WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 1, 1072-1084 Research Article ISSN 2278 – 4357

ENZYMATIC ACTIVITY INDUCED BY GLYPHOSATE HERBICIDE ON THE FRESHWATER FISH *CHANNA PUNCTATUS* (BLOCH)

Anamika Singh[1]* and Ajay Singh[2]

[1*]Research scholar, Natural Product Laboratory Department of Zoology D.D.U. Gorakhpur University Gorakhpur-273009 (U.P.) India.

[2]Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur- 273009 (U.P.) India.

Article Received on
10 November 2018,

Revised on 01 Dec. 2018,
Accepted on 22 Dec. 2018,

DOI: 10.20959/wjpps20191-12938

***Corresponding Author**
Anamika Singh
Research Scholar, Natural Product Laboratory Department of Zoology D.D.U. Gorakhpur University Gorakhpur-273009 (U.P.) India.

ABSTRACT

Fishes are very sensitive to aquatic pollution as they exposed directly to toxicants discharged from different sources to water bodies. They are good bio-indicator for assessing the effect of toxicant present in water bodies. Unfortunately, the use & abuse of herbicide has surfaced that it not only lethal to target but also sub lethal to non-target aquatic organism. Accumulation of toxic substances in body of aquatic organism leads to physiological and metabolic disorder. Glyphosate at low concentration damage liver, muscle, kidney and skin cells. There was a significant ($P<0.05$) negative correlation between LC value of glyphosate and exposure period. *In vivo* exposure of fish *Channa punctatus* with sub lethal doses (40% and 80% of LC_{50} of 24h) of glyphosate for 24h or 96h, significantly alter the enzyme activity like AChE, Acid and Alkaline phosphstses, Lactic dehydrogenase, Transaminases (GOT & GPT) was also time and dose dependent manner.

KEYWORDS: Glyphosate, *Channa punctatus*, Herbicide, Lactic dehydrogenase, Transaminases, Phosphatases, Acetylcholinesterase.

INTRODUCTION

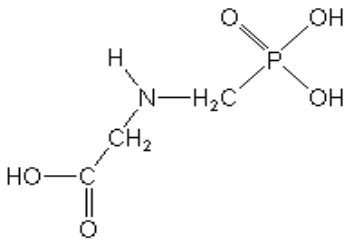
Water is the main source of living being but after industrial revolutions water bodies have been polluted by a number of pollutants including herbicides which are excessively used in the control of great variety of unwanted weeds in the agricultural field and in aquatic environment. Among the pollutants, the herbicides have been recognized as one of the major serious pollutants of the aquatic environment with deleterious effects on the living resources.

The indiscriminate use of herbicides or careless handling, accidental spillage, discharges of many other treated effluents into natural water bodies have major harmful impact on the fish population .[1, 2] According to Nehls and Segner [3] also reported that the low concentration of herbicides can't cause immediate detectable effects of aquatic organisms but, in long term exposure of herbicide can reduces their lifespan longevity. And impacts of herbicides on aquatic ecosystem depend on its mode of action, concentration as well as its own toxicity.[4] Fishes act as bio- indicators of water pollutants like in aquatic ecosystem along with this, it also occupy secondary trophic level position in food chain. Fishes are very important dietary animals for protein source in human nutrition. According to Holt *et al*.,[5] glyphosate is an organophosphorous, non-selective herbicide which play a significant role in management of unwanted weeds from agricultural field. Its action occurs by inhibition of the biosynthesis of aromatic amino acids of plants, animals and *in vitro* human lymphocytes.[6,7] The accumulation of organophosphate based herbicides in aquatic organisms (fishes) altered several physiological and metabolic enzymatic pathways in different fishes.[8,9,10,11,12,13,14] The objective of this study was to observe & analyse the effect of glyphosate herbicide (Excel Mera 71) on activity of enzymes viz. acid & alkaline phosphatase, AChE, lactic dehydrogenase and transaminases (GOT & GPT) in liver & muscle of *Channa punctatus* (Bloch).

MATERIALS AND METHODS

Chemical

Glyphosate is the isopropyl amine salt of N-(Phosphonomethyl) –glycine, glyphosate is a very effective non-selective herbicides and it is only herbicide that acts by blocking the Shikimate pathway through inhibition of S-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). The commonly used glyphosate formulations in India are – Glycel, Excel Mera 71 & Veggru glyphosate. Molecular Structure: (C3H8NO5P), Molecular weight =169.09, Chemical group – Phosphinic acid, Chemical name – N-(Phosphonomethyl) glycine.



GLYPHOSATE

Experimented Animal

The experimental fish *Channa punctatus* (Bloch) belong to the Family: Channidae, Order: Perciformes were collected from different areas of Gorakhpur district (U.P.) India. The specimens had an average weight and length of 160 to 200g and 10 to 15 cm, respectively. Fish are belonging to both the sexes were used. Fish were stored in 45L capacity of glass aquaria containing de-chlorinated tap water for acclimatization under laboratory conditions for 3 week and fed with commercial fish food. The dead animals (if any) were removed as soon as possible to avoid water fouling and contamination. The physico-chemical parameter of test water was measured in the beginning of experiment by the standard method of APHA/AWWA/WPCF.[15]

Channa punctatus

Experimental designs

After proper acclimatization of fishes in laboratory conditions for 3 weeks were subjected with 40% & 80% of LC_{50} value of 24h & 96h of glyphosate with six replica of each dose in 20 fishes containing 12L de-chlorinated tap water. The liver along with muscle tissue of fishes was dissected in ice tray and was used for biochemical analysis. Some biochemical analysis steps were taken for 6 replicas of control groups also.

Phospatases (acid & alkaline) activities

Estimation of the activities of acid and alkaline phosphatase was performed by the method of Anderson and Szcypinski.[16] Homogenates (2%, w/v) were preparing in ice-cold 0.9% NaCl solution and centrifuged at 5000×g at 0^{o} C for 15 min.

Acetylcholinesterase (AChE) activies

Estimation of Acetylcholinesterase (AChE) activities was determined by the methods of Ellman *et al*., [17]. Homogenates (50 mg/ml) were preparing in 0.1 M phosphate buffer, pH8.0, for 5 min in an ice bath and centrifuged at 1000 × g for 30 min at -4^{o} C.

Lactic dehydrogenase (LDH) activities

A lactic dehydrogenase (LDH) activity was determined by the method of Anon.[18] Homogenates (50 mg/l, w/v) were made in 0.1 M Phosphate buffer (pH 7.5) for 5 min at 4^{o} C and the supernatant was taken as enzyme source.

Transaminases (GOT & GPT) activities

Transaminases (GOT & GPT) activities was determined by the method of Reitman and Frankel.[19] Homogenates (50 mg/ml, w/v) were prepared in phosphate buffer for 5 min and centrifuged at 1000 × g for 15 min and supernatant was kept for estimation of enzyme activity.

Statistical method

Two way ANOVA and student's't' test was applied between control and treated groups for significant differences Sokal and Rohlf.[20]

RESULTS

Physico-Chemical Parameters of the test water

The physico-chemical parameter of the test water is (temperature = 22-25^{o} C, pH = 7.2-7.4, alkalinity = 130-150 mg/l $CaCo_3$, DO = 6.5-7.3 mg/l, free carbon dioxide = 4.4-6.4 mg/l) measured in the beginning of experiment.

Effect on behavioural changes and poisoning symptoms

Fish is directly affected to different concentration of Glyphosate herbicide. Herbicide induced different type of toxicity and it shows various symptoms and uncoordinated behavior. After 15 min. of treatment all experimental fishes were alert stopped swimming and remain static in position in response to the sudden changes in the surrounding environment and reduced their ability to feed. After some time they tried to avoid toxicants with fast swimming and jumping, and symptoms of toxicosis observed in fish behavior with glyphosate include lack of balance; erratic swimming, air gulping, excessive secretion of mucus, rolling movement, and the treated fish became very weak, settled at the bottom and died and the skin colour was

shining that's why the behavioural changes or any mortality and colour of the control fishes was normal. The toxicity of gyphosate was time & dose dependent. There was a significant negative correlation between LC values and exposure periods.

Estimation of enzymatic activity

Exposure of sub-lethal doses of glyphosate (40% and 80% of LC_{50} of 24h) at 24h or 96h against the fresh water fish *Channa punctatus* caused significant ($p<0.05$) decrease in acid & alkaline phosphatase, AChE where as LDH, GOT & GPT activities enhanced in fishes respectively (Table 1, 2 & Figure 1, 2). After exposure of glyphosate 40% of LC_{50} shows that the level of acid phosphatase activity is depleted 87% in liver and 81% in muscle at 24h, 82% in liver and 75% in muscle tissue at 96h. Similarly the level of alkaline phosphatase activity reduces 85% and 77% in both liver and muscle tissue at 24h, and 78% in liver & 68% in muscle tissue at 96h. AChE activity also show the decreasment level 80% in liver and 71% in muscle tissue at 24h and it progressively decrease 70% and 73% in liver and muscle tissue at 96h. While the lactic dehydrogenase activity induced 117% and 111% in liver and muscle tissue at 24h and 124% in liver and 121% in muscle tissue at 96h. The transaminase activity shows increases level such as GOT becomes126% and 121% in liver and muscle tissue at 24h, and 135% in liver and 128% in muscle tissue at 96h respectively. There was increase in percentage level of GPT 119% and 129% in liver along with 118% and 127% in muscle tissue at 24h & 96h respectively.

After exposure of 80% of $LC_{50,}$ similar pattern for percentage activity of acid phosphatase reduced to 83% (liver) and 74% (muscle) at 24h with further decrease in activity from 80% (liver) and 71% (muscle) at 96h. Similarly the level of alkaline phosphatase activity reduces 80% and 74% in both liver and muscle tissue at 24h, and 70% in liver and 64% in muscle tissue at 96h. AChE activity also show the decreasment level 76% in liver and 68% in muscle tissue at 24h and it progressively decreases 63% and 59% in liver and muscle tissue at 96h.While the lactic dehydrogenase activity induced 119% and 116% in liver and muscle tissue at 24h and 132% in liver and 128% in muscle tissue at 96h. The transaminase activity shows increases level such as GOT becomes 132% and 125% in liver and muscle tissue at 24h, and 141% in liver and 133% in muscle tissues at 96h respectively. There was increase in percentage level of GPT 126% and 134% in liver along with 121% and 130% in muscle at 24h and 96h respectively.

Table 1: Changes in enzymatic activity of acid and alkaline phosphatase (µmol P – nitrophenyl substrate hydrolysed/30 min/mg protein), AChE (µmol 'SH' hydrolysed/min/mg protein), GOT and GPT (µmoles pyruvate/mg protein/h), LDH (pyruvate reduced/min/mg protein) in liver & muscle tissue of fish *Channa punctatus* exposed to 40% and 80% of LC_{50} of 24h of glyphosate after 24h.

Parameter	Tissues	Control (%age activity)	(40% of LC_{50})*£ (%age activity)	(80% of LC_{50}) *£ (%age activity)
Acid Phosphatase	Liver	1.304±0.0006 (100)	1.134±0.0005 (87)	1.084±0.0006 (83)
	Muscle	1.293±0.0007 (100)	1.043±0.0006 (81)	0.962±0.0005 (74)
Alkaline phosphatase	Liver	1.224±0.0006 (100)	1.041±0.0004 (85)	0.984±0.0003 (80)
	Muscle	1.051±0.0004 (100)	0.810±0.0004 (77)	0.781±0.0005 (74)
AChE	Liver	0.931±0.0006 (100)	0.741±0.0003 (80)	0.703±0.0005 (76)
	Muscle	0.912±0.0004 (100)	0.652±0.0003 (71)	0.622±0.0007 (68)
GOT	Liver	0.513±0.0004 (100)	0.647±0.0002 (126)	0.677±0.0003 (132)
	Muscle	0.467±0.0006 (100)	0.567±0.0003 (121)	0.582±0.0005 (125)
GPT	Liver	0.446±0.0004 (100)	0.532±0.0006 (119)	0.562±0.0004 (126)
	Muscle	0.367±0.0003 (100)	0.432±0.0007 (118)	0.443±0.0005 (121)
LDH	Liver	1.161±0.0004 (100)	1.355±0.0008 (117)	1.385±0.0004 (119)
	Muscle	1.104±0.0004 (100)	1.224±0.0005 (111)	1.283±0.0004 (116)

- Values are mean ± SE of six replicates.
- ***** Significant ($p<0.05$) when two way ANOVA was applied between control and treated groups, to see whether enzyme activity alteration was time and dose dependent.
- £ Significant ($p<0.05$) when student's't' test was applied between control and treated groups.
- Values in parenthesis are % activity of enzyme with control taken as 100%.

Table 2: Changes in enzymatic activity of acid and alkaline phosphatase (µmol P – nitrophenyl substrate hydrolysed/30 min/mg protein), AChE (µmol 'SH' hydrolysed/min/mg protein), GOT and GPT (µmoles pyruvate/mg protein/h), LDH (pyruvate reduced/min/mg protein) in different tissues of fresh water fish *Channa punctatus* exposed to 40% and 80% of LC_{50} of 24h of glyphosate after 96h.

Parameter	Tissues	Control	(40% of LC_{50}) *£	(80% of LC_{50}) *£
Acid Phosphatase	Liver	1.304±0.0006 (100)	1.072±0.0005 (82)	1.011±0.0006 (78)
	Muscle	1.293±0.0007 (100)	0.974±0.0004 (75)	0.921±0.0004 (71)
Alkaline Phosphatase	Liver	1.224±0.0006 (100)	0.952±0.0004 (78)	0.861±0.0005 (70)
	Muscle	1.051±0.0004 (100)	0.715±0.0008 (68)	0.672±0.0004 (64)
AChE	Liver	0.931±0.0006 (100)	0.656±0.0004 (70)	0.627±0.0005 (63)
	Muscle	0.912±0.0004 (100)	0.573±0.0003 (73)	0.536±0.0005 (59)
GOT	Liver	0.513±0.0004 (100)	0.693±0.0004 (135)	0.724±0.0004 (141)
	Muscle	0.467±0.0006 (100)	0.596±0.0004 (128)	0.622±0.0007 (133)
GPT	Liver	0.446±0.0004 (100)	0.575±0.0007 (129)	0.597±0.0003 (134)
	Muscle	0.367±0.0003 (100)	0.466±0.0004 (127)	0.477±0.0005 (130)
LDH	Liver	1.161±0.0004 (100)	1.445±0.0007 (124)	1.532±0.0005 (132)
	Muscle	1.104±0.0004 (100)	1.337±0.0003 (121)	1.411±0.0003 (128)

- Values are mean ± SE of six replicates.
- Significant ($p<0.05$) when two way ANOVA was applied between control and treated groups, to see whether enzyme activity alteration was time and dose dependent.
- £ Significant ($p<0.05$) when student's't' test was applied between control and treated groups.
- Values in parenthesis are % activity of enzyme with control taken as 100%.

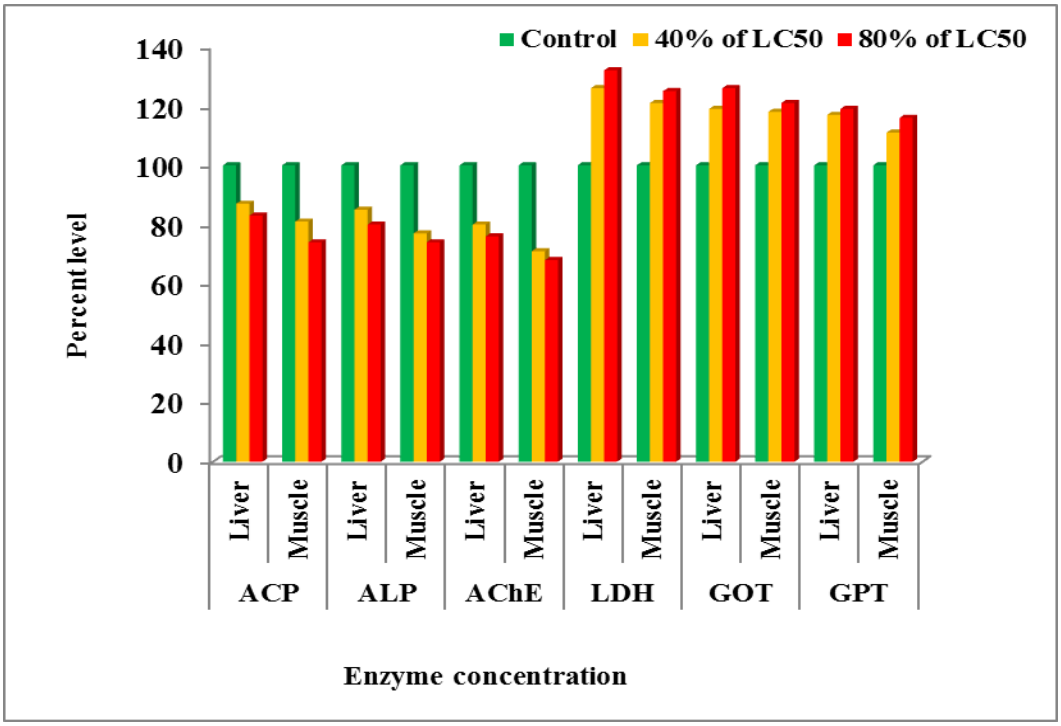


Figure 1: Changes in enzymatic activity of acid and alkaline phosphatase (µmol P – nitrophenyl substrate hydrolysed/30 min/mg protein), AChE (µmol 'SH' hydrolysed/min/mg protein), GOT and GPT (µmoles pyruvate/mg protein/h), LDH (pyruvate reduced/min/mg protein) in liver & muscle tissue of fish *Channa punctatus* exposed to 40% and 80% of LC_{50} of 24h of glyphosate after 24h.

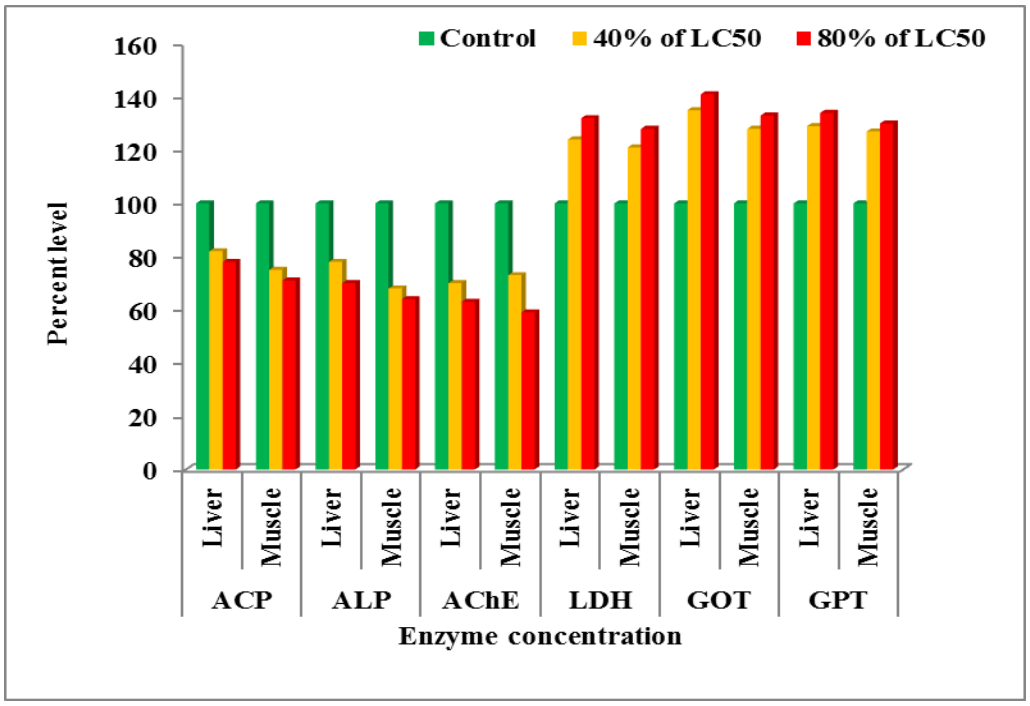


Figure 2: Changes in enzymatic activity of acid and alkaline phosphatase (µmol P – nitrophenyl substrate hydrolysed/30 min/mg protein), AChE (µmol 'SH' hydrolysed/min/mg protein), GOT and GPT (µmoles pyruvate/mg protein/h), LDH (pyruvate reduced/min/mg protein) in liver & muscle tissue of fish *Channa punctatus* exposed to 40% and 80% of LC_{50} of 24h of glyphosate after 96h.

DISCUSSION

Channa punctatus fish is a bottom dweller fish it is highly sensitive to aquatic system where the water gets contaminated with toxic chemicals. Aquatic ecosystem are mainly polluted by industrial waste, domestic sewage and different type of pesticides used in agricultural areas. In present investigation "Excel Mera 71" is a glyphosate based herbicide, and it has been reported that the exposure of glyphosate on fishes shows the symptoms of toxicant and behavioural alteration i.e. erratic swimming, air gulping, rolling movement, difficulty in respiration, excessive secretion of mucus, change in colour and changed in enzymatic activities. According to Byrene and O' Halloran,[21] the behavioural changes of fishes indicate environmental stress which affects survival. The change in behavior of non-migratory species of fishes provide index for ecosystem assessment. Some authors reported that interruption in the schooling behaviour of fish such as erratic and irregular movements, and disturbed swimming have been observed due to inhibition in activity of acetyl cholinesterase in *clarias batrachus* under exposure of propiconazole and mancozeb,[22] and exposure of glyphosate on fresh water fish *Channa punctatus.*[2] Enzymes play an important role in metabolism, and the changes in enzymatic systems may alter the metabolic processes. The significant increase in concentration & exposure time of herbicide revealed that there was decrease in activity of enzymes (acid & alkaline phosphatase and AChE) but on the contrary there was increase in the activity of enzymes (GOT, GPT & LDH) of *Channa punctatus.* According to Abou – Dania[23] also reported that the Acid phosphatase & alkaline phosphatase is hydrolytic lysosomal enzymes which are helpful in detoxification of toxicants by catabolism, pathological, necrosis, cytolosis & phagocytosis. Activity of acid phosphatase decreases may be due to histopathological changes such as necrosis, and it may be due to decrease in the rate of trans phosphorylation. Acetylcholinesterase is a serine protease that hydrolyses the neurotransmitter acetylcholine. AChE is mainly present at neuromuscular junctions and brain synapse, where its activity terminates synaptic transmission. Decreased AChE activity cause central and peripheral nervous system disorder and death this enzyme are responsible for modulating neural communication in the synaptic cleft by hydrolyzing the ubiquitous neuro-transmitter acetylcholine. According to several authors analyzed the decreased acetylcholinesterase activity inhibited the brain, liver, muscle and gill tissue of hybrid catfish *Clarias macrocephalus* and *Clarias gariepinus* exposed to sub-lethal concentration of an organophosphate, cypermethrin exposure in different fishes.[24] Exposure of diazinon in snakehead fish "*Channa striata*" integrated use of biomarkers in *Mytilus galloprovincialis* & *Mullus barbatus,*[25] effect of combination pesticide on *Danio rerio,*[26] organophosphate

pesticides, methylparation and chlorpyrifos on *Aphanius dispar*.[27] Effect of glyphosate in different fishes like *Leporinus obtusidens, Rhamdia queten, Prochilodus lineatus* and *Cyprinus carpio*.[28,29,30,31] The activity of enzyme lactic dehydrogenase (LDH) increased which resulted in hyper functioning of liver under the influence of toxicant. It also leads to muscular tissue damage. Lactate dehydrogenase is known to catalyse the biochemical process of converting pyruvate to lactate with the attendant oxidation of NADPH. Lactic dehydrogenase induced by several toxicants was reported by Das *et al.*,[9] fungicide "Mancozeb" and its metabolite "ETU" on freshwater fish, *Clarias batrachus*.[32] Significant increasement the activity of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in fish was observed in these studies. According to Tamizhazhagan *et al.*,[33] similar observation was reported that the activity of GOT & GPT increase after exposure of pesticide monocrotophos. Murugesan *et al.*,[34] also reported that there was alteration in the activities of GOT & GPT of muscle and liver in case of *Sarotherodon mossambicus* on exposure to sub-lethal & lethal concentration. Similar pattern was observed in the case of *Channa punctatus* when subjected to lethal & sub-lethal doses of glyphosate herbicide.

CONCLUSION

Thus, we should avoid excessive use of herbicide (glyphosate) in water bodies as it altered the activity of enzymes which leads to cellular damage & death of fishes and disturbing balance of aquatic ecosystem.

REFERNCES

1. Chandran R, Sivakumar AA, Mohandass S, and Aruchami M. Effect of cadmium and zinc on antioxidant enzyme activity in the gastropod, *Achatina fulica*. Comp. Biochem. and Physiol., 2005; 140: 422–426.
2. Singh A and Singh A. Studies on toxicity stress, behavioural alteration and biochemical changes induced by glyphosate herbicide on the fresh water fish *Channa punctatus* (Bloch). J. Food, Agricul. Veteri. Sci., 2017; 7(3): 39-48.
3. Nehls S and Segner H. Detection of DNA damage in two cell lines from rainbow trout, RTG-W1, using the comet assay. Environ. Toxicol, 2001; 16: 321-329.
4. Vander Werf, HMG. Assessing the impact of pesticides on the environment. Agri., Ecos. and Enviro., the Netherlands, 1996; 60: 81-96.

5. Holt J, Powles S, Holtum, J. Mechanisms and agronomic aspects of herbicide resistance. *Annual Review of Plant Physiol and Plant Mole. Biol.*, 1993; 44: 203-229.
6. Smith EA, Oehme FW. The biological activity of glyphosate to plants and animals; a literature review. Vet. Hum. Toxicol, 1992; 34: 531-543.
7. Mladinic M, Berend S, Vrdoljak AL, Kopjar N, Radic B, Zeljezic D. Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes *in vitro*. Environ. and Mole. Mutage, 2009; 50: 800-80.
8. Ramamurthy R, Nagaratnamma R, Jayasundermma B, Rama Rao P. Histopathological lesions in the gill of freshwater teleost, *Cyprinus carpio* induced by methyl parathion. Matsya, 1987; 13: 144-147.
9. Das BK and Mukherjee SC. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatical and haematological consequences. Comp. Biochem. Physiol, 2003; 134C: 109-121.
10. Bengum G. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linn.) and recovery response, Aquat. Toxicol, 2004; 66: 83-92.
11. Rao JV, Ghousia B, Pallela R, Usman PK, Nageswara Rao R. Changes in behaviour and brain acetyl cholinesterase activity in mosquito fish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos. Int J Environ Res Public Health, 2005; 2(3): 478-483.
12. Shweta Agrahari, Kashev C, Pandey, Krishna Gopal. Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). Pest. Biochemis. and Physiol, 2007; 88(3): 268-272.
13. Langiano VC, Martinez CBR. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. Comp. Biochem. Physiol, 2008; 147C; 222–231.
14. Princy Shakeet and Saroj Bakshi. Biochemical Alterations in the Gonads of *Chrotogonus trachypterus* (Blanchard) Treated with Sub-lethal Dose of Monocrotophos. Jour. Life Sci., 2010; 2(2): 107-115.
15. APHA/AWWA/WEF. Standard methods for the examination of water and waste water. 20th edition, American public health association, New York, USA, 1998.
16. Anderson MA and Szcypinski AJ. Method of Enzymatic Analysis. Bergmeyer UH (Ed.). Academic press. J Clinic, 1947; 17: 571.
17. Ellman GL, Courtney KD, Andres Jr. V, Featherstone RM. A new and rapid colorimetric determination of actylcholinesterase activity. Biol Pharmacol, 1961; 7: 88.

18. Anon. Sigma diagnostics TM Lactic dehydrogenase (quantitative, colorimetric determination in serum, urine and cerebrospinal fluid) at 400-450 nm. Procedure No. 500, Sigma, St. Louis, USA, 1984.
19. Reitman S and Frankel S. Acolorimetric method for the determination of serum glutamic oxaloacetic and glutamic-pyruvic transaminases. Am J Clin Pathol, 1957; 28: 56.
20. Sokal RR and Rohlf FJ. Introduction to biostatistics. San Francisco, W.H. Freeman and Company, 1973; 368.
21. Byrene PA and O' Halloran J. The role of bivalve molluscs as tools in estuarine sediment toxicity testing: a review. Hydrobiologia, 2001; 465: 209-217.
22. Srivastava P and Singh A. Study on some neural behavioural changes induced by carbamate (Mancozeb) fungicide on freshwater fish, *Clarias batrachus*. World J. Zool, 2013a; 8: 376-380.
23. Abou-Donia MB. Increased acid phosphatase activity in hens following an oral dose of *Leptophose*. Toxicol, 1978; 2: 199-203.
24. Banaee MS, Mirvaghefi AR, Ahmadi K. Effect of diazinon on biochemical parameters of blood in rainbow trout (*Onchorhyncus mykiss*). Pesti. Biochem. Physiol, 2011; 99: 1-6.
25. Lionetto MGR, Caricato ME, Giordano MF, Schettino T. Integrated use of biomarker (acetyl cholinesterase and antioxidant enzymes activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. Mar. Pollut. Bull, 2003; 46: 324-330.
26. Rajini A, Revathy K. Effect of combination pesticide on acetylcholine esterase activity in freshwater fish *Danio rerio.* International Journal of Pharma and Bio Sci., 2015; 6(1): 1305-1310.
27. Shoaib N, Siddiqui PJA. Impact of organophosphate pesticides, methyl parathion and chlorpyrifos on some tissue enzymes of fish (*Aphanius dispar*). Indian J. of Geo- Marine Sci., 2016; 45(7): 869-874.
28. Glusczak L, Miron DS, Crestani M, Fonseca MB, Pedron FA, Duarte MF, Vieira VLP. Effect of glyphosate herbicide on acetyl cholinesterase activity and metabolic and haematological parameters in piava (*Leporinus obtusidens*). Ecotox. Environ. Safety, 2006; 65: 237-241.
29. Glusczak L, Miron DS, Moraes BS, Simões RR, Schetinger MR, Morsch VM, Loro VL. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). Comp. Bioch. Physiol. C, 2007; 146: 519-524.

30. Modesto KA, Martinez CBR. Roundup causes oxidative stress in liver and inhibits acetyl cholinesterase in muscle and brain of the fish *Prochilodus lineatus*. Chemosphere, 2010; 78: 294-299.
31. Cattaneo R, Clasen B, Loro VL, Menezes CC, Pretto A, Baldisserotto B, Santi A, Avila LA. Toxicological Responses of *Cyprinus carpio* Exposed to a Commercial Formulation Containing Glyphosate. Bull. Environ. Conta. Toxicol, 2011; 87: 597-602.
32. Srivastava P and Singh A. In vivo study of effects of dithiocarbamates fungicide (Mancozeb) and its metabolite ethylenethiourea (ETU) on freshwater fish, *Clarias batrachus*. J. Biol. Earth Sci, 2013b; 3: 228-235.
33. Tamizhazhagan, V, Pugazhendy K. The toxicity effect of Monocrotophos 36% E. C. on the biochemical changes *Labeo rohita* (Hamilton, 1882). *Int. J. for Sci. Res. & Devlop*, 2016; 3(1): 802-808.
34. Murugesan R, Palaniswamy TN, Panneer S. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities in different tissues of *Sarotherodon mossambicus* (Peters) exposed to a carbamate pesticides, carbaryl. Pestic. Sci., 1999; 55: 1217-1221.