Effect of Ethoxyquin on Oxygen Consumption in Fresh Water Fish, *Oreochromis mossambicus*

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Abstract
Being a powerful antioxidant, ethoxyquin (EQ-1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is employed in many different foods and animal feeds. Recent studies on animals on EQ diets have shown a broad range of unintended consequences. The negative effects of EQ on vertebrates are becoming well documented, while accounts of the effects of EQ on aquatic ecosystems are very rare. In this study, the LC50 96-hour value of EQ was determined in the freshwater fish *Oreochromis mossambicus* (Tilapia) using the Probit analysis. Throughout the course of 24 hours, 48 hours, 72 hours, 96 hours, 5 days, 10 days, and 15 days, the fishes were exposed to sub lethal concentration of the toxin. The quantity of oxygen used by *Oreochromis mossambicus* and the dissolved oxygen (DO) level in the water were both monitored during the length of the exposure. The bioassay found that the median lethal dosage in fish after 96 hours (LC50-96h) was 11.37 mg/L. A higher metabolic rate and, thus, a bigger amount of energy generated correspond to a lower oxygen intake rate in the experimental fish.

Keywords: Ethoxyquin; Lethal Concentration; *Oreochromis mossambicus*; Oxygen Consumption; Dissolved Oxygen

INTRODUCTION

Across the world, water pollution is a major issue. One or more biotic species' requirements and/or human requirements are used to determine the quality of the water (Shah, 2017; Tchobanoglous & Schroeder, 1985). Water quality is deteriorating as a result of pollution from human activities. Polluted water can't be used for things like washing dishes or raising fish in a tank, therefore it ruins such potential uses. The health of aquatic life, as well as humans and other animals, is threatened by the presence of any form of contaminant in water. In the short term, we may not notice any negative effects from drinking dirty water, but over time, it might be harmful. Heavy metals may seep from nearby industrial processes into the surrounding lakes and rivers. Not only do they endanger the fish and other marine life in the vicinity, but they also pose health risks to the humans who eat those fish. Water systems are constantly being loaded with chemical xenobiotic due to environmental conditions and human involvement, which has significantly accelerated the speed of environmentally damaging changes. These shifts are the direct outcome of the persistent stress placed on the world's water infrastructure.

Biologists may examine the fish and insect life of natural waterways and assess the water quality based on a species diversity index (SDI) (Cole *et al.,* 1999; Tchobanoglous *et al.,* 1985; Alabaster & Lloyd 1984; Nathanson, 2004; Abbas *et al.,* 2014).Eco-toxicological research have shown fish to be a great model organism because they accurately represent the toxic influence on aquatic ecosystems and are the major source that are exposed to environmental toxins. Pollutants in the environment have the greatest impact on fish. Fishes are often considered to be an important food source for humans due to the high levels of calcium and phosphorus in their flesh. It's a kind of organism that serves as a bio-indicator for measuring the standard of water. The abrupt increase in fish mortality is indicative of severe pollution due to the presence of several dissolved contaminants in the water, which produce bioaccumulation in the fish's tissues and organs. Research into alterations in fish physiology gives profound insight into the deterioration of water quality measures, since fish are one of the biological sensors of water quality. Since fish are used as a biological indicator, and because of their key role in environmental contamination monitoring programmes, biomarkers have only recently been developed based on studies of organisms' responses to toxic chemicals (Korami *et al.,* 2000). Environmental poisons often enter fishes and other aquatic creatures via their respiratory systems. A shift in the breathing patterns of fish might be an early indicator of the presence of dangerous toxins in the water they inhabit. This is the body's first response. These reactions, although being less sensitive, were useful in environmental monitoring. Rates of oxygen consumption may reveal alterations in respiration, a frequent physiological response to toxicants. For assessing metabolism's impact on environmental deterioration, oxygen consumption rate is a common metric.

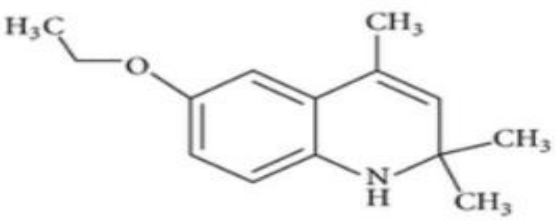
Fish bioassay techniques assess both acute toxicity and oxygen consumption caused by environmental stress (Subrahmanyam, 2004). However, a more direct assessment of stress may be done by monitoring the quantity of oxygen the fish consume, which is one of the biological early warning systems that looks for aberrant movement of the operculum as an indication of respiratory stress (Dube and Hosette, 2010). The energy expenditure in reaction to environmental changes may be estimated via studying oxygen consumption, which also helps in monitoring the impact of toxicants on aquatic organisms (Franklin *et al.*, 2010; Logaswamy and Remia, 2009; Francesco *et al.*, 2008; Somaraj *et al.*, 2005). The metabolic activities and overall health of a fish are affected by the total quantity of oxygen it consumes. As an added advantage, it may also be used to analyse an organism's behaviour, as well as to examine its physiological condition, measure its sensitivity, and determine its resistance. All of these factors may help pinpoint exactly what kind of functional disruptions the organism is experiencing. As they are able to eat both plant and animal matter, fish are well-suited for use in biological early warning systems as stress indicators related to pollution because of their heterotrophic diet. Hence, variations in oxygen consumption may act as an indicator in biological early warning systems for stress caused by pollution.

Molecular oxygen (O_2) is required for several aerobic metabolic activities. Fishes, like other aerobic organisms, are vulnerable to reactive oxygen; biotic and abiotic variables may influence the effectiveness of their antioxidant defences. Oxygen may cause harm to fish (Martinez-Alvarez *et al.*, 2005). Fish oxygen consumption may be used as a proxy for the health of the fish and the effectiveness of any environmental contaminants. Fish die when they absorb enough toxins to be fatal, and the oxygen needs of the survivors rise (Tilak *et al.*, 2007). Respiration rate changes are a metabolic response indicator; these changes are triggered by stress (Chebbi and David, 2010). It is well established that pesticides may disrupt the brain's respiratory centres and tissue, leading to respiratory discomfort or even respiratory failure. Many studies have shown that fish can maintain a constant level of oxygen consumption by regulating the amount of oxygen they take in along a gradient of ecological oxygen concentrations in response to various pesticides, including *Cirrhinusmrigala* (Mushigeri and David, 2003), *Labeorohita* (Patil and David, 2008), *Oreochromis mossambicus* (Logaswamy and Remia, 2009), *Ctenopharyngodonidell*. This continues until a certain threshold oxygen concentration is achieved, after which oxygen consumption drops down dramatically. The fish are less able to adapt to the new conditions because of the stress, and the outcome is a rise in this significant oxygen. Injuries may either increase or decrease an animal's oxygen absorption capacity, therefore monitoring its oxygen intake may act as a biodetector. Hence, the primary goal of this study was to see how a sublethal dose of ethoxyquin altered the oxygen consumption of freshwater *Oreochromis mossambicus*.

Because of climate change, variations in nutrients, acidification, habitat loss, overfishing, and invasive species, the freshwater environment has seen a dramatic decline in species richness in recent years. Discharges of waste products, both solid and liquid, that contain chemical contaminants, are the other main contributors to water contamination. Heavy metals including lead, cadmium, nickel, and mercury may increase the risk of cancer and other birth problems even after short exposure. Reduced immunity, acute poisoning, and pregnancy complications are other possible side effects. Slow decomposition of heavy contaminants leads to a build-up of a chemical that is harmful to both fish and humans. Heavy contaminants, unlike lighter ones, are not broken down in the environment by biological processes, therefore they may have a long-lasting impact on fish populations. Those who depend on aquatic products for sustenance are thus badly impacted by the presence of these toxicants. Ethoxyquin, is an antioxidant used in animal feeds. It is also known as EQ;6-ethoxy-1,2-dihydro-2,2,4-trimethyl quinoline;. A potential danger has been found with its usage in high enough quantities.

Ethoxyquin

The synthetic antioxidant ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is used to maintain the integrity of fat-soluble vitamins and lipids in a wide range of foods for human and animal consumption. 1, 2-Dihydroquinoline ethoxyquin (shown in Figure 1) contains an ethoxy at position 6, three methyls at positions 2, 2 and 4.



Ethoxyquin
6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline

Figure 1: Ethoxyquin Structure

Many unfavourable consequences in animals fed EQ-containing diets have been reported in recent years (Little,1990). Workplace exposure to this material has been linked to a variety of negative outcomes for those who come into contact

with it (Little, 1990; Drewhurst, 1998). In the 1980s, scientists found evidence of negative consequences in animals and humans exposed to it, prompting them to launch a fresh round of toxicity studies. Dicentrics, anomalous translocated chromosomes, and chromatid swaps are only some of the chromosome abnormalities that have been seen in *in vitro* chromosomal experiments using EQ (Gille *et al.,* 1991; Baszczyk *et al.,* 2003). Animals fed diets containing EQ showed a wide variety of negative consequences, prompting researchers to perform toxicity investigations. While studying the acute toxicity of ethoxyquin, *Oreochromis mossambicus* are often utilised as test subjects.

Oreochromis mossambicus (Tilapia)

Most commercial fish breeding operations in southern and eastern Africa focus on the *Oreochromis mossambicus* species (Tilapia). Mozambique tilapia survives a pH between 5 and 9, with a minimum tolerance of 7. High-salt-tolerance tilapia strains have been developed. They quickly adjusted to the much higher salt levels in the water. Mozambique tilapia thrives between 22 to 30 degrees Celsius, which is perfect for their growth and reproduction. Yet, the species may keep going even when the thermometer reads 16 to 39 degrees Celsius. Tilapia can survive in water with just 2 mg/L of dissolved oxygen and 50 mg/L of ammonium. Mozambique tilapia is a large fish species with spines on their tails. Native species are often very finely banded and have a pale yellow or green coloration. The average adult is a little over 15 inches in height and 1.1 kilogram (2.5 lb) in weight. Species may vary in size and colouring due to both breeding and environmental variables. In aquaculture settings, male tilapia matures sooner than female tilapia. Gaining in popularity is the mislabelled "Florida Red" tilapia, which is really a Mozambique blue. Being an aquaculture-friendly and hardy fish that may be easily grown in a home aquarium, the Mozambique tilapia is a popular choice. Mozambique tilapia are easily recognised by their massive, spine-covered dorsal fins. They are opportunistic omnivores that feed on everything within their reach, including algae, plant detritus, organic particles, fish, and microscopic invertebrates (Australian Centre Tropical Freshwater Research, 2007). Polygynous Mozambique tilapia have been seen engaging in mouth brooding, in which they transport embryos and young fry in their mouths before to, during, and after delivery. They may reproduce in a variety of habitats and climates. Reproductive abilities of Mozambique tilapia may vary greatly amongst populations due to their ability to delay their own growth. As they are capable of stifling their own development, this is the case. The Mozambique tilapia has several uses, including as food, in aquaculture, as a decorative fish, and to a lesser extent as a biological control agent (Canonico *et al.,* 2005).

MATERIALS AND METHODS

Experimental fish

Oreochromis mossambicus, a kind of freshwater fish, were collected at the Aquafish Aquarium fish farm on B.H. Road in Kottakal in the Malappuram District, Kerala, India. The fishes were average 12 gram in weight and 8 centimetres in length. The fishes were kept in the lab for a week prior to the experiment, where they were provided with a constant supply of water and a well-lit environment. This was accomplished by carefully delivering them to the lab in plastic bags with as little disruption as possible. The glass aquariums were cleaned with 1% $KMnO_4$ and dried in the sun to eliminate the possibility of fungus infection. Fish were fed the recommended quantity of commercial fish pellets three times a day and housed in thirty-liter glass aquariums with plenty of oxygen throughout the acclimation period. Light and dark cycles were managed at a ratio of twelve hours of light to twelve hours of darkness, and the aquarium water was dechlorinated and replaced regularly with new water. Researchers monitored the health of the fish and instantly eliminated those that seemed to be in bad condition from the aquariums. Preliminary screening and standardisation of physicochemical properties of tap water, including water temperature (28 to 29°C), pH (6.5 to 7.5), and oxygen saturation (70 and 100%), were carried out in accordance with the criteria established by the American Public Health Association (APHA, 1998). Features such as water temperature (between 28 and 29 degrees Celsius), pH (between 6.5 and 7.5), and oxygen saturation (between 70 and 100 percent) are all taken into account.

Chemicals

The chemicals used were of analytical purity and purchased from reliable local vendors. They were used without being refined further. Ethoxyquin (1,2-dihydro-2,2,4-trimethylquinolin- 6-yl ethyl ether) with a 75% purity was obtained from Sigma Aldrich in Germany.

Determination of median lethal concentration (LC50-96h)

In order to estimate the median lethal concentration (semi-static; 96 h-LC50) over a period of 96 hours, acclimatised fish were divided into six separate tanks. To reduce the quantity of food and trash that got into the test solution, fish were not fed for twenty-four hours prior to the commencement of the experiment. Five different concentrations of ethoxyquin (5, 10, 15, 20, and 25 mg/ L) were tested in fish. There were fifteen thriving fish in each of the 50-liter tanks. Triplicates were kept in perfect condition. A group of fishes who had not been exposed to any toxicants served as a control group together with the treatment groups. Every 24 hours during the study's duration of 96 hours, monitored the mortality and behavioural changes of fish in both the experimental and control groups. Median lethal concentration (96 h-LC50) is the concentration at which 50% of the fish population dies; this value was verified using the Probit tool of regression analysis with a 5% confidence limit (Finney, 1971).

Selection of sublethal concentrations
Sublethal doses, comprising one-tenth of the 96 h-LC50, were selected for further toxicological evaluation based on the median lethal concentration for 96 h duration.

Experimental design
This section describes the methodology used in the toxicity induction studies. The fishes were divided into seven groups with nine individuals in each group. The unfavourable "control group" was Group I (without toxicant). Sublethal doses of 96h LC50 (i.e. one tenth of the median lethal concentration) were given to animals in groups II to VII, who were subsequently exposed to the substance for varying amounts of time (24 hours, 48 hours, 72 hours, 96 hours, 5 days, 10 days, and 15 days, respectively).

Analysis of oxygen consumption
In order to calculate how much oxygen was utilised, we turned to Winkler's method (Welsh and Smith, 1961). First samples were collected from each group as soon as feasible before the trial began. None of the animals in these collections were injured in the course of this study. Every 24 hours, a sample was obtained from each of the test groups, and the oxygen consumption rate was calculated by multiplying the sample's oxygen content by the net weight of the fish, as described by Winkler's approach. The oxygen consumption rates (in millilitres of oxygen per litre of sample mass per hour) were determined for both untreated and treated samples. There were three separate runs of each experiment.

The samples to be tested was collected in a 300 mL BOD(Biological Oxygen Demand) bottle, with great care taken to avoid contaminating the sample with air. The bottle's stopper was then taken out, and one millilitre of a manganous sulphate solution was poured on top of the existing liquid. Next, 1 mL of alkaline potassium iodide sodium azide solution was added. The bottle was tipped upside down and forcefully shaken after the cork was replaced in such a manner as to avoid the formation of air bubbles. Then one millilitre of concentrated sulphuric acid was added into the bottle such that it would trickle down the neck of the container above the surface of the liquid, the bottle was shaken violently until the precipitate was fully dissolved. A titration was performed on the treated sample, and the volume was determined to be comparable to 200 mL of the original sample. By adding the chemicals, some of the sample was lost, and this result takes that into account. The volume was calculated using the formula: mL of sample to titrate = 200 x [300/(300-2)] = 201 mL Then 201 ml of sample was poured from the BOD bottle into a flask and titrated against 0.0250 N sodium thiosulfate to the first disappearance of the blue color and the total number of mL of sodium thiosulfate used was recorded and the dissolved oxygen was estimated by the formula mg/L oxygen consumption = (mL titrant x normality of titrant x 8000)/equivalent volume of sample titrated. The difference in dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish.

RESULT AND DISCUSSION
The amount of oxygen that a fish needs is called its "oxygen consumption." The amount of oxygen used is shown by the difference between the amount of dissolved oxygen in the first and last water samples. A lot of aquatic life depends on oxygen that has been mixed into water. Table 1 and Figure 2 show what happened when fish were exposed to ethoxyquin at different times and how much oxygen they used.

Exposure time	Initial water DO (mg/L)	Final water DO (mg/L)	O2 consumption (mg/L)
control	9.7	8	1.7
24hr	9.5	7.9	1.6
48 hr	9.1	7.7	1.4
72hr	8.85	7.62	1.23
96hr	8.7	7.58	1.12
5 days	8.5	7.41	1.09
10 days	8	6.97	1.03
15 days	7.7	6.81	0.89

Table 1: oxygen consumption in fish on EQ toxicity induction

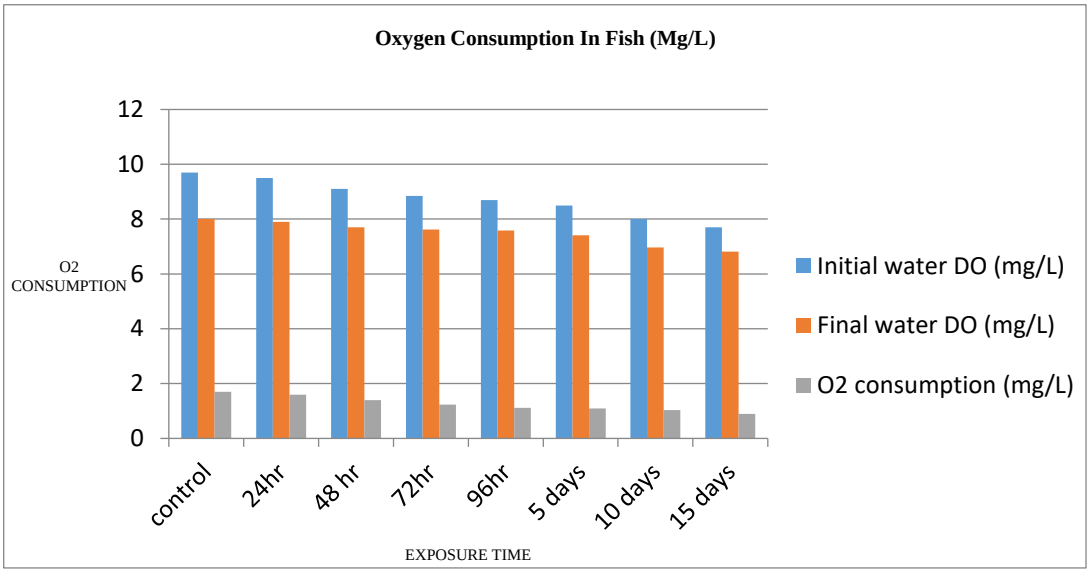


Figure 2: Bar graph showing DO levels & O2 consumption

Exposure of fish to the sublethal dose resulted in a gradual but consistent decrease in oxygen consumption from the beginning of the experiment to its end. Alterations in oxidative metabolism may cause respiratory distress and lead to changes in oxygen demand (Neelima *et al.*, 2016).Oxygen utilisation decreases as EQ molecules enter or accumulate in the body. When exposed to non-lethal levels of EQ, oxygen intake drops. This may be an adaptive or strategic reaction, since it reduces the amount of energy needed to keep the body functioning normally (Tilak and Vardhan, 2002). Furthermore, the fish may have undergone detoxification in order to recover from the EQ poisoning. Lowered oxygen consumption might be due to animals' failure to adapt to EQ's toxicity. Hence, as expected, fish activity reduced precipitously as oxygen levels plummeted. Fish given EQ for 24 hours displayed abnormal behaviour, including slow locomotion, frequent air intake, and dilated pupils. Together with these traits came protruding eyes. It is possible that a respiratory illness caused by the breakdown of the respiratory epithelium in the gill tissue is to blame for the low oxygen consumption seen in this study. Scientists have shown that toxicants cause fish to breathe less oxygen (Tilak and Swarnakumari, 2009). Reduced oxygen levels in toxic environments enhance the potency of existing poisons and the cumulative doses of poison that animals must endure. Compared to other animals, fish have a more rapid and robust breathing pattern. Fish should be able to tolerate chemical stress for the duration of sublethal exposure, the findings suggest.

SUMMARY AND CONCLUSION

Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is employed in many different foods and animal feeds due to its powerful antioxidant activity. Recent studies on animals on EQ diets have shown a broad range of unintended consequences. Those whose employment includes regular exposure to this drug have also been demonstrated to suffer negative effects. The entire metabolic rate and, by extension, the energy production of the experimental fish are reflected in the reduced quantity of oxygen consumption. This was one of the hypotheses tested in the present research, along with variations in dissolved oxygen and fish oxygen consumption. Reduced oxygen intake in EQ-exposed fish may be due to the stress on the respiratory system and the oxidative metabolism. Research shows that ethoxyquin is very toxic to the *Oreochromis mossambicus* fish, even at very low concentrations.

REFERENCES

1. Abbas SH, Ismail IM, Mostafa TM, Sulaymon AH. Biosorption of heavy metals: A review. Journal of Chemical Science and Technology. 2014;**3**:74-102
2. Alabaster JS, Lloyd RS. Water Quality Criteria for Freshwater Fish. 2nd ed. Cambridge: Butterworths; 1984
3. APHA.,1998,Standard methods for the examination of water and waste water, 20th Edition, Washington, DC.
4. Australian Centre Tropical Freshwater Research (2007). *Pest fish profiles – Oreochromismossambicus. James CookUniversity*. See http://www.jcu.edu.au/vhosts/actfr/Projects/Pestfish/Profiles/ProfileOmossambicus.htm.
5. Barbieri, E., Ferreira, L.A.A. 2010. Effects of the organophosphate pesticide Folidol 600® on the freshwater fish, Nile Tilapia (*Oreochromisniloticus*). *Pesticide Biochem. Physiol.*, 99: 209–214.
6. Błaszczyk, A., Osiecka, R. and Skolimowski, J. Induction of chromosomeaberrations in cultured human lymphocytes treated with ethoxyquin. **Mutat.Res.** 542 (2003) 117-128.
7. Canonico GC, Arthington A, McCrary JK and Thieme ML (2005). The effects of introduced tilapias on native biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems15:463–483.*
8. Chebbi, S.G., David, M. 2010. Respiratory responses and behavioural anomalies of the carp *Cyprinuscarpio*under quinalphos intoxication in sublethal doses. *Sci. Asia,* 36: 12–17.

9. Cole S, Codling I, Parr W, Zabel T, Nature E, Heritage SN. Guidelines for Managing Water Quality Impacts within UK European Marine Sites; 1999
10. Drewhurst,,1998, Ethoxyquin. JMPR Evaluationshttp://www.inchem.org/documents/jmpr/jmpmono/v098pr09.htm
11. Dube, B.N., Hosetti, B.B. 2010. Respiratory distress and Behavioural anomalies of Indian Major carp *Labeorohita*(Hamilton) exposed to Sodium cyanide. *Recent Res. Sci. Technol.*, 2(2): 42–48.
12. Francesco, P., Francesca, I., Umile, G.S., Giuseppe, C., Manuela, C., Nevio, P. 2008. Polymer in Agriculture: a Review. *American J. Agri. Biol. Sci*., 3: 299–314.
13. Franklin, R.K., Loo, H.S., Osumanu, H.A. 2010. Incorporation of Bentazone with *Exserohilumrostratum*for controlling *Cyperusiria*. *American J. Agri. Biol. Sci.*, 5: 210–214.
14. Gille, J.J.P., Pasman, P., vanBerkel, C.G.M. and Joenje, H. Effect of antioxidants on hyperoxia-induced chromosomal breakage in Chinese hamster ovary cells: protection by carnosine. Mutagenesis6 (1991) 313-318
15. Korami, D., Eric, H., Charles, G. 2000. Concentration effects of selected insecticides on brain acetylcholinesterases in the common carp (*Cyprinuscarpio*L.). *Ecotoxicol. Environmental Safety*, 45: 95–105
16. Little, A.D. Chemical Committee Draft Report, Ethoxyquin, CAS Number 91-53-2, Submitted to National Toxicology Program, Executive Summary of Safety and Toxicity Information (1990)
17. Logaswamy, S., Remia, K.M. 2009. Impact of cypermethrin and ekalux on respiratory and some biochemical activities of a freshwater fish, *Tilapia mossambica*. *J. Curr. Biotica*, 3(1): 65–73.
18. Martínez-Álvarez, R.M., Morales, A.E., Sanz, A. 2005. Antioxidant defenses in fish: Biotic and abiotic factors. *Reviews in Fish Biology and Fisheries*, 15: 75–88
19. Mushigeri, S.B., David, M. 2003. Assessment of Fenvelerate toxicity on oxygen consumption and ammonia excretion in the freshwater fish, *Cirrhinusmrigala*. *J. Ecotoxicol. Environ. Monitoring*, 13: 191–195.
20. Nathanson JA. Basic Environmental Technology: Water Supply. New Delhi: Printice-Hall of India; 2004
21. Neelima P, Gopala Rao N, srinivasa Rao and Chandra Sekhara Rao, "A Study on Oxygen Consumption in a Fresh water Fish *Cyprinuscarpio* Exposed to Lethal and Sublethal Concentrations of Cypermethrin (25% Ec), Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur, AP, India;International Journal Of Current Microbiology and Applied Sciences,volume 5,pp.338-348, 2016.
22. Patil, V.K., David, M. 2008. Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeorohita*(Hamilton). *Turkish J. Fisheries and Aquatic Sci.*, 8: 233–237.
23. Shah C. Which Physical, Chemical and Biological Parameters of Water Determine Its Quality?; 2017
24. Singh, K.S., Singh, S.K.S.,Yadav, R.P. 2010. Toxicological and biochemical alterations of cypermethrin (synthetic pyrethroid) against freshwater teleost *Colisa fasciatus* at different seasons. *World J. Zool.*, 5(7): 25–32.
25. Somaraj, I.R., Ranjitsingh, A.J., Pushparaj, A., Ramathilagam, G. 2005. Pesticidal stress influenced respiratory alterations in the fresh water fish, *Mystusvittatus*. *Indian J. Environ. Ecoplanning*, 10: 803–806.
26. Subramanian, M.A. 2004. Toxicology, M J P Publishers, Chennai. p. 202.
27. Tchobanoglous G, Peavy HS, Rowe DR. Environmental Engineering. New York: McGraw-Hill Interamericana; 1985
28. Tchobanoglous G, Schroeder E.Water Quality: Characteristics, Modeling, Modification. 1985.
29. Tilak, K.S., Swarnakumari, R. 2009. Acute toxicity of Nuvan, an organophosphate to freshwater fish, *Ctenopharyngodonidellus*and its effect on oxygen consumption. *J. Environ. Biol.*, 30(6): 1031–1033.
30. Tilak, K.S., Vardhan, S.K. 2002. Effect of fenvalerate on oxygen consumption and haematological parameters in the fish, *Channa punctatus* (Bloch). *J. Aquatic Biol.*, 17: 81–86.
31. Tilak, K.S., Veeraiah, K., Thathaji, P.B. 2007. Histopathological changes in the kidney of the fish *Channa punctata* exposed to sublethal concentration of Butachlor and Machete. *J. Ecotoxicol. Environ. Monitoring*, 17(2): 129–134.
32. Welsh, J.H., and Smith, R.I., 1961, Laboratory exercise in invertebrate physiology. Burgess Publishing Co., Minneapolis.