ESJ Natural/Life/Medical Sciences

Negative Effect of Lambda-cyhalothrin (Insecticide) on Alazani River Fish, Luciobarbus Mursa

Marine Nikolaishvili, Full Professor
Gogi Jikia, Assistant Professor
Ivane Beritashvili Center of Experimental Biomedicine, Georgia
Magda Davitashvili, Full Professor
Darejan Margalitashvili, Assistant Professor
Tamar Nadiradze, Full Professor
Lamara Zuroshvili, Associate Professor
Iakob Gogebashvili Telavi State University, Georgia

Doi:10.19044/esj.2022.v18n27p1

Submitted: 01 March 2022
Accepted: 29 July 2022
Published: 31 August 2022

Cite As:
Nikolaishvili M., Jikia G., Davitashvili M., Margalitashvili D., Nadiradze T. & Zuroshvili L. (2022). *Negative Effect of Lambda-cyhalothrin (Insecticide) on Alazani River Fish, Luciobarbus Mursa*. European Scientific Journal, ESJ, 18 (27), 1.
https://doi.org/10.19044/esj.2022.v18n27p1

Abstract

Pesticides are complex organic substances that are an important source of water pollution. Using pesticides is one of the important actions for harvest protection. This, however, does not negate the fact that its usage can result to negative consequences as well. The study of toxic action of lambda-cigalotrin (the piretroid of the insecticides which is included in icons, karate, commodore, samurai) on quantitative contents of fatty acids and structural lipids in muscle, gills, liver, and in the brain of fishes in river Alazani was carried out. Based on the actual material, one can conclude that intoxication of the fish tissues with lambda-cyhalotrin processes appear, which stimulates oxidation of free radicals of structural lipids. The peroxides of the initiators of free radicals are formed, which stimulate lipid per oxidation. Exposure to insecticides caused an induction of antioxidant enzyme activities indicating the activation of the insecticides detoxification pathways and the antioxidant defenses. The non-protein sulfhydryl groups increased to 1,75 ± 0,109 compared to the control (1,04 ± 0,123), while the total sulfhydryl groups statistically increased to 39,16 ± 1,44 compared to the control (34,00 ± 1,22).

This indicates an increase in the concentration of protein and total sulfhydryl groups compared to controls. The study also identified that excess amounts of insecticides in the tissues disrupts the biochemical parameters. These changes may be potentially disruptive for the survivability of fish in natural resources. This fact should thus be taken into consideration when this insecticide is used for pest control in agriculture fields surrounding surface water and ground water resources.

Keywords: Pesticides, fatty acids, sulfhydryl groups, oxidative stress, fish

Introduction

Water pollution is an important environmental, ecological, and microbiological problem. As a result of anthropogenic influence, various biogenic or toxic compounds that are discharged into reservoirs destroy the balance in ecosystem which reduces their self-cleaning ability (Sabinova, 2003). Pesticides are complex organic substances that are an important source of water pollution. Most modern pesticides are highly toxic compounds for the components of ecological systems (Chantladze, 1997). Due to environmental pollution, water consumed by humans can contain both a large amount of toxic chemicals (pesticides) and organic compounds used as fertilizers. The river Alazani is the largest river in eastern Georgia. It partially flows through Azerbaijan, along the border of this country. The length of it is 390 km, basin area is 11, 800 m, and average water consumption is 98 cubic/sec (Supatashvili, 2003; Beruchashvili, 2000). The river Alazani possesses a rich diversity of fish species such as catfish, gobi, trout, chanari, carp, river goby, and Barbus mursa.

While monitoring waters of Alazani River in eastern Georgia, it was found that some pesticide products used in the fields to protect agricultural crops and grapes from pests get into the water (Finenko et al., 2001; Zhou, 2007). Furthermore, the effect of the combined pesticide Lambda –cyhalothrin on quantitative distribution of lipids in tissues, gills, brain, and liver of fish was studied. The study was carried out using fish mursa (Barbus mursa), which is easily found in Alazani river and is characterized by slow zonal movement (Buchgraber et al., 2004) Biochemical processes occurring in fish due to water contamination with mineral fertilizers and pesticides were also investigated. Some uncharacteristic processes in fish organism and changes in lipid concentration caused by pesticide effect were revealed (Anudurga, Gajendiran & Jayanthi, 2018; Amit Kumar et al., 2008). The level of changes in lipid components and fatty acids in the fish organs were determined also (Ralf et al., 2011). Therefore, it is established that toxins lead to oxidative processes and the formation of free radicals in various tissues, resulting in oxidative stress (Sameeh et al., 2009).

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

The knowledge of oxidative stress in fish has a great importance for environmental and aquatic toxicology (Konstantinou et al., 2006). This is because oxidative stress is evoked by many chemicals, including some pesticides. More so, the action of pro-oxidant factors in fish organism can be used to assess specific area pollution (Köprücü et al., 2004). Since the fish brain contains a large amount of lipids, lipid oxidation often proceeds through oxidative mechanisms. In addition, oxidative stress damages cellular components and acts at the molecular level, thereby changing the structure of nucleic acids, lipids, proteins, and carbohydrates.

It is known from the literature that protein cysteine thiols respond to the cellular redox state. They can oxidize and inhibit thiol-proteins and enzymes and therefore have antioxidant action. Since the brain contains a large amount of lipid content, lipid oxidation is more common in oxidative mechanisms (Jin et al., 2013).

The use of insecticides blocks the action of enzyme acetylcholinesterase, which results in signs and symptoms of intensive cholinergic stimulation (Orlov, 2002; Schulz, 2004). Organochlorines are neurotoxins which have an effect on sodium and potassium channels in neurons. Decrease of potassium permeability and inhibition of cadmodulin, as well as Na/K and Ca-ATPase activity occur by organochlorine insecticides. Toxicity of insecticides results in alterations of the metabolic and vital functions of the cells.

Materials and Methods

Fishing was performed at Khaxeti region in summer River alazani. Fish catching was done with a fyke net (double 3-m wings, funnel 3.04 m, height 0.69 m, 4-mm nylon mesh), and it was set during daytime (length 40 cm, height 25 cm, width 25 cm, 4-mm nylon mesh, mouths on both sides with 6-cm diameter, fish sausages for bait) .The fishes were about 200 to 250 g in weight and 24 to 30 cm in total length (Elanidze, 1983; Shankar et al., 2013). They were collected seasonally during a period extending from August 2017 - 2018. This is as a result of the possible levels of pollution and water use in the selected control areas when there was an accumulation of toxicants in the liver, brain, and gills of fish tissues.

The chemical reagents which used antioxidative activity, structural lipids, and fatty acids in the current study were purchased from Sigma aldrich St. Louis, MO, USA. These include lipid standards [dodecanoic (lauric, C12:0), hexadecanoic (palmitic, C16:0), octadecanoic (stearic, C18:0), 9(Z)-octadecenoic (oleic, C18:1), 9(Z),12(Z)-octadecadienoic (linoleic, C18:2), 9(Z),12(Z),15(Z)-octadecatrienoic (linolenic, C18:3) and 4(Z),7(Z),10(Z),13(Z),16(Z),19(Z)-docosahexaenoic (cervonic, C22:6) acids], monoacylglycerols (MAGs; monoolein), diacylglycerols (DAGs; 1,3-

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

dilinolein), and TAGs (tripalmitin and trilinolein). All lipid standards were stored at −80 °C. Normal-phase high-performance liquid chromatography (NP-HPLC) method is introduced for the identification and quantitative estimation of lipid classes, including free fatty acids and cholesterol (Schaefer et al., 2003; Nikolova et al., 2001). The HPLC separation is carried out on a LiChrospher Diol (100 A, 5 microm, 125 mm x 3 mm) column with gradient elution (isooctane/0.1% acetic acid in tert-butyl methyl ether) and evaporative light scattering detection (ELSD). The method has been calibrated with representatives of each class in working ranges of about 5-150 mg/l, depending on the lipid class (Fuchs, 2011; Lin, 2007).

According to the method of Sedlak and Lindsay (1968), fish brain nonprotein sulfhydryl (NP-SH) groups were measured after homogenization in ice- old ethylenediaminetetraacetic acid (EDTA, 0.02 M). The homogenate (5 mL) was mixed with distilled water (4 mL) and TCA (50%, 1 mL), which was shaken for 10 minutes and then centrifuged. A supernatant (2 mL) was mixed with Tris buffer (4 mL, 0.4 mol/L, pH 8.9) and 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.1 mL) was then added and shaken. The assay is based on a colorimetric method with a tetrazolium salt that is reduced by superoxide anions ($O2-$) to form a formazan dye.

Data Processing and Analysis

The significance between control and results of the biochemical analysis was statistically analyzed by Student's "t" Test. The data values were expressed as mean ± standard error (M±SE) (Catherine, 2001).

Results and Discussion

The following components reveal different sensitiveness in the intoxication process: phospatidylcholine, lysophosphatidylcholine, strearins, sphingomyelin, phospatidilethanolamines, glycerides in the gills and fatty acids, glycerides, stearins, sphingomyelin, and phosphatidylcholine in the liver (Buchgraber et al., 2004). As shown in Table 1, Figure 1, and Figure 2, lipid components are characteristic of all organs. Nonetheless, ceratin regularity for Lambda-cyhalotrin is seen in certain amounts of fatty acids, stearins, phospadidilethanolamine, and lysophosphatidylcholine.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

Table 1. Quantitative changes of lipid components in the gills and liver *p<0.001*

organs of fishs	phospatidylcholine		Lysophosphatidylcholine		strearins		phospatidilethanolamines		Glycerides	
	Contr.	Exp.	Contr.	Exp	Contr.	Exp	Contr.	Exp	Contr.	Exp
Gills	20±0.001	23±0.001	15±0,003	18±0.001	20±0,001	25±0,001	22±0,001	27±0,003	16±0,001	20±0,002
Liver	26±0,001	30±0,002	20±0,002	25±0,002	27±0,001	35±0,004	25±0,003	37,5±0,005	23±0,001	33±0,002

*Note: **p<0.001. n=28 (14 in each group)*

On the basis of the obtained data, it can be noted that in the case of lambda-cigalitrin intoxication, the lipid modification process was not limited to the target of any particular organ. However, an adaptive reaction of the organism was represented. It is also known from the literature that saturated and unsaturated fatty acids take part in free radical oxidation processes. Therefore, the study of changes in the ratio of unsaturated and saturated fatty acids during fish intoxication with phenol was significantly important. The amount of unsaturated fatty acids increases, and the amount of saturated fatty acids decreases. In addition, unsaturated fatty acids are devoid of structural stabilization and are easily subjected to free radical oxidation, resulting in products such as lipid residues that cause periodic convulsions in fish and partial loss of orientation reflexes. This is because the gills increase 1.8 times and 2 times in the liver. Thus, it can be stated that during the intoxication by lambda-cyhalotrin, initiators of phospholopid peroxidation become more active. Also, it is quite possible for superoxide forms of oxygen to be initiators of cytomembrane lipid peroxidation.

Table 2. Conversion of unsaturated and saturated fatty acids in fish liver *p<0.05*

Fish liver	Control	Experiment
Lauric acid	1,9± 1.01	5,5 ± 1.01
Myristic acid	4,4 ± 1.3	6,6 ± 2.01
Palmitic acid	6,4 ± 2.1	8,1 ± 2.02
Stearic acid	3,1 ± 1.01	4,7 ± 1.01
Oleic acid	18,2 ± 3.01	21,2 ± 3.02
Linoleic acid	42, 6 ± 4.03	51,4 ± 4.01
Araachic acid	2,5 ± 1.01	3,1 ± 1.01

*Note: **p<0.05. n=28 (14 in each group)*

Based on the actual material, one can conclude that intoxication of the fish tissues with lambda-cyhalotrin processes appear, which stimulate the oxidation of free radicals of structural lipids. Hence, peroxides of the initiators of free radicals are formed, which also stimulate lipid peroxidation (Christopher et al., 2010). Activation of free radical oxidation takes place and as a result of decrease of the regulation system activity, the lipoperoxidants are formed. They are uncharacteristic of normal vital functions, which

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

negatively affect hydrobiontmetabolic processes and results in cell lysis of fish.

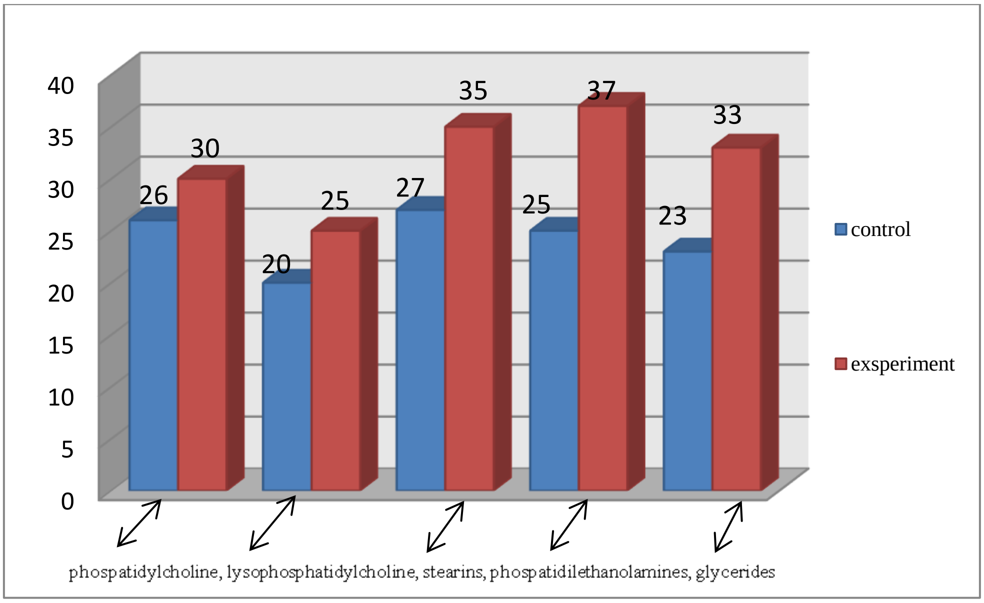

Figure 1. Quantitative change of lipid components fish liver (M±SE)

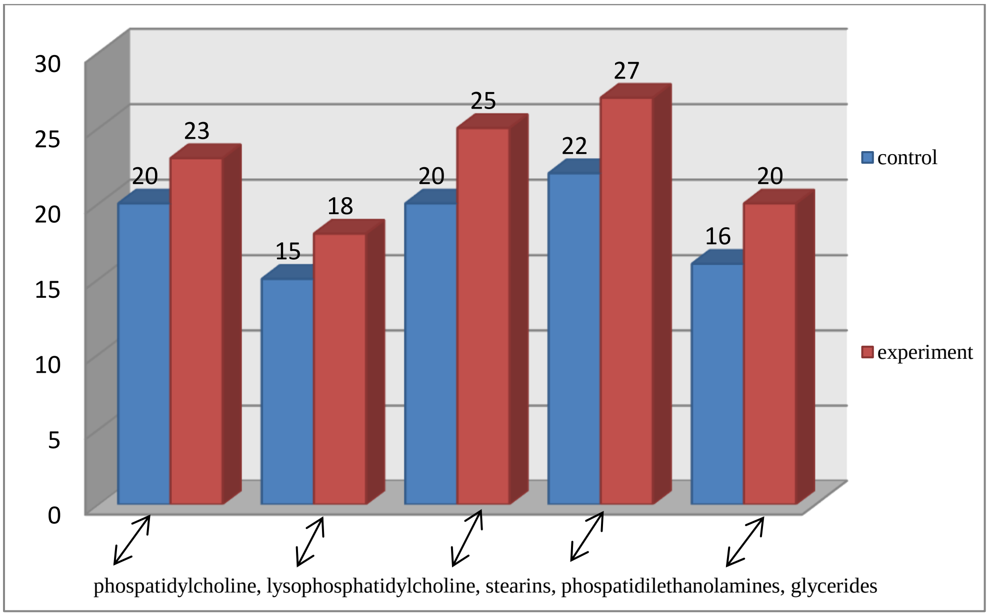

Figure 2. Quantitative change of lipid components fish gills (M±SE)

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

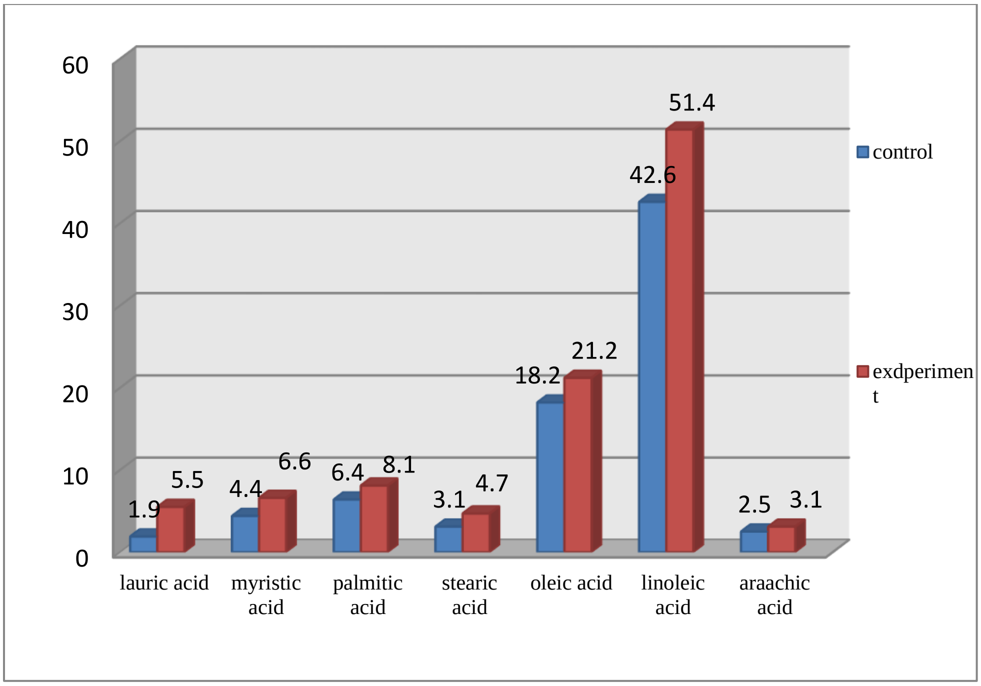

Figure 3. Quantitative change of saturated and unsaturated fatty acids in liver (M±SE)

Consequently, when oxidants increase in the cell, thiol-disulfide is involved in redox regulation. These redox-sensitive mechanisms are involved in redox various changes, including cell hypoxia. Under hypoxic conditions, the concentration of thiols decreases. This is due to the association of metabolites produced during the recovery of hypoxia with glutathione (GSH), a cellular nonprotein thiols (NPSH). Thus, the metabolites react with GSH instead of oxygen. When cellular thiols are depleted, peroxide is produced and excessive oxidative stress leads to cell death (Yang et al., 2020; Winston, 1991).

This study focused on identifying the quantitative changes in non-protein and total (protein) sulfhydryl groups. The concentration of SH-groups in the fish brain was also determined, which is shown in Table 3 below.

Table 3. Non-protein and Common sulfhydryl groups

Fish brain	Control	Studylambda-cyhalothrin
Non-protein sulfhydryl groups	1,04 ± 0,123	1,75 ± 0,109
Common sulfhydryl groups	34,00 ± 1,22	39,16 ± 1,44

*Note: **p<0.05. n=28 (14 in each group)*

Consequently, non-protein sulfhydryl groups increased to 1,75 ± 0,109 compared to the control (1,04 ± 0,123). On the other hand, the total sulfhydryl groups increased statistically to 39,16 ± 1,44 compared to the control (34,00

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

± 1,22). This indicates an increase in the concentration of protein and total sulfhydryl groups compared to controls.

The use of insecticides blocks the actions of enzyme acetylcholinesterase, which results in signs and symptoms of intensive cholinergic stimulation (Niamul et al., 2019). Organchlorines are neurotoxins which have an effect on sodium and potassium channels in neurons. Decrease of potassium permeability and inhibition of cadmodulin, as well as Na/K and Ca-ATPase activity occur by organchlorine insecticides. Pyrethroids can block Na channel and affect the function of GABA-receptors in nerve fiber. Oxidative stress is another mechanism for toxicity of insecticides resulting in cell death, cellular necrosis and apoptosis, and dysfunction in cellular physiology. Alterations can further develop in metabolic and vital functions of the cells.

Conclusion

Pesticides were introduced by the population under the violation of standardization. Accumulate in the soil and bottom sediment negatively affects the food cycle of living systems existing in the river. Pesticides also affect the hydrobiont, which causes intoxication and oxidative stress. This damages the cellular components and acts at the molecular level, thereby changing the structure of nucleic acids, lipids, proteins, and carbohydrates. Intoxication of the fish barel with lambda-cyhalothrin forms the conditions that stimulate free radical oxidation of structural lipids, which leads to the development of lipoperoxidates that are not typical for normal vitality. This in turn negatively influences the metabolic processes of hydrobionts and causes cellular lysis that finally leads to death. Also the number of non-protein and total sulfhydryl groups (SH group) increased significantly in experimental group compared to control group.

Exposure to insecticides, firstly, caused an induction of antioxidant enzyme activities indicating the activation of the insecticides detoxification pathways and the antioxidant defenses. However, excess amount of insecticides in the tissues disrupts the biochemical parameters. These changes may be potentially disruptive for the survivability of fish in natural resources. Therefore, this fact should be taken into consideration when this insecticide is used for pest control in agriculture fields surrounding surface water and ground water resources.

References:

1. Amit, K., Bechan, S., & Shankar, R. (2008). Pandey Cypermethrin and λ-cyhalothrin induced alterations in nucleic acids and protein contents in a freshwater fish, *Channa punctatus.* Fish Physiology and Biochemistry volume 34, pages 331–338.

2. Anudurga, G. & Jayanthi, A. (2018). An overview of pyrethroid insecticides. Frontiers in Biology volume 13, pages79–90.
3. Beruchashvili, N. L. (2000). Georgia's biodiversity against a global background. In: Biological and landscape diversity of Georgia. WWF, Tbilisi, pp 7–20.
4. Buchgraber, M., Ulberth, F., Emons, H., & Anklam, E. (2004). Triacylglycerol profiling by using chromatographic techniques. Eur. J. Lipid Sci. Technol., 106, 621-648 (DOI: 10.1002/ejlt.200400986).
5. Chantladze, Z. I. (1997). Hydrochemistry of river waters of the Georgian SSR under conditions of anthropogenic impact. Hidrometeoizdat, Leningrad. p p. 56-61.
6. Christopher, D.N., Wazir, S.L., Naresh, S.N., Ravindra, K., Basdeo, K., & Satish, KS. (2010). 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 and Public Health. Vol. 7 Issue 8. Pp. 3298–3312. https://doi.org/10.3390/ijerph7083298.
7. Catherine, A. P. (2001). Statistics for Analysis of Experimental Data. Department of Civil and Environmental Engineering .Princeton University . Princeton, NJ 0854.
8. Damyanova, B. & Momchilova, S. (2001). Silver ion HPLC for the analysis of positionally isomeric fatty acids .Journl of Liquid Chromatography and Related Technology.– Vol. 24. – P.p 1447-1466
9. Elanidze, R. F. (1983). Ichthyofauna of rivers and lakes of Georgia. Tbilisi: Metsniereba. 318 p.
10. Fuchs, B., Süß, R., Teuber, K., Eibisch, M., & Schiller, J. (2011). "Lipid analysis by thin-layer chromatography—a review of the current state," *Journal of Chromatography A*, vol. 1218, no. 19, pp. 2754–2774
11. Finenko, G., Anninsky, B., Romanova, Z et al. (2001). Chemical composition, respiration and feeding rates of the new alien ctenophore, Beroe ovata, in the Black Sea. Hydrobiologia, Vol. 451, 1-3: 177–186.
12. Jin, Y., Wang, J., Pan, X., Wang, L., & Fu, Z. (2013). "cis-Bifenthrin enantioselectively induces hepatic oxidative stress in mice," Pesticide Biochemistry and Physiology, vol. 107, no. 1, pp. 61–67.
13. Köprücü, K. & Aydın, R. (2004). The toxic effects of pyrethroid deltamethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae. Pesticide Biochemistry and Physiology 80:47–53. doi:10.1016/j.pestbp.2004.05.004
14. Konstantinou, I. K., Hela, D.G., & Albanis, T.A. (2006). The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

I. Review on occurrence and levels. Environmental Pollution 141(3): 555–570.

15. Lin, J.T. (2007). HPLC Separation of Acyl Lipid Classes Journal of Liquid Chromatography & Related Technologies, Volume 30, - Issue 14. doi.org/10.1080/10826070701435020.
16. Md Niamul, H., Hye-Jin, E., Sang-Eun, N., Yun Kyung, S., & Jae-Sung, R., (2019). Chlorothalonil induces oxidative stress and reduces enzymatic activities of Na+/K+-ATPase and acetylcholinesterase in gill tissues of marine bivalves. PLoS ONE 14(4 DOI: 10.1371/journal.pone.0214236.
17. Orlov, D., Sadovnikov, L., & Lozanovskaya, I. (2002). Ecology and biosphere protection *at* chemical pollution. Higher School, Moscow, pp 116–143.
18. Ralf, B., Schäfer, I., Paul, J., van den Brink, & Matthias L. (2011). Impacts of Pesticides on Freshwater Ecosystems Ecological Impacts of Toxic Chemicals, pp 111-137.
19. Schaefer, A., Küchler, T., Simat, T. J., & Steinhart, H. (2003). Migration of lubricants from food packaging's. Screening for lipid classes and quantitative estimation using normal-phase liquid chromatographic separation with evaporative light scattering detection. Chromatogr. Journal of Chromatography A. Volume 1017, Issues 1–2, 31 Pp. 107-116.
20. Shankar Murthy, K., Kiran, B.R., & Venkateshwarlu, M. (2013). A review on toxicity of pesticides in Fish. International Journal of Open Scientific Research Vol.1, No.1, pp15-36.
21. Sabinova, Z.F., Fattakhova, N.F., Karchava, G.V., & Pinigin, M.A. (2003). Assessment of the potential danger to public health of environmental pollution. Journal of Hygiene and Sanitation.vol 2 .pp 74-76.
22. Sedlak, J. & Lindsay, R. H. (1968). Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25:192–205
23. Supatashvili, G. D. (2003). Hydrochemistry of Georgia. Tbilisi State University press.
24. Sameeh, A.M. & Abdel-Tawab, H.M. (2009). "Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc," Pesticide Biochemistry and Physiology, vol. 93, no. 1, pp. 34–39, doi.org/10.1016/j.pestbp.2008.09.004.
25. Schulz, R. F. (2004). studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: Journal of Environmental Quality 33(2):419-48 DOI:10.2134/jeq2004.0419

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

26. Winston, G., Richard, W., & Di Giulio, T. (1991). Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology. Volume 19, Issue 2 Pages 137-161.
27. Yang, C., Lim, W., & Song, G. (2020). Mediation of oxidative stress toxicity induced by pyrethroid pesticides in fish. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology,*234*,[108758]. https://doi.org/10.1016/j.cbpc.2020.108758.
28. Zhou, R., Zhu, L., & Kong, Q. (2007). Persistent chlorinated pesticides in fish species from Qiantang River in East China. Chemosphere.Vol 68(5): pp. 838-847. DOI: 10.1016/j.chemosphere.2007.02.021.