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Effects of agro-chemicals on fishes: With reference to changes in circulating biochemical parameters in *Clarias gariepinus* induced with Paraquat dichloride

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ABSTRACT

The response of *Clarias gariepinus* to Paraquat dichloride, a herbicide that is in high demand in the fragile Niger Delta ecological zone was investigated. The herbicide had been detected in most of the water bodies in the region. The fishes were exposed to the range of concentrations observed in the field (0, 2, 4, 6 and 8) µg/L for 28 days, conducted under the Organization for Economic Co-operation and Development (OECD) test guidelines. Signs of stress were monitored and blood samples were taken from the caudal vein located behind the backbone for biochemical study. Biochemical parameters were measured spectrophotometrically. There were moderate changes in cortisol secretion, glucose levels and fluctuation in protein levels. No mortalities occurred during the test. These findings indicate that Paraquat dichloride can be tolerated by the tropical fish. Nevertheless, it should be used with care as incessant usage can increase its concentrations in the environment. The herbicide binds rapidly and tightly to clay materials and can easily leach into water, which could be lethal to aquatic and terrestrial flora and fauna. It can also magnify along the trophic level, which could be detrimental to humans.

Keywords: *Clarias gariepinus*, cortisol, fish, glucose, Niger Delta, Paraquat dichloride, protein.

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Introduction

Paraquat dichloride is an organochlorine herbicide that is effectively used to control many species of weeds and can be used with most crops. Though, once sprayed, it kills weeds and then becomes dormant in the soil (1). However, it causes severe, acute, and chronic poisoning when it is waterborne because it very readily dissolves and dissociates in aqueous solutions (2). It is toxic to fish, aquatic plants and significantly affects tadpole mortality (3). The herbicide inhibits biochemical and haemathological parameters in fishes and other aquatic organisms (4). Feeding rates of the shrimp, *M. nipponense*, decreased when exposed to increasing concentrations of the pesticide (5). Current studies revealed that numerous fish species experience compromised reproductive fitness and endocrine disruptors that altered immunity and decreased disease resistance, especially during embryonic and fetal development as a result of chronic exposure to Paraquat (6).

These chemicals can also alter hormone metabolism resulting in elevated levels of androgens, which may lead to pseudo hermaphrodism, developmental abnormalities, and reproductive impairment. The impacts of endocrine disruptors may become one of the most serious environmental problems faced by humans. Attempts to protect species diversity within the remaining natural habitats of the world will become difficult, if the health and reproductive capabilities of wildlife are seriously affected by widespread exposure to endocrine disruptors (7). An integrated examination of the parallels between human and wildlife health with respect to exposure to organochlorine chemicals yielded greater insights, greater awareness, and modified public policies, plus increased activity to mitigate adverse effects.

Clarias gariepinus was chosen for this study because it is a good sentinel for the study of toxicity. It feeds on detritus, it inhabits the streams, rivers, lakes, estuarine and brackish lagoons, found all year round (8). Its feeding habits reflect the local contamination simply because it is a bottom dweller.

Although the endocrine-disrupting, oxidative stress, cytotoxic potential, and other biochemical alterations of pesticides such as mancozeb, diazinon, atrazine and endosulfan have been assessed in teleost and amphibian *in vitro* (9-12); it is not known whether chronic field exposures of mild organochlorine pesticides, such as Paraquat impair adrenal function, damage pancreas and alter protein level in teleosts. Thus, the objectives of the present study was to investigate the effects of Paraquat dichloride (agrichemicals) on protein level, glucose production and cortisol secretion and to validate these parameters as markers of chronic exposure to agrichemicals, and to test the hypothesis that endocrine-disrupting effects of chronic exposure to agrichemicals can inhibit or induce protein and glucose production.

Materials and Methods

Experimental Design

Eight– week old juveniles of *C. gariepinus* of similar size and weight were collected from a private fish farm in Delta State, Nigeria and allowed to acclimatize to laboratory conditions in glass tanks for one month, before exposure in experimental tanks containing dechlorinated tap water. The holding tanks were aerated with the help of air pump, cleaned and the water was renewed daily. Fish were fed twice daily with TAIYO feeds (Ingredient composition- fish meal,

wheat flour, soybean meal, corn meal, yeast, vitamins and mineral salt: Proximate analysis- crude protein mineral; 32%, crude fat mineral; 4%, crude fibre, maximum; 5% and moisture, maximum; 10%). Water quality of the test and reference solutions was monitored throughout the duration of the experiment.

Toxicity Test

Fish were divided into five groups each containing five fish. Group I was held in an insecticide free container (served as the control), and the other groups were exposed to range of concentrations observed in the field (0, 2, 4, 6, and 8) µg/l Paraquat dichloride for 28 days. Throughout the experiments, the control and experimental fish were fed daily with TAIYO feeds, at approximately 3% of their body weight. Fish were maintained in semi static renewal conditions, where water was aerated using an air pump. Water and pesticide were completely replaced every 24h, tanks cleaned daily and fish were transferred to freshly prepared solutions containing toxicants.

Serum preparation and analysis

At the end of the 28 days, the fish were removed from aquaria and immediately anesthetized with MS222 (Ethyl 3-aminobenzoate methanesulfonate salt, Sigma), and blood samples were taken from the caudal vein just behind the backbone of each fish as described by (13). This blood was collected in anticoagulant-free centrifuge tubes. Serum was obtained by centrifugation of blood at 3.000 rpm for 10 min. Serum samples were then stored at −80°C until analysis.

Biochemical parameters in the serum samples were analyzed using biochemical analyzers (Modular Roche DPP, Modular Roche E170, Hitachi Ltd, Tokyo, Japan). Reactants for all the measurements were obtained from Sigma Chemicals, St Louis, MO, USA for analyses.

Electrochemiluminometric assay was used in the determination of the cortisol levels. The test kit was prepared in accordance with the method described by (14) as modified by (15). The serum cortisol assay is a competitive polyclonal antibody immunoassay that employs a magnetic separation step followed by electrochemiluminescence quantitation.

The enzymatic UV test was used for glucose level determination. The principle of the method is as described by (16). The enzyme hexokinase catalyzes the reaction between glucose and adenosine triphosphate to form glucose-6-phosphate and adenosine diphosphate. In the presence of NAD (Nicotinamide adenine dinucleotide), the enzyme glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to 6-phosphogluconate. The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

Bovine serum albumin (BSA) used for the determination of protein quantity was purchased from Sigma Chemical Company. Protein determination was performed using the original Lowry method (17). Fifty micro liters of supernatant were incubated with 1130 µL of Folin reagent (130 µL of A and 1000 µL of B). The content was then mixed and after 30 min of incubation, the absorbance was read at 750 nm using a Shimadzu 160A UV spectrophotometer at 25°C.

Data analysis

One-way analysis of variance SPSS (16.0 version), SPSS Inc, Chicago, USA, was employed to calculate the significance of the differences between control and experimental means and within various treatments. P values of 0.05 or less were considered statistically significant (18). Multiple line graphs were also used in this study for the pictorial representation of assessment endpoints.

Results

Cortisol: Alteration in the cortisol concentration in the control and induced fish is shown in Figure 1, with further illustration in Table 1. The cortisol concentration were lower in the control fish than the treated fish but was not significantly different (P> 0.05). The level of variation increased with increase in the concentration of the toxicant.

Glucose. Changes in the concentration of glucose in *C.gariepinus* exposed to different treatment of Paraquat dichloride at 28 days is shown in Figure 2, with further illustration in Table 1. There was no significance between the control and various treatments and within the treatments (P> 0.05). However, glucose concentration increased with increase in toxicant concentration.

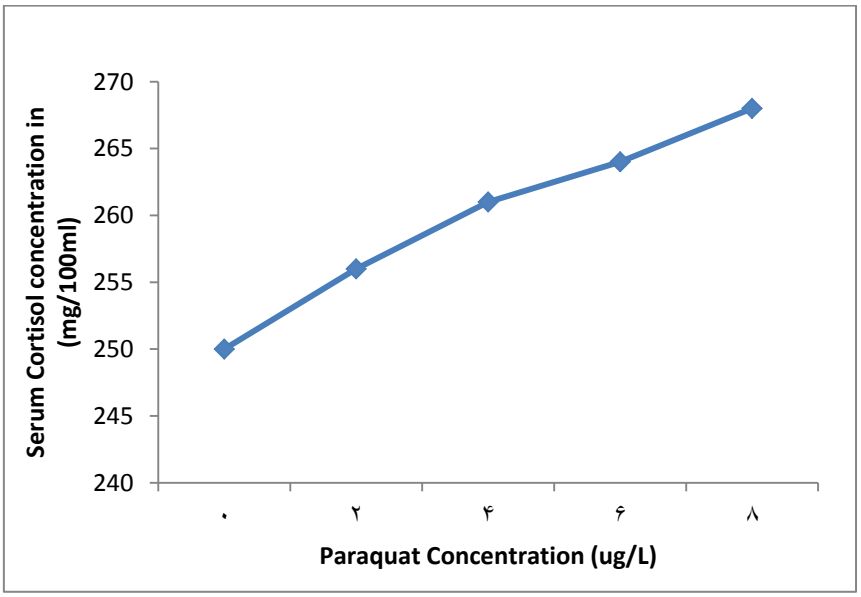

Figure 1. Changes in serum cortisol concentration in *C. gariepinus* exposed to sub-lethal concentrations of Paraquat dichloride at 28 days

Table 1. Biochemical indices in *C. gariepinus* at 28 days sub lethal exposure to different concentrations (µg/L) of Paraquat dichloride

Conc. (ug/L)	0.00	*2.00*	4.00	6.00	8.00
	Mean ± SD	*Mean± SD*	Mean ± SD	Mean ± SD	Mean ± SD
Cortisol	250.00± 1.2[a]	*256.60±1.08[a]*	261.00± 1.20 [a]	264.67±1.15 [a]	268.00± 0.30[a]
Glucose	189.60±3.23[a]	*192±7.16[a]*	197±7.30 [a]	206± 1.72 [a]	210.67±3.06[a]
Protein	20.5± 1.9[a]	*20.63±1.24 [a]*	24.30±1.02[a]	23.15±1.11 [a]	20.10± 1.30 [a]

[a] Mean with the same superscript in the row are not significantly different (P< 0.05)

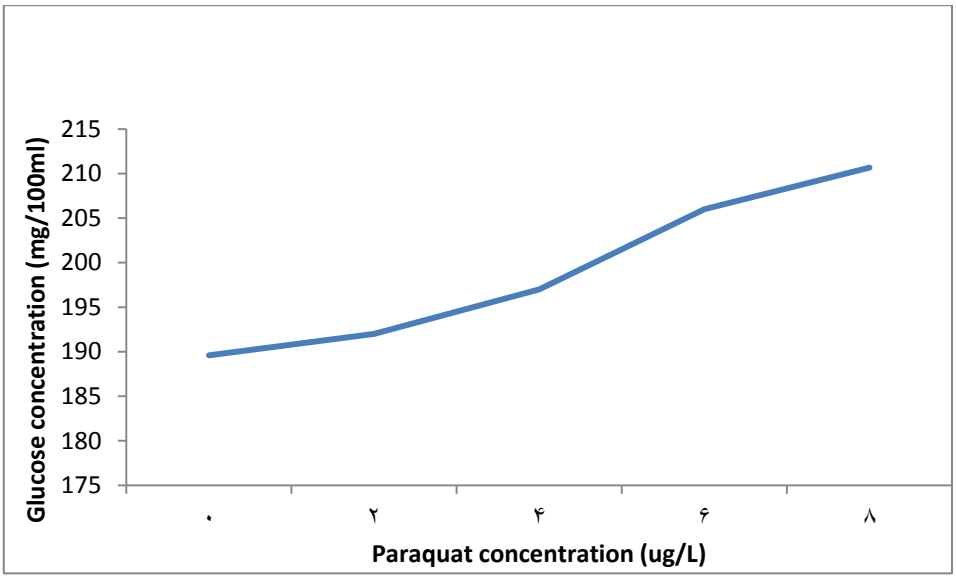

Figure 2. Changes in glucose concentration in *C. gariepinus* exposed to sub-lethal concentrations of Paraquat dichloride at 28 days

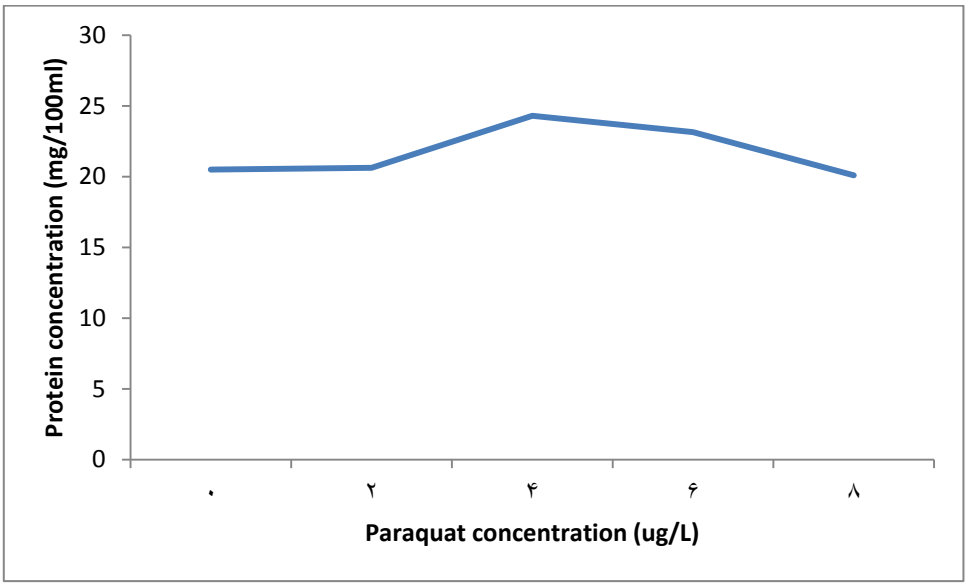

Figure 3. Changes in protein level in *C. gariepinus* exposed to sub-lethal concentrations of Paraquat dichloride at 28 days

Protein. The protein level was not significantly (P>0.05) affected. However, there were fluctuations in protein concentration in the exposed fish and were slightly higher than the control, irrespective of the concentrations of the toxicants. The changes are shown in Figure 3 and Table 1.

Discussion

The physicochemical parameters of the study river are within the water quality standard range, found in the natural environment in the tropic and are within the approved limit set by standard organization of Nigeria (19).

The treated fish did not show any morphological changes during the study period except during early periods of exposure which then stabilized after 3 min post exposure. Normal swimming was observed and there was no sign related to toxicity such as physical disorientation,

partial jerking, violent behaviors, short movements, sluggishness or excitations. The behavioral changes observed indicated that *C. gariepinus* was not susceptible to the test chemical at a minimal concentration, as reported in most rivers in the Niger Delta ecological zone (20).

However, this study provided evidence that serum cortisol, glucose and protein levels were altered in fish induced with Paraquat dichloride. Therefore, it can be deduced that endocrine-disrupting effects were associated with hyperglycemia and liver impairment.

This finding revealed the endocrine-disrupting effects of Paraquat dichloride. The cortisol concentration was lower in the control fish than the treated fish but was not significantly different (P> 0.05). The slight decrease may be attributed to stress caused by the herbicide. Stress is considered as an adaptive mechanism that allows the fish to cope with real or perceived stressors, in order to maintain its normal or homeostatic state. Quite simply, stress can be considered as a state of threatened homeostasis that is re-established by a complex suite of adaptive responses (21). Similarly, the release of catecholamines from chromaffin tissue (22), and the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis culminates in the release of corticosteroid hormones (23). Comparably, endosulfan, another organochlorine pesticide, was recently identified *in vitro* as a chemical that increases cortisol secretion by teleost adrenocortical cells (24, 25). Cortisol measurement thus may be useful for assessing the environmental impact of pollutants in the field, by measuring exposure and providing a reliable indication of toxic effects such as endocrine dysfunction. Thus, providing evidence that endocrine-disrupting effects were associated with hyperglycemia and liver impairment.

The increase in blood glucose in this study is insignificant (P> 0.05). A change in glucose concentration is known as a general secondary response to stress and is considered as a reliable indicator of environmental stress (26, 27). Its high blood concentrations, indicate that the fish is in stress and is intensively using energy reserves i.e., glycogen in liver and muscles (28). On the other hand, in the pancreas of animals, the organochlorine pesticide is toxic to the Beta-cells of the islet of Langerhans, which produces insulin necessary to reduce blood sugar levels, thereby causing a rise in blood sugar levels (29). The observed value in this investigation corroborated the finding of (30). Hyperglycemic response illustrated in the present study is an indication of a disruption in carbohydrate metabolism, possibly due to enhanced glucose 6-phosphatase activity in the liver, elevated breakdown of liver glycogen, or the synthesis of glucose from extrahepatic tissue proteins and amino acids. Raja *et al*. (31) suggested that the increase in blood glucose by pesticide treatment may indicate disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by increase in adrenocorticotrophic and glucagon hormones and/or reduced insulin activity. Omoregie *et al*. (32) reported that tilapia showed marked hyperglycemic response to stressed environmental conditions, as a result of incomplete metabolism of the blood sugar due to impaired osmoregulation. Similarly, cypermethrin-induced hyperglycemia has been recorded in *L. rohita* (33) and *S. schlegeli* (34).

Total serum protein, which is synthesized in the liver, is used as an indicator of liver impairment (35). In this investigation, there were no significant changes in total protein levels in all toxicants exposed fish and the control (P> 0.05). However, there were fluctuations in protein concentration in the exposed fish and was slightly higher than the control, irrespective of the concentrations of

the toxicants. Increase in protein levels may be attributed to the stress mediated immobilization of these compounds, leading to an increase in energy demands by the fish to cope with the environmental condition caused by the toxicant (36). On the other hand, Neff (37) opined that changes in protein content may be related to impaired metabolic activities, increased energy cost of homeostasis, tissue repair and detoxification mechanism during stress. It is therefore logical to presume that in the case of prolonged and continued exposure to the pesticide, the deleterious effects of this substance on protein synthesis and kidney function may alter the concentration of total serum protein.

Biochemical indices are highly sensitive to various environmental factors and can provide substantial diagnostic information about the internal environment of the organism. On this basis, biochemical studies would be useful to predict the physiological state of fish in natural water bodies. Lipophilic pesticides are known to accumulate in fatty tissues and have a capacity for accumulation in the food chain. Therefore, the environmental guideline on the use of pesticides such as Paraquat should be adhered to, because the presence of Paraquat even in small amounts in fish muscles, can lead to their bioaccumulation in organisms at higher levels of the food chain. Also, chlorinated pesticides are known to exert a neurotoxic effect and can cause many diseases. Additional field and laboratory studies are required to investigate the potential of recovery of the affected parameters after the exposure period.

REFERENCES

1. Tomlin, Clive (ed). (1994) The Pesticide Manual, incorporating The Agrochemicals Handbook, Tenth Edition, British Crop Protection Council and the Royal Society of Chemistry, Farnham UK.
2. Amdur, M.O., Doull, J. and Klaassen, C.D. (1991) Toxicology, the basic science of poisons. 4th ed Pergamon Press, New York, pp 602-603.
3. Bauer, C. and Dial, N. (1995) Lethal effects of the consumption of field levels of Paraquat-contaminated plants on frog tadpoles. *Bull. Environ. Contam. Tox*., **55**,870-877.
4. Yuan, Y.C., Chen, H.C. and Yuan, Y.K. (2004) Sublethal Effects of Paraquat and Malathion on the Freshwater Shrimp, *Macrobrachium nipponense. Acta Zoologica. Taiwanica.,* **14**, 87-95.
5. Campbell, S. (1968) Paraquat poisoning. *Clin. Toxicol*., **1**, 245-249.
6. Tanner, C.M., Freya, K.G., Webster, R., Jane, A., Hoppin, S.M., Goldman, M., Korell, C., Marras, G.S., Bhudhikanok, M., Kasten, A.R. *et al*. (2011) "Rotenone, Paraquat and Parkinson's Disease". *Environmental Health Perspectives*, **119**, 866–72.
7. WHO. (1991) International Programme on Chemical Safety, Paraquat Health and Safety Guide No. 51 Geneva.
8. Idodo, U.G. (2003) Fresh water fishes of Nigeria (Taxonomy, Ecological notes, Diet and Utilization). Idodo Umeh publications, Benin City, Nigeria. pp.250.
9. Leblond, V.S., Bisson, M. and Hontela, A. (2001) Inhibition of cortisol secretion in dispersed head kidney cells of rainbow trout (*Oncorhynchus mykiss*) by endosulfan, an organochlorine pesticide.*Gen. Comp. Endocrinol.,* **121**,48–56.
10. Lorenzen, A., Moon, T.W., Kennedy, S.W. and Fox, G.A. (1999) Relationships between environmental organochlorine contaminant residues, plasma corticosterone concentrations, and intermediary metabolic enzyme activities in Great Lakes herring gull embryos. *Environ. Health Perspect.,* **107,**179–186.
11. Hontela, A. (1998) Interrenal dysfunction in fish from contaminated sites: In vivo and in vitro assessment. *Environ. Toxicol. Chem.,* **17,** 44–48.
12. Bisson, M. and Hontela, A. (2002) Cytotoxic and endocrine-disrupting potential of atrazine, diazinon, endosulfan, and mancozeb in adrenocortical steroidogenic cells of rainbow trout exposed in vitro. *Toxicol. Appl. Pharmacol.,* **180**,110–117.
13. Congleton, J.L. and LaVoie, W.J. (2001) Comparison of blood chemistry values for samples collected from juvenile Chinook salmon by three methods. *J. of Aquatic. Animal Health*, **13**, 168–172.
14. Barseghian, G., Rachmiel, L. and Epps, P. (1982) "Direct Effect of Cortisol and Cortisone on Insulin and Glucagon Secretion". *Endocrinology*, **111**, 1648.
15. Chiu, S.K., Collier, C.P., Clark, A.F. and Wynn-Edwards, K.E. (2003) Salivary cortisol on ROCHE Elecsys immunoassay system: pilot biological variation studies. *Clin. Biochem*., **36,**211–214.

16. Schmidt, F.H. (1961) Enzymatic determination of glucose and fructose simultaneously. *Klin Wochenschr*, **39**, 1244–1250.

17. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with folin phenol reagent. *J. of Biol. Chem.,* **193**,265-267.

18. Fisher, R.A. (1950) Statistical methods for research workers. 11th edition. Oliver and Boyd, London 75 pp.

19. Standard Organisation of Nigeria (2007) Drinking water quality guidelines, Nigeria. Department of Petroleum Resouces, Federal Ministry of Environment, Nigeria.

20. Erewa, O. (2013) Occurrence and distribution of pestides residue in major river, Niger Delta, Nigeria, M.Sc. thesis submitted to the Department of Animal and Environmental Biology, University of Benin Nigeria,109pp.

21. Reid, S.G., Bernier, N.J. and Perry, S.F. (1998) The adrenergic stress response in fish: Control of catecholamine storage and release. *Comp. Biochem. Physiol*., **120**, 1–27.

22. Chrousos, G.P. (1998) Stressors, stress, and neuroendocrine integration of the adaptive response. *Ann. N.Y. Acad. Sci.*, **851**, 311–335.

23. Mommsen, T.P., Vijayan, M.M. and Moon, T.W. (1999) Cortisol in teleosts, dynamics, mechanisms of action, and metabolic regulation. *Rev. Fish Biol. Fish*., **9**,211–268.

24. Dorval, J., Leblond, V.S. and Hontela, A. (2003) Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. *Aquat Toxicol.,* **63**,229–241.

25. Dorval, J. and Hontela, A. (2003) Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*).*Toxicol Appl Pharmacol.,* **192**,191–200.

26. Luskova, V., Svoboda, M. and Kolarova, C. (2002) The effects of diazinon on blood plasma Biochemistry in carp, Cyprinus carpio. *Acta Vet.Brno.*, **71**, 117–123.

27. Sepici-Dinçel, A. Benli, A.Ç., Selvi, M., Sarıkaya, R., Şahin, D., Özkul, I.A. and Erkoç, F. (2009) Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: biochemical, hematological, histopathological alterations. *Ecotoxicol. Environ. Saf*., **72**,1433–1439.

28. Vosyliene, M.Z. (1999) The effects of heavy metals on haematological indices of fish. Act Zool. Lit. Hydro., **9,**76–82.

29. Kalender, Y., Kalender, S., Uzunhisarcikli, M., Ogutcu, A., Acikgoz, F. and Durak, D. (2004) Effects of endosulfan on B cells of Langerhans islets in rat pancreas. *Toxicology.,* **200**, 201–205.

30. Cicik, B. and Engin, K. (2005) The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L., 1758). *Turk. J. Vet. Anim. Sci.*, **29**,113–117.

31. Raja, M., Al-Fatah, A., Ali, M., Afzal, M., Hassan, R.A., Menon, M. and Dhami, M.S. (1992) Modification of liver and serum enzymes by Paraquat treatment in rabbits. *Drug Metab. Drug Inter*.,**10**,279–291

32. Omoregie,E.,Ufodike,E.B.and Keke, I.R. (1990) Tissue chemistry of *O. niloticus* exposed to sublethal concentrations of Gammalin 20 and Actellic 25EC. *J. of Aquatic Science*. **5,**33-36.

33. Das, B.K. and Mukherjee, S.C. (2003) Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. *Comp. Biochem. Physiol.*, **134**, 109–121.

34. Jee, J.H., Masroor, F. and Kang, J.C. (2005) Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquac. Res***.,** **36**, 898–905.

35. Yang, J.L. and Chen, H.C. (2003) Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere, **53,**877–882.

36. Jenkins, F. and Smith, J. (2003) Effects of sublethal concentration of endosulfan on haematological and serum biochemical parameters in the carp, *Cyprinus carpio*. *Bull. Environ.Contam. Toxicol*., **70**, 993–947.

37. Neff, J.M. (1985). Use of biochemical measurements to detect pollutant– mediated damage to fish. In Carwel, R.D., Purdy, R. Bahner, R.C. Eds. Aquatic toxicology and hazard assessment: Philadelphia: *America Society for Testing Material*, 155–181.