VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 516-523 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Haematological and Biochemical Parameters of Endosulfan Exposed Female Nile Tilapia *Oreochromis niloticus*

Rathika R.[1*], Bhuvaneshwari S.[2], Gowri Lakshmi S.[3], Srinivasan K.[1], Mahmoodah Parveen K.[4] and Suganthi P.[5]

[1]PG and Research Department of Zoology, Government Arts College, Affiliated to Periyar University, Dharmapuri 636705, Tamil Nadu, India
[2]Department of Zoology, Bishop Heber College (Autonomous), Tiruchirappalli 620017, Tamil Nadu, India
[3]Department of Zoology, PSG College of Arts and Science, Coimbatore - 641014. Tamil Nadu, India
[4]PG and Research, Department of Chemistry, Jamal Mohamed College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli – 620020, Tamil Nadu, India
[5]KIRND Institute of Research and Development Pvt. Ltd., Tiruchirappalli – 620020, Tamil Nadu, India

**Corresponding Author*

Received: 19th May, 2023; **Accepted:** 22nd July, 2023; **Published online:** 7th September, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.059

Abstract: Endosulfan is the most commonly used polychlorinated pesticide against leptoteran and dipteran insects in cotton, grains and vegetable cultivations. Three different concentrations of Endosulfan (120 ppm, 150 ppm and 180 ppm) toxicity was studied on freshwater adult female fish *Oreochromis niloticus* (n=6, 96 h). Erythrocytes, leucocytes, lymphocytes, haemoglobin and haematological indices (MCH, MCHC, MCV) were studied by standard procedures. Liver and muscle tissues biochemical (Total protein, Total glucose, Total cholesterol) and tissues damaging enzymes (AST and ALT) were analyzed. Triplicate results were statistically analyzed. Endosulfan treated *O. niloticus* hematological parameters revealed the significantly decreased blood cells and their indices than compared to the control fish. Decreased WBC and lymphocytes ($P<0.05$) with increased neutrophil in treated groups were due to the high impact stage in the tissues which resulted in the migration of phagocytic leucocytes to the affected tissues. Endosulfan treated fish biochemical levels were significantly ($P<0.01$) decreased due to cellular stress in liver and muscles. Increased tissue damaging enzyme indicated the high degree of cellular damages especially on the membrane system as well as altered cellular metabolism. Our study concluded that Endosulfan concentrations (120 ppm, 150 ppm and 180 ppm) showed toxic effect on the blood cells, liver and muscle tissues of freshwater female fish *Oreochromis niloticus*.

Keywords: *Oreochromis niloticus*, Endosulfan, Blood, Liver, Transaminase, Erythrocytes, Leucocytes, Lymphocytes, Haemoglobin

Citation: Rathika R., Bhuvaneshwari S., Gowri Lakshmi S., Srinivasan K., Mahmoodah Parveen K. and Suganthi P.: Haematological and biochemical parameters of endosulfan exposed female Nile tilapia *Oreochromis niloticus.* Intern. J. Zool. Invest. 9(2): 516-523, 2023.

https://doi.org/10.33745/ijzi.2023.v09i02.059

Introduction

The primary cause of the pollution is by commercial, household, and agricultural operations (Christobher *et al.*, 2016). For instance, each year, freshwater systems are exposed to nearly 300 billion kilograms of synthetic chemicals used in businesses and farming goods (Fernandes, 2011). Pesticides are used to prevent, reduce, eliminate, or limit the development of biological creatures that are not desirable. Sunlight (photodecomposition), elevated air or water temperature (thermal degradation), moisture, biological activity (microbial decay), and soil conditions can all cause pesticides to degrade. Some herbicides are more hazardous because they are accessible to aquatic creatures for a longer period of time and are therefore more persistent (long-lasting) (Kavita *et al.*, 2021). Pesticides are readily mobile and transformable, allowing them to travel from one ecosystem to another (degradation) (Stahlschmidt *et al.*, 2022).

Whiteflies, aphids, leafhoppers, Colorado potato beetles, and cabbage worms are among the insect pests that are controlled with the help of the polychlorinated xenoestrogen poisonous pesticide endosulfan, which also has a high mortality rate (Santosh Kumar, 2022). Endosulfan acts as an aromatase inhibitor and endocrine disruptor in humans, causing persistent convulsions, headache, vertigo, and ataxia through either deliberate or unintentional consumption (Martin and Arivoli, 2008; Morcillo *et al.*, 2017). Because of their widespread use, these chemicals may have toxic impacts on the environment, the public, industrial employees, and outdoor applicators (Suganthi *et al.*, 2015). The significant contamination of surface waterways by these contaminants has been well documented worldwide and is a significant problem on a local, regional, national, and international scale (Spalding *et al.*, 2012). Fish make up a significant portion of the world's food supply, it is important to watch and safeguard them (Dhara *et al.*, 2021).

The most popular animal model for toxicological, metabolic, and chromosomal research is the freshwater fish *Oreochromis niloticus* (Casas-Solis *et al.*, 2007). It demonstrated a variety of resilient traits with high toleration capacities for illness and environmental shifts (Giron-Perez *et al.*, 2008). Fish have blood that makes up between 1.3 and 7% of their body weight (Fazio *et al.*, 2013), and the haematological profile is frequently used as a marker of physiological stress responses in fish caused by endogenous or exogenous factors as well as being useful in determining the health status (Clauss *et al.*, 2008; Moosaviand Shamushaki, 2015; Stalin *et al.*, 2019). They react particularly to the quantity and kind of contaminants, fish biochemical markers were also used for tracking the aquatic environment and contamination (Glenn *et al.*, 2010; Suganthi and Sadiq Bukhari, 2016). This study examined the impact of endosulfan pesticides on the haematological andtissue biochemical characteristics of the female fish Nile tilapia *Oreochromis niloticus*.

Materials and Methods

Experimental animal and treatment:

Adult freshwater female fish *Oreochromis niloticus* (18.2±1.5 g) was collected locally from a nearby waterway and acclimated to the laboratory conditions for one week. During acclimation period fish were given an artificial fish food (ground nut oil cake and rice bran). Every day, refuse products like faeces and remaining food were siphoned off. After acclimation, fish were divided into four groups (3 experimental and 1 control group) each containing 6 female *Oreochromis niloticus*.

Pesticide endosulfan (5%W/V) was bought from a neighbourhood pest control store. Experimental groups of female *O. niloticus* were exposed to endosulfan (120, 150, and 180 ppm). The control group (n=6) was not exposed to pesticide. The leftover food and faeces were siphoned off every day. The fish were chosen at random for further examination after 96 h.

Collection of blood and tissue samples:

The cardinal vein's blood was drawn into eppendorf tubes (containing 0.1 ml of 2% EDTA) by using an insulin needle. After collection of blood, fish were dissected and tissues (liver and muscle) were collected from each group for further analysis of various parameters.

Haematological and biochemical analysis:

Standard techniques were used to analyse the RBC, WBC, lymphocytes, Hb, and haematological markers MCH, MCHC, and MCV of the control and treated fish (Chandrasekara and Pathiratne, 2005; Yonar, 2013; Ramesh *et al.*, 2017). Standard methods were used to analyse the amounts of tissue damaging enzyme transaminase (AST and ALT) as well as biochemical parameters (total protein, total cholesterol and total glucose) in liver and muscle tissues (Almeida *et al.*, 2001; Chowdhury *et al.* 2004; Bedi and Kenan 2005). All samples were estimated in triplicates. Using SPSS software (17.0), the triplicate results were examined for mean, SD and also to determine whether there was a difference between the control and treatment groups by ANOVA.

Results

Haematological parameters:

Endosulfan treated *O. niloticus* heamatological parameters (mean±SD) revealed the significantly varied blood cells and their indices as compared to the control fish. Decreased RBC count was noticed in endosulfan treated groups as 1.10±0.02 (120 ppm), 1.06±0.04 (150 ppm) and 1.00±0.09 (180 ppm) million cells/cu.mm as compared to control (1.41±0.05 million cells/cu.mm) (Table 1). The packed cell volume (PCV) of the treated fish decreased progressively (120 ppm- 7.33±1.21, 150 ppm - 6.66±1.36 and 180 ppm-5.50±1.87%) (Table 1) as compared to the control fish (8.50±0.83%). The mean cell volume (MCV) was 73.33±12.11 fl, 60.56±12.52 fl, 39.29±13.32 fl and mean cell haemoglobin (MCH) was 22.95±3.11, 23.63±4.83, 21.40±3.52 pg after treatment with endosulfan-- 120, 150, 180 ppm, respectively. When compared to the control, treated fish MCV were greatly reduced whereas MCH were significantly increased (Table 1). The MCHC levels were observed as 31.75±4.85, 40.95±14.78 and 63.41±34.74% for 120, 150 and 180 ppm endosulfan exposed fish, respectively.

Increased Hb levels were recorded as 2.29±0.31, 2.60±0.52 and 2.99±0.49 g/d for 120, 150 and 180 ppm endosulfan treated fish, respectively. Oxygen carrying capacity of the fish RBC were measured as 2.86±0.18, 3.25±0.00 and 3.73±0.00 mlO_2gHb for 120, 150 and 180 ppm endosulfan exposed fish, respectively (Table 1). Decreased WBC levels were observed as 48.11±0.001, 16.00±0.001 and 10.40±0.003 10^{-3}cells/cu. mm and lymphocyte levels were 98.66±0.51, 95.16±1.72 and 94.83±1.32% for 120, 150 and 180 ppm endosulfan treated fish respectively (Table 1). Increased neutrophils were observed in fish treated with endosulfan 120, 150 and 180 ppm as 2.450±0.42, 2.96±0.34 and 5.330±1.03%, respectively (Table1).

Biochemical parameters:

Various parameters such as biochemical (Total Protein, Total Glucose and Total Cholesterol), tissues damaging enzymes -- Aspartate transaminase and Alkaline transaminase) were analyzed in liver and muscle tissues of control and treated fish. In 120, 150 and 180 ppm endosulfan treated fish total protein levels were 0.70±0.01, 0.50±0.01 and 0.29±0.01 g/dl for liver tissues; and 0.40±0.01, 0.31±0.03 and 0.29±0.02 g/dl for muscle tissues, respectively (Fig. 1).

In 120, 150 and 180 ppm endosulfan treated fish liver, total glucose levels were 70.15±4.16, 44.21±0.14 and 29.87±0.79 mg/dl and cholesterol levels were 39.00±0.21, 29.33±2.05 and 22.18±1.39 mg/dl, respectively which showed decreased values as compared to control (Fig. 1). In 120, 150 and 180 ppm endosulfan treated fish muscle total glucose levels were 38.19±0.46, 31.99±0.35 and 25.06±0.22 mg/dl and cholesterol levels were 21.71±1.92, 18.09±0.71 and 10.16±0.23 mg/dl, respectively (Fig. 1). AST (GOT) and ALT (GOT) levels in liver and muscles were

Table 1: Haematological analysis (mean±SD) of control and endosulfan treated female fish *O. niloticus*

Parameters	Endosulfan treatment				P value
	Control	120 ppm	150 ppm	180 ppm	
Total RBC count (million cells/cu. mm)	1.41±0.05	1.10±0.02	1.06±0.04	1.00±0.09	0.39 ($P<0.05$)
PCV (%)	8.50±0.83	7.33±1.21	6.66±1.36	5.50±1.87	0.22 ($P<0.05$)
MCV (fl)	85.0±8.36	73.33±12.11	60.56±12.52	39.29±13.32	1.24 ($P<0.05$)
MCH (pg)	20.03±4.36	22.95±3.11	23.63±4.83	21.40±3.52	1.09 ($P<0.01$)
MCHC (%)	23.49±3.97	31.75±4.85	40.95±14.78	63.41±34.74	3.28 ($P<0.05$)
Haemoglobin (gm/dl)	2.00±0.43	2.29±0.31	2.60±0.52	2.99±0.49	1.11 ($P<0.05$)
Blood O_2 Carrying Capacity (mlO_2 gHb)	2.50±0.08	2.86±0.18	3.25±0.00	3.73±0.00	1.20 ($P<0.05$)
Total WBC count (10^{-3} cells/cu. mm)	71.67±0.001	48.11±0.001	16.00±0.001	10.40±0.003	2.00 ($P<0.05$)
Lymphocytes (%)	98.50±0.83	98.66±0.51	95.16±1.72	94.83±1.32	13.21 ($P<0.05$)
Neutrophils (%)	1.15±0.19	2.45±0.42	2.96±0.34	5.33±1.03	0.98 ($P<0.05$)

increased after exposure of the fish to 120, 150 and 180 ppm endosulfan (Fig. 1).

Discussion

Erythropoietin, which is made in the kidney, controls the erythropoietic activity in the majority of vertebrates, including fish (Gluszak *et al.*, 2006). By encouraging hemopoietic stem cells to develop into erythroblasts, erythropoietin further encourages erythropoiesis which resulted in the increased RBC during stress conditions. According to Saha and Kaviraj (2009), the action of pesticide on the freshwater catfish *Heteropneustes fossilis* blood-forming organ may be the cause of the drop in Hct in fish exposed to pesticides. This decrease in Hct was caused by a decrease in RBC count. Sub-lethal lindane exposure to freshwater teleost *Cyprinus carpio* resulted in a declining trend in RBC, Hb, and Hct values (Ural, 2013).

The condition of erythrocytes and other components required for the synthesis of haemoglobin can be determined by erythrocyte count and other red cell markers, which were used in toxicological assessments of fish (Ramesh and Saravanan, 2008; Oner *et al.*, 2008; Nwani *et al.*, 2013). Congenital sphaerocyteosis causes changes in the MCHC in fish, which serve as a marker of haemoglobin variants in the blood (Sarma, 1990; Sobecka, 2001). Hypochronic mycrocytic anaemia is thought to be indicated by an increase in MCHC and a reduction of MCV (Prusty *et al.*, 2011). According to Gallaugher and Farrell (1998), the haematocrit number, which reflects the blood's ability to transport oxygen, is related to the oxygen intake. A lower haemoglobin count indicates a drop in the production of haemoglobin. Fish with higher haematocrit levels have greater erythrocyte lysis rates as well (Martinez and Souza, 2002).

Moosavi and Shamushaki (2015) reported the acute toxicity of nickel to the freshwater fish *Carassius auratus* and noticed decreased white

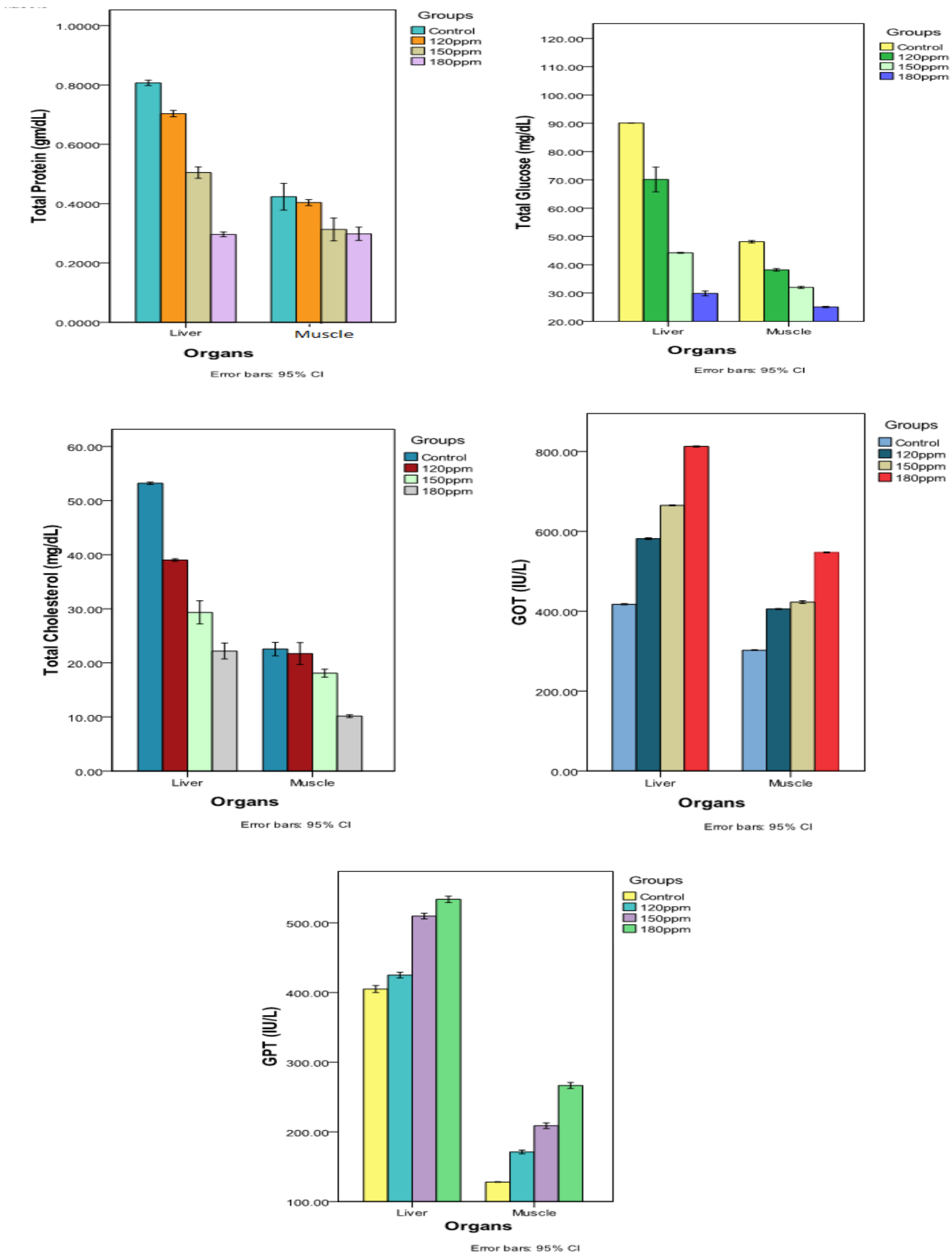

Fig. 1: Biochemical and Transaminase enzymes analysis in liver and muscle of endosulfan treated fish.

blood cell (WBC) count, haemoglobin (Hb), and hematocrit (Hct). Nickel-treated groups significantly outperformed the control group in terms of red blood cell (RBC) count, mean corpuscular haemoglobin (MCH), cortisol, and glucose levels, but significant differences in mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were not observed.

The current study also noted alterations in blood cell indices including MCH, MCH, MCHC and MCV. This might be as a result of the fact that they have a high sensitivity and can alter fish homeostasis irreversibly. RBC count, hemoglobin concentration, and packed cell volume changes are directly correlated with changes in these indicators.

Long-term (4 weeks) exposure of endosulfan (6.72 ppb) caused alterations in blood and organ chemistry parameters of the fish *Barbus conchonius*. Total lipids, cholesterol and protein levels in blood were reduced compared to unexposed controls (Gill *et al.*, 1991). Reduced glucose and cholesterol levels in the pesticide treated freshwater fish organs were reported by Neeraj Kumar *et al.* (2011), Rahman *et al.* (2020) and Dawood *et al.* (2020).

Protein reduction may be brought on by protein synthesis inhibition, protein denaturation, or a break in the production of amino acids. Additionally, the reduced protein levels in fish exposed to chlorpyrifos may be caused by a decrease in feed intake and subsequent degradation of these molecules as energy substrates to handle chlorpyrifos-induced stress (Cheema *et al.*, 2014).

In this study liver function, liver enzymes, and plasma proteins were all adversely affected by endosulfan. Fish treated with pesticides showed significant increases in serum transaminase levels. Serum ALT and AST levels are increased due to oxidative stress induced by pesticides treatment, which destroys the cell membrane of hepatocytes and releases these enzymes into the blood. ALT and AST levels showed significant increases in fish treated with pesticides profenofos (Rahman *et al.*, 2020).

Based on the lethal concentrations studies (results not included in this work), three different concentrations were used in this study. Méndez-Rivera (2022) reported the median lethal concentration (96 h) for β-endosulfan for the fish *I. pseudopuma* as 123.6 μg/l. Younis *et al.* (2012) reported significantly increased GOT and GST levels in zinc treated *O. niloticus* with short and long term sublethal exposure which indicates hepatic damage due to zinc accumulation which in turn releases these enzymes into the bloodstream.

The rates of the tissue-damaging enzymes GOT, GPT, ALP, ACP, G6PHD, and LDH significantly elevated in *O. mossambicus* after paracetamol exposure (24 h) (Kavitha *et al.*, 2011). Enzymes involved in the breakdown of amino acids include AST and ALT. Significantly increased ALP levels may be a sign of both renal and liver injury. An increase in AST levels has been shown to represent liver damage (Gill *et al.*, 1990; Bhattacharya *et al.*, 2005). Similar findings were also observed in serum of *C. carpio, Oreochromis niloticus* exposed to endosulfan, Lufenuron, Deltamethrin (Hussein *et al.*, 2019; Dawood *et al.*, 2020; Ghelichpour *et al.*, 2020).

Conclusion

It is concluded that Endosulfan (120 ppm, 150 ppm and 180 ppm) treatment caused toxic effect on the liver and muscle tissues of freshwater female fish *Oreochromis niloticus*. Treated fish tissues showed significantly reduced macro biomolecules (protein, glucose and cholesterol) which resulted in the altered cellular metabolism. Similarly, increased tissue damaging enzymes also revealed the endosulfan impact on fish tissues.

Acknowledgements

Authors are thankful to the authorities at PG and Research Department of Zoology and Botany, Government Arts College, Dharmapuri for the support during this study.

References

Almeida JA, Novelli EL, Das PSM and Junior RA. (2001) Environmental cadmium exposure and metabolic responses of Nile tilapia, *Oreochromis niloticus*. Environ Poll. 114(2): 169-175.

Attaullah M, Yousuf MJ, Amin M, Buneri ID, Rahim A, Anjum SI and Ilahi I. (2019) Endosulfan concentrations in association with serum biochemical parameters and risk of cancer. Appl Ecol Environ Res. 17: 5235-5244.

Bedi C and Kenan E. (2005) The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio*. Turk J Vet Anim Sci. 29: 113-117.

Bhattacharya H, Lun L and Gomez RGD. (2005) Biochemical effects to toxicity of CCl_4 on Rosy Barbs (*Puntius conchonius*). Our Nat. 3: 20-25.

Chandrasekara HU and Pathiratne A. (2005) Influence of low concentrations of Trichlorfon on haematological parameters and brain acetylcholinesterase activity in common carp, *Cyprinus carpio* L. Aquat Res. 36: 144-149.

Cheema N, Bhatnagar,A and Yadav AS. (2014) Changes in growth performance and biochemical status of fresh water fish *Cirrhinus mrigala* exposed to sublethal doses of chlorpyrifos. Int J Agricult Food Sci Technol. 2: 619-630.

Chowdhury MJ, Pane EF and Wood CM. (2004) Physiological effects of dietary calcium acclimation and waterborne cadmium challenge in rainbow trout: respiratory, ionoregulatory and stress parameters. Comp Biochem Physiol C Toxicol Pharmacol. 139(1-3): 163-173.

Christobher S, Periyasamy M, Suganthi P and Saravanan TS. (2016) Effect of phosphamidon on haematological and biochemical parameters in freshwater fish *Labeorohita*. Int J Fauna Biol Stud. 3(1): 114-116.

Clauss TM, Dove AD and Arnold JE. (2008) Hematologic disorders of fish. Vet Clin North Am Exot Anim Pract. 11(3):445-462.

Dawood MA, AbdEl-Kader MF, Moustafa EM, Gewaily MS, Abdo SE. (2020) Growth performance and hemato-immunological responses of Nile tilapia (*Oreochromis niloticus*) exposed to deltamethrin and fed immunobiotics. Environ Sci Pollut Res. 2020:1-10

Dhara K, Saha S, Mukherjee D and Saha NC. (2021) Comparative acute toxicity of mercury to air breathing fish, *Channa gachua* (Ham.) and non-air breathing fish *Cyprinus carpio* (Linn.): Ethological and haematological consideration. Ind J Ecol. 48(5): 1243-1253.

Fernandes VC, Domingues CF, Delerue-Matos C and Mateus N. (2011) Determination of pesticides in fruit and fruit juices by chromatographic methods. An overview. J Chrom Sci. 49(9):715-730.

Gallaugher P, Thorarensen H, Kiessling A and Farrell AP. (2001) Effects of high intensity exercise training on cardiovascular function, oxygen uptake, internal oxygen transport and osmotic balance in chinook salmon (*Oncorhynchus tshawytscha*) during critical speed swimming. J Exp Biol. 204: 2861-2872.

Ghelichpour M, Mirghaed AT, Hoseini SM and Jimenez AP. (2020) Plasma antioxidant and hepatic enzymes activity, thyroid hormones alterations and health status of liver tissue in common carp (*Cyprinus carpio*) exposed to lufenuron. Aquaculture 2020; 516.

Gill TS, Pande J and Tewari H. (1991) Effects of endosulfan on the blood and organ chemistry of freshwater fish, *Barbus conchonius* Hamilton. Ecotoxicol Environ Saf. 21(1): 80-91.

Gill TS, Tewari H and Pande J. (1990) Use of the fish enzyme system in monitoring water quality: effects of mercury on tissue enzymes. Comp Biochem Physiol C Comp Pharmacol Toxicol. 97(2): 287-292.

Glenn JL, John AM and Clive WE. (2010) Evaluating the impact of environmental pollution on fish in McMurdo Sound, Antarctica: A biomarker approach. Polar Sci. 3(4): 246-253.

Gluszak L, Santos MD, Crestani M, da Fonseca MB, de Araujo Pedron F, Duarte MF and Vieira VLP. (2006) Effect of glyphosate herbicide on acetylcholinestrase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). Ecotoxicol Environ Saf. 6: 237-241.

Hussein MM, Elsadaawy HA, El-Murr A, Ahmed MM, Bedawy AM, Tukur HA, Swelum AAA and Saadeldin IM. (2019) Endosulfan toxicity in Nile tilapia (*Oreochromis niloticus*) and the use of lycopene as an ameliorative agent. Comp Biochem Physiol C Toxicol Pharmacol. 224: 108573.

Kavita G, Shaila K, Mansi P, Aishwarya M, Niraj K, Aaditee C, Pallavi S and Shreya I. (2021) Exposure risk and environmental impacts of glyphosate: Highlights on the toxicity of herbicide co-formulants. Environ Challen. 4: 100-149.

Kavitha P, Ramesh R, Bupesh G, Stalin A and Subramanian P. (2011) Hepatoprotective activity of *Tribulus terrestris* extract against acetaminophen-induced toxicity in a freshwater fish (*Oreochromis mossambicus*). In Vitro Cell Develop Biol Ani. 47(10): 698-706.

Martin PDP and Arivoli S. (2008) Biochemical study of freshwater fish *Catla catla* with reference to mercuric chloride. Iran J Environ Health Sci Eng. 5(2): 109-116.

Martinez CBR and Souza MM. (2002) Acute effects of nitrite on ion regulation in two neotropical fish species. Comp Biochem Physiol A 133: 151-160.

Méndez-Rivera M, Freylan M, Margaret P-B, Clemens R, Marco DB, Randall RJ and Gilbert A. (2022) Effects of the insecticide β-endosulfan on tadpoles of *Isthmohyla pseudopuma* (Anura: Hylidae), Aquat Toxicol. 250: 106231.

Moosavi MJ and Shamushaki VAJ. (2015) Effect of sub-acute exposure to nickel on hematological and biochemical indices in gold fish (*Carassius auratus*). J Clin Toxicol. 5: 228.

Morcillo P, Esteban MA and Cuesta A. (2017) Mercury and its toxic effects on fish. Toxicol in vitro (3): 386-402.

Neeraj Kumar, Antony Jesu Prabhu P, Pal AK, Remya S, Md. Aklakur, Rana RS, Gupta Subodh, Raman RP and Jadhao SB. (2011) Anti-oxidative and immunohematological status of Tilapia (*Oreochromis mossambicus*) during acute toxicity test of endosulfan, Pesticide Biochem Physiol. 99(1): 45-52.

Nwani CD, Ama UI, Okoh F, Oji UO, Ogbonyealu RC, Ibiam AA and Udu-Ibiam O. (2013) Acute toxicity of the chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zillii*. Afr J Biotechnol. 12(5): 499-503.

Oner M, Atli G and Canli M. (2008) Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. Environ Toxicol Chem. 27(2): 360-366.

Prusty AK, Kohli MPS, Sahu NP, Pal AK, Saharan N, Mohapatra S and Gupta SK. (2011) Effect of short term exposure of fenvalerate on biochemical and haematological responses in *Labeo rohita* (Hamilton) fingerlings. Pesticide Biochem Physiol. 100: 124-129.

Rahman ANA, Mohamed AAR, Mohammed HH, Elseddawy NM, Salem GA and El-Ghareeb WR. (2020) The ameliorative role of geranium (*Pelargonium graveolens*) essential oil against hepato-renal toxicity, immunosuppression, and oxidative stress of profenofos in common carp, *Cyprinus carpio* (L.). Aquaculture 517: 734777.

Ramesh M and Saravanan M. (2008) Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. Int J Integ Biol. 3(1): 80-83.

Ramesh M, Anitha S, Poopal RK and Shobana C. (2017) Evaluation of acute and sublethal effects of chloroquine ($C_{18}H_{26}ClN_3$) on certain enzymological and histopathological biomarker responses of a freshwater fish *Cyprinus carpio*. Toxicol Rep. 5: 18-27.

Saha S and Kaviraj A. (2009) Effects of cypermethrin on some biochemical parameters and its amelioration through supplementation of ascorbic acid in freshwater catfish *Heteropneustes fossilis*. Chemosphere 74: 1254-1259.

Santosh Kumar K, Aditya U and Awanish K. (2022) Biomonitoring of endosulfan toxicity in human. Biocell 46(7): 1771-1777.

Sarma PR. (1990) Red Cell Indices. In: Clinical Methods: The History, Physical, and Laboratory Examinations, (eds.) Walker H.K., Hall W.D., Hurst J.W., 3rd ed. Boston: Butterworths, pp. 720-723.

Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U and Wehrli B. (2006) The challenge of micropollutants in aquatic systems. Science 313(5790): 1072-1077.

Sobecka E. (2001) Changes in the iron level in the organs and tissues of wels catfish, *Silurus glanis* L. caused by nickel. ActaIchthy Pisca. 31: 127-143.

Spalding MD, Agostini VN, Rice J and Grant SM. (2012) Pelagic provinces of the world: a biogeographic classification of the world's surface pelagic waters. Ocean Coast Manag. 60: 19-30.

Stahlschmidt ZR, Whitlock J, Vo C, Evalen P and Bui D. (2022) Pesticides in a warmer world: Effects of glyphosate and warming across insect life stages. Environ Poll. 307: 119508.

Stalin A, Suganthi P, Mathivani S, Paray BA, Al-Sadoon MK, Gokula V and Saiyad Musthafa M. (2019) Impact of chlorpyrifos on behaviour and histopathological indices in different tissues of freshwater fish *Channa punctatus* (Bloch). Environ Sci Poll Res. 26(17): 17623-17631.

Suganthi P and Sadiq Bukhari A. (2016) Effect of CuO nanoparticles on haematological parameters of *Oreochromis mossambicus*. J Sci Technol Invest. 1(1): 18-27.

Suganthi P, Soundarya N, Stalin A and Nedunchezhiyan S. (2015) Toxicological effect of cobalt chloride on freshwater fish *Oreochromis mossambicus*. Int J Appl Res. 1(3): 331-340.

Ural MS. (2013) Chlorpyrifos-induced changes in oxidant/antioxidant status and haematological parameters of *Cyprinus carpio* ameliorative effect of lycopene. Chemosphere 90: 2059-2064.

Yonar SM. (2013) Toxic effects of malathion in carp, *Cyprinus carpio carpio*: protective role of lycopene. Ecotoxicol Environ Saf. 97: 223-229.

Younis EM, Abdel-Warith AA and Al-Asgah NA. (2012) Hematological and enzymatic responses of Nile tilapia *Oreochromis niloticus* during short and long term sublethal exposure to zinc. Afr J Biotech. 11(19): 4442-4446.