Available online at http://www.urpjournals.com

International Journal of Research in Environmental Science and Technology

Original Article

ISSN 2249–9695

BIOCHEMICAL PROFILE OF CLARIAS GARIEPINUS EXPOSED TO SUB-LETHAL CONCENTRATIONS OF CHEMICAL ADDITIVES EFFLUENT.

Dahunsi, S.O*; Oranusi, S.U and Ishola, R.O.

Biological Sciences Department, Covenant University, Ota, Ogun State, Nigeria
*Correspondent Author: - Dahunsi, S.O.
E-mail;- dahunsi_olatunde@yahoo.com.
Phone no: +2347032511675

Received 05 October 2011; accepted 20 October 2011

Abstract

Chemicals such as industrial effluents induces some level of alterations in the naturally occuring chemical composition of aquatic phase which in turn alters the behavioural, biochemistry, and general physiology of aquatic fauna among which is catfish, *Clarias gariepinus.* Chemical additives effluent was analysed to determine its physicochemical parameters. Part of the result conforms to the Federal Environmental Protection Agencies standard specification for effluent discharge into the aquatic environment while other parameters like TDS, TSS, and Alkalinity deviated from the standard. The fish, *Clarias gariepinus* was exposed to 0.25mg/L, 0.30mg/L, 0.35mg/L and 0.40mg/L concentrations of the effluent for 96 hours and the LC_{50} value for the acute toxicity was found to be 0.335223mg/L. The impact of long term exposure to the effluent was also evaluated through changes of selected biochemical parameters using the 20%, 10%, 5% and 2.5% of the 96-h LC_{50} value for 42 days.the parameters measured are glucose, total protein, cholesterol, albumin and globulin. All the parameters recorded a significant difference in their values as against the control except cholesterol. The alteration in all parameters was significantly concentration and time dependent and this could be attributed to stress behavioural response as a result of the toxicity of the effluent.

Keywords: Effluent, biochemical, Toxicity, Concentration, Chemical additive.

1. INTRODUCTION

Toxic substances may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of water and making it unsuitable for aquatic life. When the concentration of the toxic substance is higher than what the homeoistasis of the aquatic organisms can control, it results in death/organ damages. In fish, organs such as opercula, the skin, liver and gill could be impared [1]. A toxic substance is a chemical pollutant that is not a naturally occuring substance in aquatic ecosystems. The greatest contributors to toxic pollution are herbicides, pesticides and industrial compounds [2]. The effects of waste waters discharged into water bodies can be acute which occurs rapidly and are clearly defined as fatal and rarely reversible or may be chronic which normally have lingering effects after long period of exposure and may ultimately cause death [3]. The entry of toxicants into aquatic media may affect the water quality parameter which in turn lesds to changes in the haematological variables of fish, due to its close association with the external environment [4,5]. It has been reported that biological monitoring techniques like haematological and biochemical variables are attractive and useful for monitoring environmental quality, water pollution, and the health conditions of aquatic organisms [6-10]. Biochemical biomarkers like glucose, protein, and enzymes are frequently used as an indicator of the general state of health and early warning of stress in fish under stressful conditions [11,12,13]. Heavy metals have been reported to

have negative impact on all relevant parameters and caused histo-pathological changes in fish [14].

Pesticides are major causes of concern for aquatic environment because of their toxicity, persistency and tendency to accumulate in the organism [15]. These pesticides are posing a great threat to aquatic fauna especially to fishes, which constitutes one of the major sources of protein rich food for mankind [16]. Metals are transported in the blood by binding to specific plasma protein [15]. The fishes serves as bio-indicators of water quality and the impact of the pesticides can be well understood by analysing either blood or serum of the fish, because blood is a pathophysiological reflector of whole body [17].

Oil pollution is one of the environmental constraints that produce aqua-toxicological effects which are deleterious to aquatic life [18]. Martin-Skilton et al., [19] demonstrated that acute exposure of juvenile turbot; *Scophthalmus maximus* to the prestige fuel oil elicits alterations in some hepatic biotransformation enzymes with different sensitivities, and leads to decreased levels of testosterone in plasma of juvenile turbot which might threaten reproductive capability of exposed individuals. Many laboratory studies have shown the toxicity of plant extract to fish and changes in haematological and biochemical profiles leading to death of fish [20,21]. Botanical products when used extensively may enter aquatic systems such as streams,rivers, and lakes, which may have an effect on non-target organisms in due course of time [22-26].

Fish haematology is known to be an essential tool to the fisheries biologist, as it acts as a frontline sensitive indicator of vital physiological and biochemical functions as well as status of nutrition, health, diseases and stress responses of the organism subjected to changes in environmental conditions. Therefore, the striking alterations in the blood parameters and associated pathological changes in fishes under influence of various toxic agents have attracted the attention of workers in the field [27]. Bhatia et al., [28] reported that fish are highly sensitive to very low concentrations of endosulfan and that blood is the primary target of pesticides action. Blood being the medium of intercellular and intracellular transport, which comes in direct contact with various organs and tissues of the blood, the physiological state of an animal at a particular time is reflected in its blood [29].

The objectives of this study therefore are to investigate the toxic effect of chemical additives effluent on the biochemical indices of *Clarias gariepinus.*

2. MATERIALS AND METHODS

2.1. *Test Chemical*

The effluent used for this reseach was obtained from a chemical additive and synthetic resin producing factory located in, Sango-Ota Industrial Estate, Ogun State, Nigeria. The effluent after collection was refrigerated immediately to prevent further microbial growth.

2.2. *Test Organism*

Clarias gariepinus of sizes between 120g and 130g were procured from a commercial Agricultural farm in Ogbomoso, Oyo State, Nigeria. Aerators were employed for proper oxygen dissolution during the exposure period.

2.3. *Physicochemical Analysis*

This was carried out prior to the laboratory experiment to quantify the concentrations of the metals and other parameters in the effluent. The methods of [30] were adopted.

2.4. *Toxicity Test*

The test organisms were acclamatized for two weeks during which the water was renewed daily using a renewal bioassay procedure while the fishes were fed twice daily. The toxicity test was then carried out in two phases i.e the acute and the chronic evaluations.

2.4.1. *Acute Evaluation*

After acclamatization, range finding following the method of [31] was conducted to determine the definitive concentrations to be used for the acute test. Four different concentrations were set up in replicates; these are 0.25mg/L, 0.30mg/L, 0.35 and 0.40mg/L. A total of ten fishes were introduced into each concentration including a control experiment. Mortalities were recorded at intervals and at the end of 96 hours, the following were determined;

a. Total number of death (mortality) after 96 hours

b. The percentage mortality at 96 hours

c. The LC_{50} which is the concentration at which half or 50% of the test organism died on exposure

Since the organisms were exposed for 96 hours, the 96-h LC_{50} was determined from the graph of percentage mortality against concentration. Arithmetic Graphic method was used to determine the 96-h LC_{50}.

2.4.2. *Chronic Evaluation*

After the 96hrLC_{50} was evaluated, four different c oncentrations were set up on the basis of the LC_{50} value obtained. They are the 2.5%, 5%, 10% and the 20% of the LC_{50} value respectively and these are. A control experiment was also set up. The solutions were renewed every 48 hours and the entire exposure period was 42 days. This was to allow the RBC of the test organisms undergo a complete cycle of maturation during the exposure period.

2.5. *Biochemical Analysis*

At the 42nd day, three organisms per concentration were randomly selected for biochemical analysis. Blood used was collected from the fish heart through cardiac puncturing with a needle and syringe, spun in a centrifuge for 5minutes at 5000rpm and biochemical indices like Total Cholesterol, Total serum protein, Serum Albumin, Globulin and Total Glucose level were analysed. The total Plasma protein was determined by the method of [32], Plasma Glucose by the method of [33]. Serum albumin was determined using the method of [34] while Total Cholesterol level was determined by the method of [35]. The Globulin content was measured by subtracting the value of Albumin from that of Total protein.

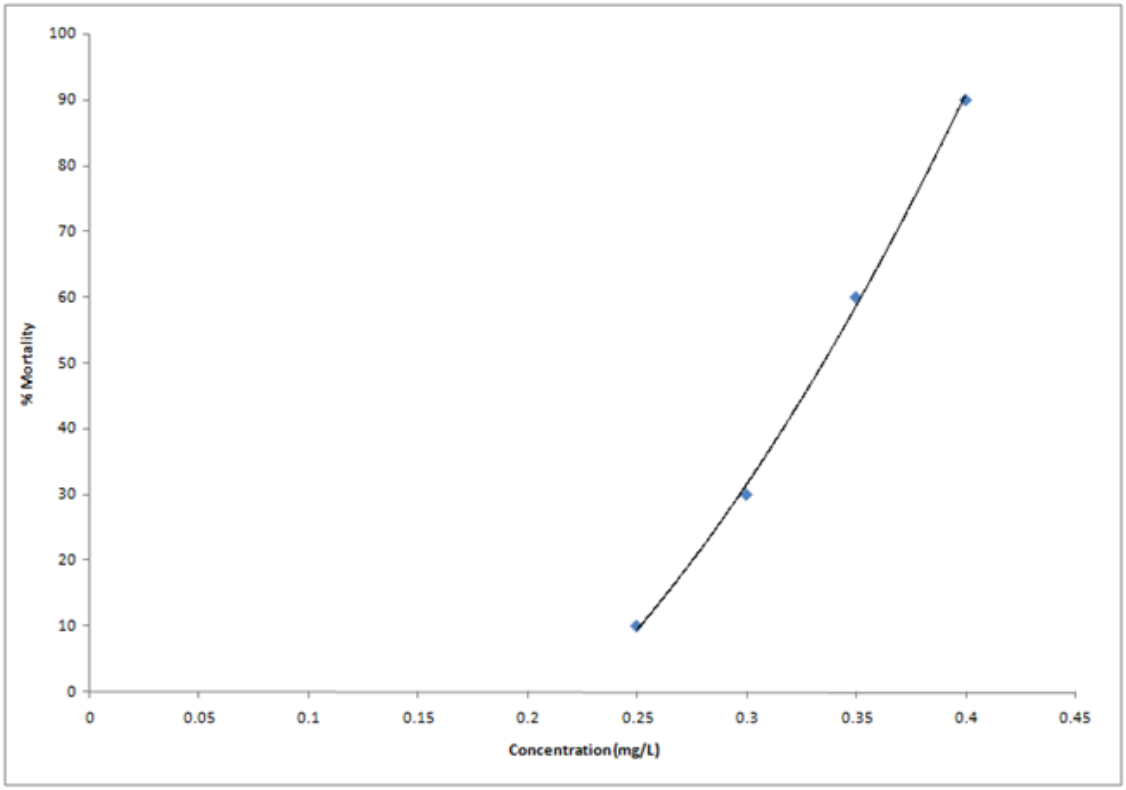

Fig 1. LC 50 for Clarias gariepinus exposed to sub-lethal concentrations of chemical additives effluent.

Table 1: Physicochemical characteristics of chemical additives effluent

Parameters	**Experimental Values**	**FEPA 1991 Specification**
Ph	6.7	6.5-8.5
D.O mg/L	2.6	5.0
B.O.D mg/L	0.4	50
T.S.S mg/L	72	30
IRON µg/L	0.6387	<1.00
CADMIUM µg/L	N.D	5.00µg/L
CHROMIUM µg/L	0.05	<1.00
SULPHIDE	0.25	0.2
NITRATE	3.3	10
CYANIDE µg/L	14µg/L	5.0µg/L
LEAD µg/L	9.5765	<1.00
COPPER µg/L	0.0775	5.8-6.0µg/L
ZINC µg/L	0.3484	<1.00
TOTAL HARDNESSmg/L	52.0	N.D
Ca^{2+}mg/L	20.8	N.D
Mg^{2+}mg/L	0.5953	N.D
T.D.Smg/L	32.4	2000
T.Smg/L	3.96	N.D
OIL AND GREASE	12.5	10
ALKALINITY	65	N.D
MANGANESE µg/L	0.0250µg/L	100µg/L
PHENOPTHALENE	N.D	20
METHYL ORANGE	65	20

N.D = Not Detected

Table 2. Mean and standard deviations for biochemical parameters of *Clarias gariepinus* exposed to different concentrations of chemical additives effluent for 42 days

Parameters	Concentration				
	Control	0.020952	0.041904	0.083808	0.167617
Glucose	43.333 ±8.819^{b}	40.000 ±11.547^{a}	40.000 ±5.773^{b}	66.666 ±13.333^{b}	80.000 ±23.094^{b}
Total Protein	2.4333 ±0.088^{c}	3.3000 ±0.264^{a}	2.6667 ±0.033^{bc}	3.1000 ±0.057^{ab}	3.0000 ±0.152^{ab}
Albumin	1.5667 ±0.088^{b}	2.2333 ±0.176^{a}	1.5333 ±0.176^{b}	2.0000 ±0.057^{ab}	1.8000 ±0.251^{ab}
Globulin	0.8667 ±0.088^{b}	1.0667 ±0.088^{a}	1.1333 ±0.145^{ab}	1.1000 ±0.000^{ab}	1.2000 ±0.100^{a}
Cholesterol	150.000 ±15.275^{a}	147.000 ±13.316^{a}	139.333 ±41.462^{a}	140.666 ±21.827^{a}	134.667 ±20.827^{a}

Note: mean or values with the same alphabet for same parameter are not significantly different ($P<0.05$)

2.6. *Statistical Analysis*

The statistical analysis of the biochemical parameters was done using the SPSS 10 package. The values obtained were confirmed using one-way ANOVA at 0.05 level of significance. Further test on those found to be significant was done using Duncan Multiple Range Test (DMRT).

3. RESULTS

Physicochemical Characteristics of chemical additives Effluent:- This is shown in table 1. The data obtained have some of its values conforming to [36] specifications for maximum limits allowed for effluent discharge into water bodies while the values for lead, cyanide, total hardness, calcium, oil and grease and alkalinity do not conform to the standard.

3.1. *Behavioural Responses*

During the toxicity test, *Clarias gariepinus* exhibited distress behavioural responses due to the effects of the chemical additives effluent. These were noticed by the sudden change in the organism's response to the environment such as erratic swimming, gasping for breath and frequent surfacing which increases as the concentration increases. As the experiment progressed, the test organisms were seen to get weaker and those that couldn't tolerate the concentrations any longer went into comatose. Normal behaviour was however observed in the control.

3.2. *Mortality*

The result of the acute toxicity shows the absence of mortality in the lower concentrations while maximum mortality was observed in the highest concentration.

Figure 1 shows the arithmetic graph of percentage mortality against concentration for the acute evaluation. The 96-h LC_{50} was calculated to be 0.335223mg/L

Table 2 reveals the biochemical parameters of C. Gariepinus after 42 days exposure. The glucose and total protein increases with concentration, while the cholesterol decreases with increase in concentration.

4. DISCCUSSION

The work shows the chemical additives effluent to be high in total suspended solid (TSS), lead and cyanide, low biochemical oxygen demand (BOD), lower dissolved oxygen (DO), lower total dissolved solids (TDS) and high alkalinity content which shows the effluent to be toxic for discharge into our immediate environment. This corresponds to the findings of (Adewoye et al., 2005) that the observed characteristics features may have resulted from the organic loads in the wastewater. The abnormalities observed prior to mortality are an indication of depleted oxygen content due to higher demand for oxygen. Consequently, it was observed in this study that the abnormal behaviour and mortality rate of the test organism's increaded with increase in the concentrations of pollutant. This corresponds to the findings of [37] that the behaviour and mortality rate of *C. catla* during experimentation was found to depend on both duration of exposure and concentration of the toxicant.

The introduction of the effluent at different concentrations impair the swimming pattern, skin colouration, feeding rate and general behaviour of fish. Also, the variation in the behavioural responses and mortality in the sub-lethal test compareds with the Acute test can be attributed to the low level of accumulation of the effluent. This suggests that fish can tolerate low concentrations of pollutants with reduced mortality.

The significant ($P < 0.05$) increase in glucose which was concentration and time dependent may be considered to be manifestations of stress induced by the chemical additives effluent. Glucose increase is a general response of fish to acute and sub-lethal pollutant effects [38]. Increase in serum glucose levels in fish under stress was reported by [39,40,41].

This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis [39].
There is an increase in serum protein recorded in this work. is in agreement with [42] who reported increase in liver protein followin exposure to 2,4 Diamine for 30 days. There is a significant decrease in serum protein observed in the 0.041904mg/L and this may be due to the toxic stress which may reduce protein content in tissues. This is supported by [43,44] that proteins are mainly involved in the architecture of the cell. During chronic period of stress, they are also a source of energy. During stress condition, fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amount of carbohydrate, the next alternative source of energy is protein to meet the increased energy demand. The depletion of protein may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Shobha et al., [37] also observed that decrease in the protein content as observed in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free aminoacids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation. It could also be due to the production of heat shock proteins or destructive free radicals or could be a part of heavy metal induced apoptosis. [45] also recorded serum protein decrease in fish exposed to phenol.

Cholesterol was found to decrease considerably in this work which may be due to utilization of stored and circulatory cholesterol and other lipid fractions in the treated fish to counteract toxic effects produced. This result conforms closely with [46] who observed a decrease level of cholesterol in *Channa punctatus* exposed to phorate. Rani, et al., [47], and Shankar and Kulkarni [48],also observed the same trend in *Notopterus notopterus* during stress. There is also time dependent significant ($P < 0.05$) serum albumin and globulin elevation due to the effluent exposure.

5. CONCLUSION

In conclusion, it's evident from this study that increasing concentration of the chemical additives effluent when present in any water body could lead to abnormal bahavioural responses, haematological and biochemical dysfunction in fish health and general condition. There is therefore a need for preventive measures to be taken in order to prevent the indiscriminate discharge of this effluent into nearby streams and ponds. Man is the final reciepient of toxic bioaccumulated chemicals via the food chain and environment, effective application of hazard analysis critical control point (HACCP) monitor is stressed. It is recommended that the application of appropriate effluent technology be adopted by the concerned industries and individuals.

6. REFERENCES

1. F. Oyedapo, I. Akinduyite, Acute Toxicity of Aqueous *Morinda lucida* leaf extracts to Nile Tilapia, *Oreochromis niloticus* (Linnaeus 1857). Proceedings of the Ninth International Symposium on Tilapia in Aquaculture. Shanghai, China. April 22nd-24th, 2011. 52-59.
2. A. Agrawal, S.P. Ravi, S. Bechan, Water pollution with special reference to Pesticide Contamination in India. *J. Water Resources and Protection* 2: 432-448. 2010
3. S.O. Adewoye, O.O. Fawole, O.D. Owolabi, J.S. Omotosho, Toxicity of cassava wastewater effluent to African catfish: *Clarias gariepinus*. *Ethiop. J. Sci.,* 28 (7): 189-194. 2005
4. C.S. Carvalho, M.N. Fernandes, Effects of temperature on Copper toxicity and haematological responses in the Neotropical fish, *Prochilodus scrofa* at low and high pH. *Aquaculture* 25(10): 9-17. 2006.
5. C. Kavitha, A. Malarvizhi, K.S. Senthil, M. Ramesh, Toxicological effects of arsenate exposure on haematological, biochemical and liver transaminase activity in an Indian major carp, *Catla catla. Food Chem. Toxicol*. 48(28): 48-54. 2010.
6. E.S. Celik, Blood chemistry (electrolytes,lipoproteins and enzymes) values of black scorpion fish (*Scorpaena porcus*) in the Dardanelles. *Turkey J. Biol. Sci.* 4(6):716–719. 2004.
7. H.R. Kohler, C. Sandu, V. Scheil, E. M. Nagy-Petrica, H. Segner, I. Telcean, Monitoring pollution in River Mures, Romania, Part 111: Biochemical effects markers in fish and integrative reflection. *Environ. Monit. Assess*., 127: 47-54. 2007.
8. O. Kori-Siakpere, E.O. Ubogu, Sublethal haematological effects of zinc on the freshwater fish, *Heteroclarias spp.* (Osteichthys: Clariidae). *Afr. J. Biotech.*, 7(20): 68-73. 2008.
9. M.O. Olufayo, Hematological characteristics of *Clarias gariepinus* (Burchell 1822) juveniles exposed to *Derris elliptica* root powder. *Afr. J. Food Agric. Nutr. Sci*. 9(3):920–33. 2009.
10. C. Kavitha, R. Mathan, S.K. Satyanarayanan, Toxicity of Moringa oleifera seed extract on some haematological and biochemical profiles in a freshwater fish, *Cyprinus carpio. Exp. Toxicol. Pathol.* (2011), doi:10.1016/j.

etp.2011. 01.001.

11. I.E.J. Barnhorn, J.H.J. Van-Vuren, The use of different enzymes in feral freshwater fish as a tool for the assessment of water pollution in South Africa. *Ecotoxicol Environ. Safety*. 59:180–5. 2004.

12. E.H. Abou El-Naga, K.M. El-Moselhy, M.A. Hamed, Toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish *Mugil seheli*. *Egyptian J. Aquat. Res.,* 31(2): 60–71. 2005.

13. A.G.M. Osman, M. Koutb, A.E.D. Sayed, Use of hematological parameters to assess the efficiency of quince (*Cydonia oblonga,* Miller) leaf extract in alleviation of the effect of ultraviolet—iradiation on African catfish, *Clarias gariepinus* (Burchell, 1822). *J. Photochem. Photobiol. B. Biol.* 99:1–8. 2010.

14. S. Maity, S. Roy, S. Chaudhury, S. Bhattacharya, Antioxidant responses of the earthworm *Lampito mauritii* exposed to Pb and Zn contaminated soil. *Environ. Pollut*. 151: 1–7. 2008.

15. B. Joseph, J.S. Raj, Effects of curacon toxicity on the total serum protein content of *Cyprinus carpio*. *Toxicol. Environ. Chem.* 92: 1889-1893. 2010.

16. G. Sharma, S. Singh, Effects of indofil toxicity on MCHC of *Channa punctatus* (Bloch). *J. Environ. Res. Dev.,* 1: 261-263. 2007.

17. G. Sharma, S. Singh, Studies on the effects of intoxicant indofil on the blood morphology of *Channa punctatus* (Bloch). *Bionotes* 6:20-30. 2004.

18. O.M. Agbogidi, B.C. Okonta, D.E. Dolor, Socio-economic and environmental impact of crude oil exploration and production on agricultural production: a case study of Edjeba and Kokori communities in Delta in Nigeria. *Global Journal of Environmental Sciences*. 4: 171-176. 2005

19. R. Martin-Skiltona, F. Saborido-Rey, C. Portea, Endocrine alteration and other biochemical responses in juvenile turbot exposed to the Prestige fuel oil. *Science of the Total Environment* 404: 68 – 76. 2008.

20. S. Tiwari. A. Singh, Piscicidal and anti-acetylcholinesterase activity of *Euphorbia royleana* stem bark extracts against fresh water common predatory fish *Channa punctatus*. *Environ Toxicol Pharmocol*. 18:47–53. 2004.

21. E. Ayotunde, B.O. Ofem, Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to adult Nile Tilapia (*Oreochromis niloticus*). *Afr. J. Biotech*. 7(3): 22675-32274. 2008.

22. D. Singh, A. Singh, The toxicity of four native Indian plants: effect on AChE and acid/alkaline phosphatase level in fish *Channa marulius. Chemosphere*. 60(1):35–40. 2005.

23. E. Dongmeza, P. Siddhuraju, G. Francis, K. Becker, Effects of dehydrated methanol extracts of moringa (*Moringa oleivera,* Lam.,) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile Tilapia ((*Oreochromis niloticus* L.) *Aquaculture* 261(40): 7-22. 2006.

24. V. Tiwari, A. Singh, Biochemical stress response in freshwater fish, *Channa punctatus* induced by aqueous extracts of *Euphorbia trucalli* plant. *Chemosphere* 64: 36-42. 2006.

25. E.U. Winkaler, T.R.M. Santos, J.G. Machado-Neto, C.B.R. Martinez, Acute lethal and sublethal effects of neem leaf extract on the Neotropical freshwater fish, *Prochilodus lineatus*. *Comp. Biochem. Physiol*., 145(2): 36-44. 2007.

26. U.U. Gabriel, F.G. Obomanu, O.S. Edori, Haematology, Plasma enzyme and organ indices of *Clarias gariepinus* after transmuscular injection with aqueous leaves extracts of *Lepidagathis alopecuroids*. *Afr. J. Biochem. Res.*, 3(9): 312-316. 2009.

27. S. Nair, Toxic effects of Mercury on the haematological parameters of *Oreochromis mossambicus*. *Poll. Res.,* 1993: 399-402. 2002.

28. N.P. Bhatia, G.S. Sandhu, M.S. Johal, Haematological alterations in *Heteropneus fossilis* upon exposure to endosulfan. *Poll. Res.* 23(4):633-636. 2004.

29. G. Sharmila, C. Maruthanayagam, Haemato-biochemical response to sumaach (a crude form of HCG) in fish, *Notopterus notopterus* Pallas under pesticides treatment. *Geobios* 20:255-259. 2004.

30. APHA/AWWA/WEF, American Public Health Association/American Water Works Association/Water Environment Federation Standard Methods for Water and Wastewater Analysis. 1999

31. ASTM E729-96, Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. 2007.

32. D.C. Cannon, I. Olitzky, A. Iukpen, Proteins. In: Clinical Chemistry, Principles and Techniques, 2nd Edition, New

York. Pp 407-421. 1972.

33. G.R. Cooper, V. McDaniel, The determination of glucose by the O-toluidine method. *Stand Meth. Clin. Chem.* 6(1):59–70. 1970.

34. J.E. Gustafsson, Improved specificity of Serun Albumin determination and estimation of 'acute phase reactants' by use of the bromocresol green reaction. *Clinical Chemistry*. 22: 616-622. 1976.

35. G.R. Warnick, Compact analysis for cholesterol; Triglyceride and high density lipoprotein cholesterol. *Curr. Opn. Lipidol.,* 2:343. 1991.

36. FEPA, Federal Environmental Protection Agency's guidelines and standards for environmental pollution in Nigeria. 1991.

37. K. Shobha, A. Poornima, P. Harini, K. Veeraiah, A study on biochemical changes in the fresh water fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant Cadmium chloride. *Kathmandu University Journal of Science, Engineering and Technology*. 1(4): 1-11. 2007.

38. J.J. Ceron, E. Sancho, M.D. Ferando, C. Gutierrez, E. Andreu, Changes in carbohydrate metabolism in the eel *Anguilla anguilla*, during short term exposure to diazinon. *Toxicol. Environ. Chem.,* 60: 201-210. 1997.

39. J.A. Almeida, E.L. Novelli, P. Das, M. Silva, R.A. Junior, Environmental Cadmium exposure and metabolic responses of the Nile Tilapia, *Oreochromis niloticus*. *Environmental Pollution.* 114(2): 169-175. 2001.

40. M.J. Chowdhury, E.F. Pane, C.M. Wood, Physiological effects of dietary Cadmium acclimation and waterborn Cadmium challenge in rainbow trout: respiratory, ionoregulatory, and stress parameters. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol. ,* 139(1-3): 163-173. 2004.

41. C. Bedii, E. Kenan, The effects of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L., 1758). *Turkish Journal of Veterinary Animal Science.* 29: 113-117. 2005.

42. E.O. Oruc, N. Uner, Effects of 2,4-Diamine on parameters of protein and carbohydrate metabolisms in the serum, muscle and liver of *Cyprinus carpio*. *Environ. Poll.,* 105: 267-272.1999.

43. S. Singh, A. Khare, Effects of pesticides on protein metabolism in liver of *Clarias barachus*. *Rec. Ad. App. Env. Zool*., 1,21. 1999.

44. H.S. Desai, Toxicological effects on some biochemical parameters of fresh water fish *Channa punctatus* under the stress of Nickel. *J. Environ., Biol.,* 23(3): 275-277. 2002.

45. H.A. Nassr-Alla, Physiological and Histopathological Alterations induced by Phenol Exposure in *Oreochromis aureus* juveniles. *Turkish Journal of Fisheries and Aquatic Sciences.* 7:131-138. 2007.

46. P.S. Anand, S. Surendra, B. Prabhat, Y. Khushbu, Toxic effects of Phorate on the serum Biochemical Parameters of Snake Headed Fish, *Channa punctatus* (Bloch). *Advances in Bioresearch*. 1(1): 177-181. 2010.

47. A.S. Rani, R. Sudharsan, T.N. Reddy, P.U.M. Reddy, T.N. Raju, Effects of arsenite on certain aspects of protein metabolism in freshwater teleost, *Tilapia mossambica* (Peters). *Journal of Environmental Biology*. 22(2): 101-104. 2001.

48. D.S. Shankar, R.S. Kulkarni, Tissue cholesterol and Serum cortisol level during different reproductive phases of the female freshwater fish, *Notopterus notopterus. Journal of Environmental Biology*. 28(1):137-139. 2007.

Source of support: Nil; Conflict of interest: None declared