

Quantitative Determination of Lethal Concentration LC_{50} of Atrazine on Biochemical Parameters; Total Protein and Serum Albumin of Freshwater Fish Grass Carp (*Ctenopharyngodon idella*)

Ahsan Khan^{1*}, Nazish Shah¹, Muhammad², Mian Sayed Khan¹,
Munawar Saleem Ahmad¹, Muhammad Farooq², Muhammad Adnan⁴,
Sahibzada Muhammad Jawad³, Hayat Ullah⁵, Ali Muhammad Yousafzai³

¹Zoology Department, University of Swabi, Khyber Pakhtunkhwa (KP), Pakistan

²Microbiology Department, University of Swabi, Khyber Pakhtunkhwa (KP), Pakistan

³Zoology Department, Islamia College Peshawar, Khyber Pakhtunkhwa (KP), Pakistan

⁴Department of Agriculture, University of Swabi, Khyber Pakhtunkhwa (KP), Pakistan

⁵Department of Microbiology, Hazara University, Mansehra, Khyber Pakhtunkhwa (KP), Pakistan

Received: 24 November 2015

Accepted: 18 February 2016

Abstract

The indiscriminate use of herbicides has led to the contamination of water bodies, possibly affecting the health of aquatic biota, especially fish. Atrazine is considered as toxicants for aquatic fauna, due to its high persistence in soil, high half-life and high mobility toward aquatic bodies as well as high solubility in water. The objective of the present study was to determine (LC_{50}) and to evaluate the acute and chronic toxicity of atrazine on the biochemical parameters; total protein and serum albumin of freshwater grass carp (*Ctenopharyngodon idella*). Above $15 \mu\text{L}^{-1}$, the LC_{50} was recorded revealing sensitivity of grass carp to atrazine. Grass carp was exposed to atrazine for 01 ($15 \mu\text{L}^{-1}$), 02 ($13 \mu\text{L}^{-1}$), 03 ($10 \mu\text{L}^{-1}$), and 04 ($08 \mu\text{L}^{-1}$) days/concentration for scrutinizing acute toxicity. Likewise, fish were exposed to atrazine for 10 ($06 \mu\text{L}^{-1}$), 15 ($04 \mu\text{L}^{-1}$), and 25 ($02 \mu\text{L}^{-1}$) days/concentration for scrutinizing chronic toxicity. Control group concentration was 8.3 gL^{-1} and 3.5 gL^{-1} . Total protein concentration observed for acute toxicity was 7.5 gL^{-1} , 6.5 gL^{-1} , 4.6 gL^{-1} , and 3.2 gL^{-1} and serum albumin concentration was 2.7 gL^{-1} , 1.6 gL^{-1} , 1.4 gL^{-1} , and 1.1 gL^{-1} respectively. Similarly total protein concentration observed for chronic toxicity was 8.2 gL^{-1} , 6.8 gL^{-1} , and 4.3 gL^{-1} and serum albumin concentration was 2.1 gL^{-1} , 1.7 gL^{-1} , and 1.4 gL^{-1} respectively. Markedly decline (denoted by $P < 0.05$, $P \leq 0.01$ and $P \leq 0.001$) was noticed

in both the parameters concentration during acute as well as chronic toxicity, when compared with control group concentration, indicating negatively impinge of atrazine on grass carp as well as atrazine present in aquatic bodies must jeopardize the health of other aquatic fauna.

Keywords: acute toxicity, grass carp, total protein, serum albumin, atrazine

Introduction

The presence of pesticides in the environment has caused significant social and scientific development anxiety worldwide, as their extensive usage globally can create potential risks to the environment, especially aquatic bodies, resulting in extensive damage to non-target species, particularly fish [1]. The aquatic ecosystems are known to receive a wide spectrum of pollutants, which may be introduced to it directly or indirectly and herbicide are one of these pollutants representing a serious environmental problem [2-4]. The major source of contamination by these herbicides is the deposits resulting from their application to control agriculture pests and harmful aquatic herbs [5]. In modern farm practices chemicals play the most important roles and are considered as integral part of modern agriculture systems [6-7]. But it has been found that chemicals like agricultural herbicides used against undesirable herbs also have adverse side-effects on the environment [8]. It has been confirmed by environmental toxicology studies that herbicides affect non-target species in the environment [9]. Agricultural production can be improved by the use of herbicides, but unfortunately it may reach non-targeted areas and can impact non-target organisms, especially aquatic species and their environment [10]. During application their particles can volatilize and effect different areas like; adhering to wind contributing to wind pollution, effect wildlife, birds, domesticated animals via grazing, soil microorganisms, aquatic organisms particularly fish via; reducing their biodiversity, alteration in reproductive and behavioral responses, increased disease susceptibility and accumulation of toxic substances that can reach human via food chain thus affecting human being as well [11]. Specially they can effects the physical and chemical composition of aquatic bodies and can affect all the fauna present in aquatic bodies, especially fish [12]. They can disturb enzymatic and hormonal activities of fish. High doses of herbicides can kill fish while low doses can affect fish behavior, physiology, growth, and reproduction [13]. Fish exposed to any medium have toxicant showed, restlessness, rapid body movement, convulsions, difficulty in respiration, excess mucous secretion, change in color, and loss of balance [14].

Atrazine (2-chloro-4-thylamino-6-isopropylamino-striazine, ATZ) is one of the most commonly used herbicides. Its utilization is controversial worldwide, as it is currently banned in Europe but not in the U.S. and other countries [15]. Different studies have been conducted on atrazine's effects on fish, as in ATZ affecting hematological parameters, locomotor activity, immune response, metabolism, oxidative stress, osmoregulatory disturbance

and reproduction [16-22]. So in accordance with these effects, the present study was conducted having aims and objectives, to scrutinized the toxicological impinge of atrazine on grass carp (*Ctenopharyngodon idella*) following acute and chronic toxicity via undertaking the evaluation of biochemical parameters; total protein and serum albumin.

Methodology

Experimental Fish

The healthy and active specimens of grass carp (*Ctenopharyngodon idella*) were selected as a model for the present investigation and were procured from Sherabad Hatchery, Peshawar, Khyber Pakhtunkhwa, Pakistan.

Maintenance of Experimental Fish

After procuring, all fishes were carefully analyzed and treated with 0.2% KMnO₄ solution for two minutes before stocking to get rid of any dermal infection. Fish were transferred to acclimatization tanks containing tap water for two weeks for acclimatization purpose. After acclimatization fishes were transferred to experimental tanks containing tap water for experimentation. Fish were fed properly with commercial food on every alternate day in both acclimatization and experimentation tanks. The pH and temperature of the water in tanks were kept constant and checked every other day. Other parameters were additionally checked in both acclimatization and experimental tanks, including total hardness, calcium hardness, magnesium hardness, water conductivity, dissolved oxygen, total solids, total dissolved solids, total suspended solids, total alkalinity and chloride having concentrations like 95 mg/l, 61.6 mg/l, 35 mg/l, 431 µS/cm, 7.37 ppm, 321 mg/l, 221 mg/l, 100 mg/l, 163.3 mg/l and 20.3 mg/l, respectively indicating that all of these parameter concentrations were in normal range.

Experimental Design

For the research purpose, one group was considered as the control group consisted of 05 fishes and kept without treatment. For acute toxicity analysis, 26 fishes were taken and made up of four groups. Group (1 and 2) consisted of 05 fishes each which was treated under a dose of 15 µl/L and 13 µl/L for 01 and 02 days respectively. Group (3 and 4) consisted of 08 fishes each which was processed under a dose of 10 µl/L and 08 µl/L for 03 and 04 days respectively. For chronic toxicity analysis 40 fishes were

taken and divided into three groups. Group 1st, 2nd and 3rd consisted of 10, 15 and 15 fishes and was treated under dose of 06, 04 and 02 $\mu\text{L/L}$ for 10, 15 and 25 days respectively.

Blood Collection and Preservation

Blood samples were collected from the freshly anesthetized fish on the spot. For anesthetizing of fish, chemical MS 222 was used. Blood samples were collected from the caudal vein of fish and sometimes from direct puncturing of heart of fish. Blood samples were collected with the help of hypodermic syringes which were heparinized with the help of a few drops of heparin to avoid blood clotting [23, 24].

After collection, blood samples were stored in EDTA tubes having ethylenediaminetetraacetic acid and were rotated gently for mixing of blood with ethylenediaminetetraacetic acid for preventing blood clotting. Gel tubes were also utilized for storing blood samples and were found more beneficial as compared to EDTA tubes because blood biochemical parameters are determined from the serum of the blood and gel tube have a special layer of gel which is helpful in isolation of blood cells from the serum of the blood via centrifugation (3000 RPM). After assembling, all blood sample tubes were shifted to the ice box and conveyed to the lab for analysis of blood total protein and serum albumin.

Estimation of Total Protein and Serum Albumin

For analysis of total protein and serum albumin the biochemical analyzer set (Merck micro lab 300 biochemistry analyzer) protocol was followed.

Statistical Calculations

Results were reported in mean, standard deviation and standard error of mean. SPSS software was used to calculate paired T test to detect the significant (P value)

difference between control and experimental means, and represented as $P < 0.05$ = significant, $P \leq 0.01$ = highly significant and $P \leq 0.001$ = maximum highly significant and result reported above these values are represented as nonsignificant.

Results

Concentrations of Control Group

Total protein concentration for the control group was; for fish 1 = 8.2 g/L, fish 2 = 8.8 g/L and fish 3 = 8.0 g/L, with a mean value of 8.2 g/L, standard deviation value 0.4 and standard error of mean = 0.2. Likewise serum albumin concentration for the control group was; for fish 1 = 3.6 g/L, fish 2 = 3.4 g/L and fish 3 = 3.5 g/L, with a mean value of 3.5 g/L, standard deviation value 0.1 and standard error of mean = 0.05.

Concentration Obtained After Treatment

Acute Toxicity (Figs 1 and 2)

Total protein

Total protein concentration obtained after treatment of fish for 01 day was: fish 1 = 7.8 g/L, fish 2 = 7.0 g/L and fish 3 = 7.8 g/L, with a mean \pm S.D value 7.5 g/L \pm 0.46, standard error of mean = 0.26 and paired t test value = 1.58 with a significant (p) value = 0.25 (Non-Significant). Concentration obtained after treatment of fish for two days were: fish 1 = 6.5 g/L, fish 2 = 6.3 g/L and fish 3 = 6.8 g/L, with a mean \pm S.D value = 6.5 g/L \pm 0.25, standard error of mean = 0.14 and paired t test value = 4.75 with a significant (p) value = 0.04 (Significant, $p < 0.05$). Concentration obtained after treatment of fish for three days were: fish 1 = 4.0 g/L, fish 2 = 5.0 g/L and fish 3 = 4.8 g/L, with a mean \pm S.D value = 4.6 g/L \pm 0.52, standard error of mean = 0.30 and paired t test value = 12.48 with a significant (p) value = 0.006 (highly significant, $P \leq 0.01$). Concentration obtained after treatment of fish for four

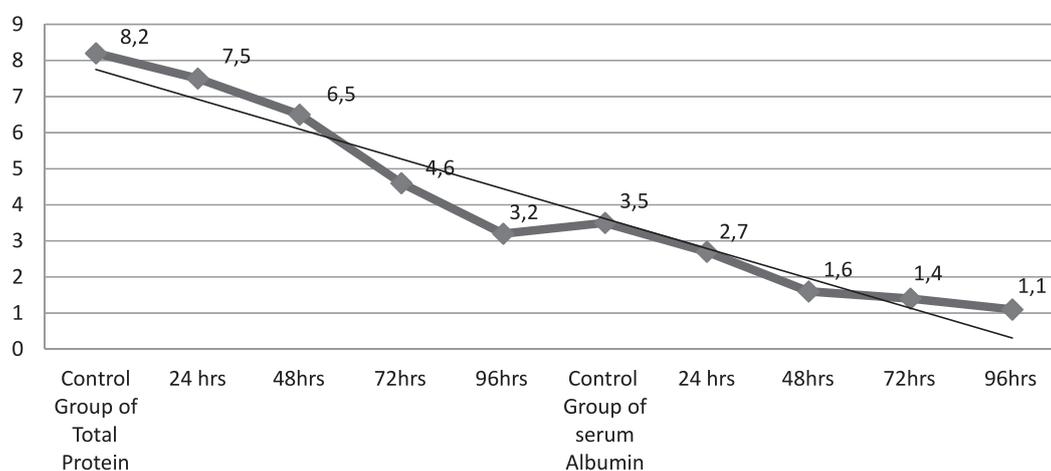


Fig. 1. Comparison of control group and treated group (Acute toxicity) of biochemical parameters: total protein and serum albumin (g/L).

days were: fish 1 = 3.6 g/L, fish 2 = 3.0 g/L and fish 3 = 3.2 g/L, with a mean \pm S.D value = 3.2 g/L \pm 0.30, standard error of mean = 0.17, and paired t-test value = 13.65 with a significant (p) value = 0.005 (highly significant, $P \leq 0.01$).

Serum Albumin

Serum albumin concentration obtained after treatment of fish for one day was: fish 1 = 2.8 g/L, fish 2 = 2.6 g/L and fish 3 = 2.9 g/L, with a mean \pm S.D value = 2.7 g/L \pm 0.15, standard error of mean = 0.08 and paired t-test value = 8.69 with a significant (p) value = 0.01 (highly significant, $P \leq 0.01$). Concentration obtained after treatment of fish for two days were: fish 1 = 1.6 g/L, fish 2 = 1.6 g/L and fish 3 = 1.6 g/L, with a mean \pm S.D value = 1.6 g/L \pm 0.00, standard error of mean = 0.00 and paired t-test value = 32.90 with a significant (p) value = 0.0009 (Maximum highly significant, $P < 0.001$). Concentration obtained after treatment of fish for three days were: fish 1 = 1.4 g/L, fish 2 = 1.3 g/L and fish 3 = 1.3 g/L, with a mean \pm S.D value = 1.4 g/L \pm 0.05, standard error of mean = 0.03 and paired t-test value = 65.00 with a significant (p) value = 65.00 (Maximum highly significant, $P \leq 0.001$). Concentration obtained after treatment of fish for four days were: fish 1 = 1.3 g/L, fish 2 = 1.2 g/L and fish 3 = 1.2 g/L, with a mean \pm S.D value = 1.1 g/L \pm 0.15, standard error of mean = 0.08 and paired t-test value = 68.00 with a significant (p) value = 68.00 (Maximum highly significant, $P < 0.001$).

Chronic Toxicity (Figs 1 and 2)

Total protein concentration obtained after treatment of fish for 10 days were: fish 1 = 8.8 g/L, fish 2 = 7.0 g/L and fish 3 = 9.0 g/L, with a mean \pm S.D value = 8.2 g/L \pm 1.10, standard error of mean = 0.63, and paired t test value =

0.07 with significant (p) value = 0.94 (Non-Significant). Concentration obtained after treatment of fish for 15 days were: fish 1 = 7.0 g/L, fish 2 = 6.7 g/L and fish 3 = 6.8g/L, with a mean \pm S.D value = 6.8g/L \pm 0.15, standard error of mean = 0.08, and paired t-test value = 5.00 with a significant (p) value = 0.03 (Significant, $P < 0.05$).

Concentration obtained after treatment of fish for 25 days were: fish 1 = 4.0 g/L, fish 2 = 4.0 g/L and fish 3 = 5.0 g/L, with a mean \pm S.D value = 4.3 g/L \pm 0.5, standard error of mean = 0.33, and paired t-test value = 7.55 with a significant (p) value = 0.01 (Significant, $P < 0.05$).

Serum Albumin

Serum albumin concentration obtained after treatment of fish for 10 days were: fish 1 = 2.3 g/L, fish 2 = 2.3 g/L and fish 3 = 1.8 g/L, with a mean \pm S.D value = 2.7 g/L \pm 0.28, standard error of mean = 0.16 and paired t-test value = 11.73 with a significant (p) value = 0.007 (highly significant, $P < 0.01$). Concentration obtained after treatment of fish for 15 days were: fish 1 = 1.9 g/L, fish 2 = 2.0 g/L and fish 3 = 1.4 g/L, with a mean \pm S.D value = 1.6 g/L \pm 0.32, standard error of mean = 0.18 and paired t-test value = 11.92 with a significant (p) value = 0.007 (highly significant, $P < 0.01$). Concentration obtained after treatment of fish for 25 days were: fish 1 = 1.5 g/L, fish 2 = 1.5 g/L and fish 3 = 1.2 g/L, with a mean \pm S.D value = 1.4g/L \pm 0.17, standard error of mean = 0.1 and student t test value = 36.37 with a significant (p) value 0.0008 (Maximum highly significant, $P < 0.001$).

Discussion

Atrazine herbicides are used to control broad-leaf weeds in crops [27]. Atrazine has high mobility so that after application it can move to untargeted areas, especially to aquatic bodies [28]. Different concentrations

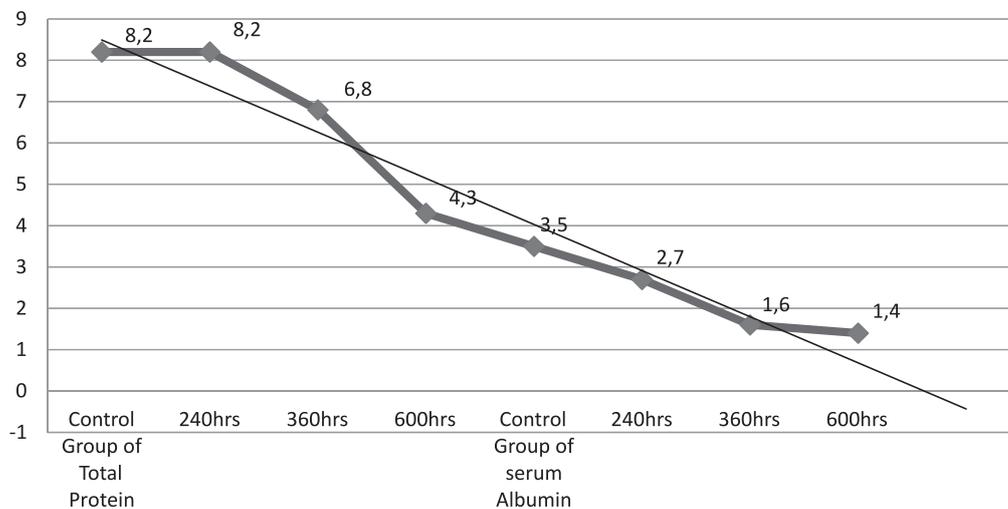


Fig. 2. Comparison of control group and treated group (Chronic toxicity) of biochemical parameters: total protein and serum albumin (g/L).

of atrazine have shown different alterations in biochemical parameters of the fish [29]. The presence of 1000 μ of atrazine in a pool has caused the death of rainbow trout (*Onchorhynchus mykiss*) [30]. Blood biochemical parameter concentrations of common carp are altered after exposure to different doses of herbicides [31]. Atrazine affected the fat oxidation and antioxidant enzymes, kidneys (including endoplasmic softening), and the egg sac of *Channa punctatus* [10, 32, 33]. Bioaccumulation of atrazine in different organs of fish have been noted and thus we concluded that environmental pollutants can contaminate fish bodies and pollutants can enter fish bodies through the gills and skin [34]. Exposing fish to different concentrations of atrazine can also bring changes in fish behavior, such as irregular movement, increased opercular movement, floating on the side, vertical movement, fast swimming, coming toward the water surface, etc., and such abnormal behavior indicates that herbicides affect the CNS of the fish [35]. Atrazine is toxic to aquatic animals and most studies have noted that exposure of fish to atrazine results in biochemical parameter alteration, behavioral abnormality, and structural deformality, plus stress on reproduction and the immune system by quantifying white blood cells [22, 36-40]. Histopathological changes in kidney, liver, gills, and other organs due to exposure to different atrazine concentrations can lead to death [41, 42, 36].

Protein is also one of the important biochemical parameters used to understand the general state of fish health and biological mechanisms of metabolism under pollutant stress [43]. In the present study the total protein concentration starts depletion from the first day, and such depletions of total protein were also observed continuously on remaining days of exposure to atrazine for acute toxicity. A decline in total protein concentration of *Oreochromis niloticus* and *Chrysichthyes auratus* against atrazine herbicide have been observed [44]. Carp exposed to 10 μ l/L atrazine for 72 hrs had significantly lower plasma protein concentrations [45]. In addition, our results showed an agreement with the results obtained by different researchers [46-50].

The reduction in protein could be attributed to adjustment of the fish to its new environmental conditions as a result of stress response [51]. Unlike mammals, fish consume protein and do not store it in the body tissue for muscle energy when a carbohydrate source is absent, hence the exposed fish meet their extra energy requirements from body proteins, which are used to produce glucose for fish by the process of gluconeogenesis [52, 53]. A decreased protein level may be attributed to stress-mediated immobilization of these compounds to fulfill an increased element for energy by the fish to cope with environmental conditions exposed by the toxicant [54]. So from this we concluded that in the present study when fish were exposed to atrazine doses for acute toxicity we observed a decrease in protein concentrations compared to the control group because the protein was converted to glucose by the process of gluconeogenesis. On the other hand, in silver catfish *Rhamdia quelen* were

observed to have increased levels of protein, which is in disagreement with our results [55].

Serum albumin plays an important role in maintaining the osmotic balance between the circulating blood and the tissue membrane [56]. A significant decrease in serum albumin observed in the present investigation was in agreement with the work of Ravichandran [57]. This is also supported by a study in which it was observed that when Nuvan toxicant exposure increased we saw further decrease in serum albumin [58]. A similar trend of serum albumin decrement was also observed in Indian catfish, *Mystus vittatus*, after Nuvan toxicant exposure [59]. The finding in *Cyprinus carpio* after exposure to monocrotophos pesticides showed similar results [60].

These findings are also supported by results observed in *Monosex tilapia* treated with cabofuran [61]. Similar results were recorded in *Cyprinus carpio* subjected to Chlorpyrifos [61]. The effect of indofil toxicity was noted on the serum albumin content of *Channa punctatus* (Bloch) [62]. Recently similar findings were interpreted in *Clarias gariepinus* on paraquat dichloride toxicity [63]. The reduced level of albumin is observed in rock fish exposed to cypermethrin [63]. Similar findings were reported in *Mystus vittatus* exposed to metasystox and sevin regarding serum albumin [64]. However, similar findings were estimated in sheet fish after exposure to 2-phenoxy ethanol [48]. Depletion of serum albumin was found in *Nile Tilapia*, *Oreochromis mossambicus* exposed to benomyl [65]. Recently, similar results of decreasing serum albumin were observed in *Channa punctatus* under indofil toxicity, and the same was witnessed in *Channa punctatus* and *Clarias gariepinus* exposed to sub-lethal concentrations of Nuvan toxicant [66].

Conclusion

The result obtained for acute toxicity and chronic toxicity in the case of total protein and serum albumin lead to the conclusion that both total protein and serum albumin show continuous decline against the different doses of the herbicide atrazine.

References

1. MORENO D.H.F, SOLER M.P, MÍGUEZ LÓPEZ MP. "Brain acetylcholinesterase, malondialdehyde and reduced glutathione as biomarkers of continuous exposure of tench, *Tinca tinca*, to carbofuran International Journal of Chemical Engineering and Applications, 4 (4), August 2013 207 or deltamethrin," Science of the Total Environment, 408, 4976, 2010.
2. JOSEPH B., RAJ S.J. Impact of Pesticide Toxicity on Selected Biomarkers in Fish. Int. J. Zool. Res. 7, 212, 2011.
3. OKAYI R.G., TACHIA M.U., ATAGUBA G.A., DIKWAHA S.H. Toxicity of the Herbicide Propanil on *Oreochromis niloticus* Fingerlings. Journal of Fisheries and Aquatic Science 8, 233-237, 2013a.
4. OKAYI R.G., TACHIA M.U., ATAGUBA G.A., DIKWAHAL S.H. Toxicity of the Herbicide Propanil on

- Oreochromis niloticus* Fingerlings. J. Fisheries and Aquatic Sci. **8**, 233, **2013b**.
5. YADAV A.S., BHATNAGAR A., KAUR M. Assessment of Genotoxic Effects of Butachlor in Fresh Water Fish, *Cirrhinus mrigala* (Hamilton). Research Journal of Environmental Toxicology. **4**, 223, **2010**.
 6. FARAH M.A., ATEEQ B., ALI M.N., SABIR R., AHMAD W. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere. **55**, 257, **2010**.
 7. XING H., LI S., WANG Z., GAO X., XU S., WANG S., Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in Common carp. Pesticide Biochemistry and Physiology. **103**, 74, **2012**.
 8. SARIKAYA R., YILMAZ M. Investigation of acute toxicity and the effect of 2,4- d (2,4-dichlorophenoxyacetic acid) herbicide on the behavior of the common carp (*Cyprinus carpio*). Chemosphere. **52**, 195, **2003**.
 9. RAO J.V. Toxic effects of novel organophosphorus insecticide (rpr-v) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Pesticide Biochemistry and Physiology. **86**, 78, **2006**.
 10. NWANI C.D., LAKRA W.S., NAGPURE N.S., KUMAR R., KUSHWAHA B., SRIVASTAVA S.K. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (bloch). International journal of environmental research public and health. **7**, 3298, **2010**.
 11. OZMEN M., AYAS Z., GUNGURDU A., EKMEKCI G.F., YERLI S. Ecotoxicological assessment of water pollution in Sariyar Dam Lake. Turkey. Ecotoxicol. Environ. Saf., **70**, 163, **2008**.
 12. ORUC E.O., SEVGILER Y., UNER N. Tissue-specific oxidative stress responses in fish exposed to 2,4-d and azinphosmethyl. Comparative Biochemistry and Physiology. **137**, 43, **2004**.
 13. KEGLEY S., NEUMEISTER L., MARTIN T. Ecological impacts of pesticides in California. Pesticides action network, California, USA. **99**, **1999**.
 14. HAIDER S., INBARAJ M. Relative toxicity of technical material and commercial formulation of malathion and endosulfan to a freshwater fish, *Channa punctatus* (bloch). Ecotoxicology and Environmental Safety. **11**, 347, **1986**.
 15. DONG X., ZHU L., WANG J., WANG J., XIE H., HOU X., JIA W. Effects of atrazine on cytochrome p450 enzymes of zebrafish (*Danio rerio*). Chemosphere. **77**, 404, **2009**.
 16. NASCIMENTO C.R.B., SOUZA M.M., MARTINEZ C.B.R. Copper and the herbicide atrazine impair the stress response of the freshwater fish *Prochilodus lineatus*. Pharmacol. Toxicol. Endocrinol., **155**, 456, **2012**.
 17. RYMUSZKA A., SIWICKI A.K., SIEROSLA W.A. Determination of modulatory potential of atrazine on selected functions of immune cells isolated from rainbow trout (*Oncorhynchus mykiss*). Central European Journal of Immunology. **32**, 97, **2007**.
 18. TIERNEY K.B., SINGH C.R., ROSS P.S., KENNEDY C.J. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in Rainbow trout exposed to three currently-used pesticides. Aquatic Toxicology. **81**, 55, **2007**.
 19. PRASAD T.A.V., REDDY D.C. Atrazine toxicity on hydromineral balance of fish, *Tilapia mossambicus*. Ecotoxicology and environmental safety. **28**, 313, **1994**.
 20. FATIMA M., MANDIKI S.N.M., DOUXFILS J., SILVESTRE F., COPPE P., KESTEMONT P. Combined effects of herbicides on biomarkers reflecting immune-endocrine interactions in goldfish immune and antioxidant effects. Aquatic Toxicology. **81**, 159, **2007**.
 21. LIONETTO M.G., CARICATO R., GIORDANO M.E., ERROI E., SCETTINO T. Carbonic anhydrase as pollution biomarker: an ancient enzyme with a new use. International Journal of Environmental Research Public Health. **9**, 3965, **2012**.
 22. TILLITT D.E., PAPOULIAS D.M., WHYTE J.J., RICHTER C.A. Atrazine reduces reproduction in fathead minnow (*Pimephales promelas*). Aquatic Toxicology. **99**, 149, **2012**.
 23. NELSON D.L., COX M.M. Lehninger, principles of biochemistry” 3rd ed. Worth publishing: New York. ISBN 1-57259-153-6, **2000**.
 24. GUYTON A.C., HALL JOHN E. Guyton and hall textbook of medical physiology with student consult online. Access (11 ed.). Philadelphia: elsevier saunders. ISBN 0-7216-0240-1. **2006**.
 25. STRICKLAND R.D., MACK P.A., PODLESKI T.R. Ultraviolet spectrophotometry of serum proteins, Anal. Chem., **32**, 199, **1960**.
 26. DUMAS Clinical Chemistry, Acta, **31**, 87, **1971**.
 27. CUI H., HWANG H.M., ZENG K., GLOVER H., YU H., LIU Y. Riboflavin photosensitized degradation of atrazine in a freshwater environment. Chemosphere. **47**, 991, **2002**. Cytopathologic reaction of liver and intestines to atrazine. Schriftenver-wasser- boden- lufthyg. **89**, 109.
 28. WARING C.P., MOORE A. The effects of atrazine on Atlantic salmon (*Salmo salar*) smolts in fresh water and after sea water transfer. Aquatic Toxicology. **66**, 93, **2004**.
 29. ELIA A.C., WALLER W.T., NORTON S.J. Biochemical responses of bluegill sunfish (*Lepomis macrochirus*) to atrazine induced oxidative stress. Bulletin of environmental contamination and toxicology. **68**, 809, **2002**.
 30. AARONSON M.J. Identification and confirmation of atrazine in pond water. Bulletin of environmental contamination and toxicology **25**, 492, **1980**.
 31. RAMESH M., SRINIVASAN R., SARAVANAN M. Effect of atrazine (herbicide) on blood parameters of common carp, *Cyprinus carpio* (actinopterygii: cypriniformes). African journal of environmental science and technology. **3**, 453, **2009**.
 32. OULMI Y., NEGELE R.D., BRAUNBECK T. Segment specificity of the cytological response in Rainbow trout (*Oncorhynchus mykiss*) renal tubules following prolonged exposure to sublethal concentrations of atrazine. Ecotoxicology environmental safety. **32**, 39, **1995**.
 33. PREEZ D.U.H.H., VUREN J.H.J. Bioaccumulation of atrazine in the banded tilapia, *Tilapia sparmanii*. Comparative biochemistry and physiology. **101**, 652, **1992**.
 34. ORTIZ J.B., GONZALEZ M.L., SARASQUETE C. Histological alterations in different tissues of fish under the impact of persistent chemical pollution. Ecotoxicology Environmental Restonion. **54**, 45, **2002**.
 35. ANTYCHOWICZ J., SZYMBOR E., ROSZKOWSKI J. Investigations upon the effects of some pesticides on carp (*Cyprinus carpio*). Bulletin of Veterinary Institute in Pulawy **23**, 124, **1979**.
 36. SOLOMON K.R., CARR J.A., PREEZ L.H.D. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. Critical reviews in toxicology. **38**, 721, **2008**.
 37. BLAHOVA J., PLHALOVA I., HOSTOVSKY M. Oxidative stress responses in Zebrafish, *Danio rerio* after subchronic exposure to atrazine. Food and chemical toxicology **61**, 82, **2013**.
 38. MELA M., GUILOSKI L.C., DORIA H.B. (Effects of herbicide atrazine in neotropical catfish (*Rhamdia quelen*). Ecotoxicology and environmental safety. **93**, 13, **2001**.

39. KREUTZ L.C., BARCELLOS L.J.G., MARTENINGHE A., DOS S., ZANATTA E.D. Innate immune response of silver catfish (*Rhamdia quelen*) exposed to atrazine. *Fish Shellfish Immunol.*, **33**, 1055, **2012**.
40. FU Y., LI M., LIU C. Effect of atrazine and chlorophyros exposure on cytochrome p450 contents and enzyme activities in common carp gills. *Ecotoxicology and environmental safety*. **94**, 28, **2013**.
41. PLHALOVA L., BLAHOVA J., MIKULIKOVA I. Effects of subchronic exposure to atrazine on zebrafish (*Danio rerio*). *Polish journal of veterinary science*. **15**, 417, **2012**.
42. PAULINO M.G., SOUZA N.E.S., FERNANDES M.N. Subchronic exposure to atrazine induces biochemical and histopathological changes in the gills of a neotropical freshwater fish, *Prochilodus lineatus*. *Ecotoxicology and environmental safety*. **80**, 6, **2012**.
43. MARTINEZ C.B.R., NAGAE M.Y., ZAIA C.T.B.V., ZAIA D.A.M. Morphological and physiological acute effects of lead in the neotropical fish, *Prochilodus lineatus*. *Brazilian Journal of Biology*. **64**, 797, **2004**.
44. HUSSEIN S.Y., EL-NASSER M.A., AHMED S.M. Comparative studies on the effects of herbicide atrazine on fresh water fish *Oreochromis niloticus* and *Chrysichthyes auratus*, Assiut, Egypt. *Bulletin of environmental contamination and toxicology*. **57**, 503, **1996**.
45. GLUTH G., HANKE W. A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. *Ecotoxicology and Environmental Safety*. **9**, 179, **1985**.
46. MEKKAWY A.A., HUSSAIN S.Y., AHMAD S.M. Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut. *Egypt Journal of Egypt German Society of Zoology*. **19**, 283, **1996**.
47. CRESTANI M., MENEZES C., GLUSCZAK L., MIRON D., LAZZARI R., DUARTE M., MORSCH V.M., PIPPI A., VIEIRA V.L. Effects of clomazone herbicide on haematological and some parameters of protein and carbohydrate metabolism of silver catfish, *Rhamdia quelen*. *Ecotoxicology. Environmental Safety*. **65**, 48, **2006**.
48. VELISEK J.Z., SVOBODOVA V., PIACKOVA L., NOVOTNY J., BLAHOVA E., SUDOV A., MALY V. Effects of metribuzin on Rainbow trout, *Oncorhynchus mykiss*. *Veterinarni medicina*. **53**, 324, **2008**.
49. VELISEK J., STARA A. ZUSKOVA E., SVOBODOVA Z. Use of biometric, hematologic, and plasma biochemical variables, and histopathology to assess the chronic effects of the herbicide prometryn on common carp. *Veternia clinical pathology*. **2013** **42**, 508, **2012**.
50. HAMED H.S. Physiological and biochemical studies on the effect of exposure to an organophosphorous compound in African catfish, *Clarias gariepinus*. M.sc. D. Thesis. Fac. Sci., women's college, ain shams Univ. Cairo. **2010**.
51. ORUC E.O., USTA D. Evaluation of oxidative stress response and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ. Toxicol. Pharmacol.*, **23**, 48, **2007**.
52. BASKARAN P., PALANICHAMY S. Impact of agricultural (ammonium chloride) fertilizer on physiology and biochemistry of freshwater teleost fish, *Oreochromis mossambicus*. *J. Ecobiol.*, **2**, 97, **1990**.
53. SHAKOORI A.R., MUGHAL A.L., IQBAL M.J. Effects of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscle of a freshwater fish *Ctenopharyngodon idella*. *Bull. Environ. Contam. Toxicol.*, **57**, 487, **1996**.
54. VASANTHI R., BASKARAN P., PALANICHAMY S. Influence of carbofuran on growth and protein conversion efficiency in some freshwater fish. *J. Ecobiol.*, **2**, 85, **1990**.
55. GLUSCZAK L., MIRON D.S., MORAES B.S., SIMOES R.R., SCHETINGER M.R., MORSCH V.M., LORO V.L. Acute effect of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish, *Rhamdia quelen*. *Comparative Biochemical and Physiology Toxicology Pharmacology*. **146**, 519-524, **2007**.
56. HARPER H.A., RODWELL V.W., MAYES P.A. Review of physiological chemistry. Lange medical publications, Los Anglos, Ca. Usa. **1997**.
57. RAVICHANDRAN S.K., MIDHUNASHANTI INDIRA N. Impact of phenol on protein metabolism in the freshwater fish, *Oreochromis mossambicus*. *Journal of ecotoxicology and environmental monitoring*. **4**, 33, **1994**.
58. MEDDA C., SARKAR S.K., GANGULY S. Basu Tk. Effects of dichlorovos on some important bioindicators of two freshwater major carps, *Labeo rohita* and *Cirrhinus mrigala*. *Journal of Inland fish society India*. **24**, 36, **1992**.
59. MARUTHANAYAGAM C., SHARMILA G. Haematobiochemical variations induced by the pesticides monocrotophos in *Cyprinus carpio* during the exposure and recovery periods. *Nature environmental Pollution Technology*. **3**, 491, **2004**.
60. SOUFY H., SOLIMAN M.K., EL-MANAKHLY E.M., GAFFAR A.Y. Some biochemical and pathological investigations on *Monosex tilapia* following chronic exposure to carbofuran pesticides. *Global veterinaria*. **1**, 45, **2007**.
61. RAMESH M., SARVANAN M. Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *International journal of integrative biology*. **3**, 82, **2008**.
62. SHARMA G., MALLA F.A., SINGH S. An assay on the effect of indofol toxicity on the total serum protein content of *Channa punctatus* (bloch). *Biology and medicine*. **1**, 25, **2009**.
63. OGAMBA E.N., INYANG I.R., AZUMA I.K. Effect of paraquat dichloride on some metabolic and enzyme parameters of *Clarias gariepinus*. *Current research journal of biological sciences*. **3**, 186, **2010**.
64. JOHN P.J. Alteration of certain blood parameters of freshwater teleost, *Mystus vittatus* after chronic exposure to metasystox and sevin. *Fish physiology and Biochemistry*. **33**, 15, **2007**.
65. MIN E.Y., KANG C. Effect of water born benomyl on the haematological and antioxidant parameters of *Nile tilapia*, *Oreochromis niloticus*. *Pesticide Biochemistry and Physiology*. **92**, 138, **2008**.
66. RANI R., GAUTAM R.K. Biochemical studies on blood of *Channa punctatus* (bloch.) After nuvan toxicity. Ph.d. Thesis department of Zoology, Dr. Bhim. Rao ambedkar University, Agra. **2009**.