilo, Toxicity of lead nitrate and crude oil on the growth of the African catfish *Clarias gariepinus*. Inter J Agric. Biosci., 2013; 2(6): 337-339.
9. U. Adiukwu. Dietary carbonhydrate utilization in. Tilapia (*Oreochromis niloticus*). J. Agric. Sci. Tech., 1992; 2(1): 33-37
10. Igwilo IO, CI Nsofor, FF Avwemoya and CS Adindu,. The Effects of feeds formulated with *Moringa oleifera* leaves on the growth of the African catfish, *Clarias gariepinus.* Res. Rev. Biosci., 2012; 6: 121-126.
11. Adeyemo, O.K., Adedeji, O.B., Adedeji O.A and Agbede S.A. Acute toxicity of diazinon to the African Catfish, *Clarias gariepinus.* African Journal of Biotechnology, 2008; 7(5): 651-654.
12. Blaxhall, P.C, Daisley K.W Routine haematological methods for use with fish blood. *J. Fish Biol*., 1973; 5: 771-781.
13. Jain, N. C. Schalm's Veterinary Haematology (ed N.C. Jain) Lea and Febiger, Philadephia USA. 4th edition. 1986: 1221.
14. Reitman, S. and Frankel, S. A Colorimetric method for the determination of serum glutamate-oxaloacetate and glutamate-pyruvate transaminases. American Journal of Clinical Pathology, 1957; 28:56.
15. MS Excel, 2001. MS USA
16. Van Vuren. The effects of toxicants on the haematology of *Labeo urubratus* (Teleosteir cyprinidae). Comp. Bioch. Physiology, 1994; 83C:155-159.
17. Fernandes, M.N. and A.F. Mazon, 'Environmental Pollution and Fish Gill Morphology. In: Val, A.L. Kapoor B.G. (Eds.), *Fish Adaptations*. Science Publishers, Enfield, USA. 2003:203-231.
18. Tawari-Fufeyin P, Igetei J., and Okoidigun, E.M. Changes in the catfish (*Clarias gariepinus)* exposed to acute cadmium and lead poisoning. Bioscience Research Communications, 2008; 20(5) Pp. 271-276.
19. Borane, V.R. and Zambare, S.P. Protective Role of Ascorbic acid on the Lead and Cadmium induced Alteration in total R.B.C. of the fresh water fish *Channa orientalis.* In Aquaculture. APH Publishing Corporation, New Delhi, India. 2006: 163.
20. Smith, G.R.W., Bortish T.M., Kubiak T.J. Assessing the exposure of fish to environmental contaminants. Information and technology Report Usas/BRO-1999 0007. US. Geological Surgery. Biological Resources Division, Columbia M.O. 1999: 547.
21. Larsen, A., Haux, C. and Sjobeck, M.L. Fish Physiology and metal pollution: Results and experiences from Laboratory and field studies. *Ecotoxicol*. *Environ. Safety,* 1985; 9: 250-281.
22. Christensen, H., Hunt, E and Fianot, J. (1977). The effect of methylmercuric chloride, cadmium chloride and lead nitrate on six biochemical factors of the brook trout, *Salvelinus fontinalis*. Toxicol. Appl. Pharm. 42: 523-530
23. Ezeri, G.N.O., Haematological responses of *Clarias gariepinus* to bacterial infection and treatment with antibiotics and prophylactic treatment with antibiotics. J.Aquat. Sci., 2001; 16:22-24.
24. Musa, S.O. and E. Omoregie, Haematological changes in *Clarias gariepinus* exposed to malachite green. J. Aquat. Sci., 1999; 14:37-42.
25. Adedeji, O.B. Acute effect of diazinon on blood plasma Biochemistry in the African Catfish *Clarias gariepinus.* Journal of Clinical Medicine and Research, 2010; 2(1): 001-006.
26. Michael, M.I., Hilmy A.M., El-Domiaty N.A. and Wershara K. Serum transaminase activity and histopathological changes in *Clarias lazera* Chronically exposed to nitrite Comp. Biochem. Physiology, 1987; 86:255-262.

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