ISSN Print/Online: 2320-9577/2320-9585
INTERNATIONAL JOURNAL OF PURE AND APPLIED ZOOLOGY
Volume 1, Issue 2, June 2013
Available online at: http://www.ijpaz.com
RISHAN PUBLICATIONS
RESEARCH ARTICLE OPEN ACCESS

SUBLETHAL EFFECT OF PROFENOFOS ON OXYGEN CONSUMPTION AND GILL HISTOPATHOLOGY OF THE INDIAN MAJOR CARP, *CATLA CATLA* (HAMILTON)

A.MAHARAJAN*, R.USHA, P.S. PARU RUCKMANI, B.S.VIJAYKUMAR, V.GANAPIRIYA AND P.KUMARASAMY

PG and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, Tamilnadu, India

*Corresponding Author Email address: athimaha@yahoo.co.in, Tel: +91 9443286900

Article History: Received: 04.05.2013, Accepted: 19.06.2013

ABSTRACT

As aquatic organisms have their outer bodies and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced. Pesticides enter into the fish mainly through gills and with the onset of symptoms of poisoning, Profenofos, a well-known organophosphate pesticide has been in agricultural use over the last two decades for controlling pests of paddy, cotton and tobacco. In the present study, an attempt has been made to study the effect of profenofos on oxygen consumption and gill histopathology of the Indian major carp, *C. catla*. The fishes were exposed to sublethal concentration ($1/10^{th}$ and $1/20^{th}$ of 96 h LC_{50}) of profenofos for a period of 15 days. The treatment of these profenofos brought about significant decrease in oxygen consumption as compared to control. Exposure to profenofos was found to result in several alterations in the histo-architecture of the gills of *C. catla.* The alterations included curved secondary gill filaments, necrosis of gill filaments and conjestion of Secondary Lamellae. The significance of this study as a bio-indicator for assessing the toxicity and economic importance of the fish is discussed.

Keywords: Profenofos, gills, oxygen consumption, histopathology, *Catla catla.*

INTRODUCTION

India is primarily an agro-based country with more than 60-70% of its population dependents on agriculture. However, 30% of its agricultural production is lost owing to pest infestation. In the absence of a better alternative, deployment of pesticides becomes inevitable despite their known hazardous effects. Utilization of pesticides in India is about 3% of the total world consumption and is increasing at the rate of 2-5% per annum (Bhadbhade *et al*., 2002). Usage of Organophosphorus (OP) pesticides is found to be increasing in recent years since they are biodegradable and therefore persist in the environment only for a short time. Because of their low persistence, repeated applications of these pesticides are being practiced for the control of pests in agricultural fields and thereby large quantities find their way into water bodies

(Jyothi and Narayan, 1999). A large numbers of pesticides for the control various agricultural pests; however, their toxicological impact also extends to non target species like fish (Naqvi and Vaishnavi, 1993). Fish is a good indicator of aquatic contamination because its biochemical stress responses are quite similar to those found in mammals (Mishra and Shukla, 2003).

Profenofos, a well known organophos-phate pesticide has been in agricultural use over the last two decades for controlling pests of paddy, cotton and tobacco. Profenofos has been classified as a moderately hazardous (Toxicity class II) pesticide by the World Health Organization (WHO) and it has a moderate order of acute toxicity following oral and dermal administration. Profenofos is extremely toxic to fishes. The acute toxic action of profenofos is the inhibition of the acetylcholine esterase activity resulting in neuro toxicity to aquatic vertebrates and also humans. Microbial degradation of organophosphate pesticides is of particular interest because of the high mammalian toxicity of such compounds and their widespread and extensive use. The most significant step in detoxifying organophosphate compounds is hydrolysis since that makes the compounds more vulnerable to further degradation. The enzyme responsible for catalyzing this reaction is referred as an esterase or phosphotriesterase.

Depletion in oxygen content occurs in the medium when pesticides, chemicals, sewage and other effluents containing organic matter are discharged into water bodies. Pesticides in sub lethal concentrations present in the aquatic environment are too low to cause rapid death directly but may affect functioning of the organisms, disrupt normal behaviour and reduce the fitness of natural population. In the aquatic environment one of the most important manifestation of the toxic action of chemical is the over stimulation or depression of respiratory activity. The changes in the respiratory activity of fish have been used by several investigators as an indicator response to environmental stress. The respiratory potential or oxygen consumption of an animal is the important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general (Prosser and Brown, 1977). Pesticides are remarkable in causing respiratory distress or even failure by affecting respiratory centers of the brain or the tissues involved in breathing.

As the aquatic organisms have their outer bodies and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced. Pesticides enter into the fish mainly through gills and with the onset of symptoms of poisoning, the rate of oxygen consumption increases (Premdas and Anderson, 1963). Holden (1973) observed that one of the earliest symptoms of serves not only as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also useful to correlate the behavior of the animal.

By cannulating the blood system of fishes, it is possible to measure the concentrations of oxygen, metabolites and pollutants and hence understand more fully the mode of action of toxic pollutants. Skidmore (1970) using cannulation techniques, found that zinc reduced the oxygen level of blood leaving the gills. It reduced the efficiency of oxygen transport across the gill membrane, so that fish die of hypoxia. Respiratory responses to lethal concentrations increase the ventilation volume and symptoms of pyrethroid intoxication suggesting that the effect on respiratory surface is lethal in fish. It is known that pyrethroids are less persistent and are effective substituents for organ chlorine (OC) pesticides. Like OC compounds, the mechanism of pyrethriod interference is with nerve membrane function through the interaction with the sodium channels. The symptoms of profenofos intoxication suggest that, besides effect on the nervous system, effect on respiratory surfaces and renal ion regulation may be associated with the mechanism of lethality in fish (Bradbury *et al.,* 1987).

Total oxygen consumption is one of the indicators of the healthy status of a fish. It may also be useful to assess the physiological condition in an organism, helps in evaluating the susceptibility or resistance potentiality and also

useful to correlate the behavior of the animal, which ultimately serve as predictors of functional disruptions of population. Hence the analysis of oxygen consumption can be used as a biodetectory system to evaluate the basic damage inflicted on the animal which could either increase or decrease the oxygen uptake. Therefore an attempt was made to study the effect of sub lethal concentrations of profenofos on oxygen consumption and gill histopathology of Indian major carp, *Catla catla.* The present investigation of toxicity of profenofos in *C. catla* is carried out. Since, it is cultured in the ponds of Delta Districts of Tamil Nadu. The culturing pond receives river runoff from paddy fields has the possibility of containing pesticides.

MATERIALS AND METHODS

Test Animal Collection and Maintenance

Live fish, *C. catla* of size ranging from 10-12g were collected from R.K, Fresh water fish farm, Orathanadu, Thanjavur Dist, Tamil Nadu. They were transported and kept for acclimatization in rectangular tank of 100 l capacity containing well aerated filtered fresh water maintained at ambient temperature (27± 2°C) for a period of one week. Before stocking, the tank was washed with clean water several times. Finally, the tank was washed with 0.1% $KMnO_4$ for disinfection. Before introducing into the tank, the fishes were screened for any visible pathological symptoms and were treated with 0.1% of $KMnO_4$.

Preparation of Stock Solution for Profenofos Toxicity Test

One milliliter of profenofos was dissolved in one liter of double-distilled water and used as the stock solution for preparing different concentrations of profenofos in rearing water. It was stored in a clean standard flask at room temperature, in the laboratory.

Exploratory Test

Exploratory tests, otherwise called range finding test, were carried out to assess the approximate effective concentration range of Profenofos required for conducting short term tests to assess the effect of Profenofos on the metabolic function of the fish, as recommended by APHA (1985). The test solutions were prepared over a wide range of concentrations. These tests were performed by exposing 10 specimens of *C. catla s* in 10 litre fresh water containing different concentrations of Profenofos. The dead animals were removed immediately. Death of each animal was recorded. Three replicates were made for short term toxicity tests, the least concentration was chosen where no mortality was recorded in 24hrs and the highest lethal concentration was where 100% mortality was recorded in 24hrs.

Acute Toxicity test

To study the toxicity of Profenofos, the Static Bioassay Method (APHA, 1985) was followed. The test individuals were exposed to selected and serially diluted Profenofos concentrations. For acute toxicity test, 10 active animals each were exposed to various concentrations of the Profenofos (0.04, 0.06, 0.08, 0.10, 0.12, 0.14 and 0.16 ppm) using fresh water as control. The manifestation time and survival time of fish were observed. Fishes were exposed to the above said concentrations along with common control. Experimental animals were starved for 1 week. The experiments were conducted in three replicates at room temperature. No feed was given during the test period.

Sublethal toxicity tests

For sublethal toxicity tests, the fishes were grouped into three batches. Each batch had 10 animals and had 3 replicates.

Group I: Fishes maintained in normal Fresh water and served as control.

Group II: Fishes exposed to the sublethal concentration of 0.0008ppm ($1/10^{th}$ of LC_{50} value for 96 hours) of Profenofos in fresh water.

Group III: Fishes exposed to the sublethal concentration of 0.0016 ppm ($1/20^{th}$ of LC_{50} value for 96 hours) of Profenofos in fresh water.

The media were renewed every alternate day. Fishes were fed daily with artificial feed .Two specimens each from the groups I, II and III were sacrificed after 0, 5^{th} 10^{th} and 15^{th} days of the experiment. After their respective exposure

period, oxygen content was estimated by Winker's method (Welch, 1952).

Evaluation of Histopathology

Fishes were exposed to profenofos at two sublethal concentrations for 15 days. Sampling was done on the 5th and 15th day of exposure; five fishes in each group were sacrificed. The gills of representative fish from each test and control group were dissected out and fixed in Davidson's fixative for 24 h. The preserved tissues were processed by a routine histological method (Humason, 1972), dehydrated in alcohol series and embedded in paraffin wax. They were cut into sections of 6 mm thickness by a rotary microtome (Weswox, MT1090:1090A, India). The thin sections of the tissues were stained by haematoxylin and eosin for observation by the Nikon Bright field transmission microscope with Koechler illumination, and an automatic exposure unit was used.

RESULTS

Acute Toxicity Test

Acute toxicity study was done to find out the impact of profenofos on *C. catla* within a short period. In the present study the 96hrs LC_{50} value was found to be 0.0079ppm. Among the test concentrations prepared from the preliminary toxicity test the mortality of 50% of the population after 96hrs exposure was observed on 0.0079ppm concentration of profenofos.

Rate of Oxygen Consumption

Fishes are quickly responding to the pollutants by altering their metabolic rate. The rate of metabolism is direly proportional to the oxygen consump-tions of the fish exposed to the sub lethal concentrations of profenofos showed significant variations. After 5th, 10th and 15th days of toxicants there is a sudden slash down in oxygen consumptions decreased with increase in concentrations of profenofos (Figure 1).

Histopathology of Gills

The transverse section of gill tissue of normal fish shows branching form the central axis called the primary gill lamellae. Each of the primary lamella further divides into secondary gill lamellae or filaments. Within each division of the gills are the adjacent efferent vessels and afferent vessels with hemocytes. The primary and secondary gill filaments are separated by a thin septum. The secondary non branching filament lamella possesses epithelial pillar cells separated by large lacunae (Plate 1).

After 5 days of exposure to 0.0008 ppm concentration of profenofos, the gill tissues revealed large inter-lamellar space, necrosis, lamellar fusions enlarged secondary gill lamellae, curved secondary gill filaments resulting in distension of the lamellae (Plate 2). Similarly, after 15 days of exposure, further swelling of tip of secondary gill lamellae and their erosion were observed. Rupture of capillaries at the tip of secondary gill lamellae releasing blood cells was also seen at some places. Hemorrhage between gill filaments, dilation in blood vessels of gill filaments and Telangiectasis in secondary lamellae were observed (Plate 3).

After 5 days of exposure to 0.0016 ppm concentration of profenofos, a large number of hemocytes accumulated in secondary gill lamellae and resulted in enlargement of gill lamellae. Consequently the separation of epithelial cells from the basement membrane and fusion of secondary gill lamellae were also observed (Plate 4). Similarly, after 15 days of exposure, the gills also exhibited lamellar fusion at some places as a result of filamentary epithelium proliferation. In addition, a few aneurisms were also observed in gill lamellae. In some areas, epithelial hyperplasia, swelling of epithelial cells and bronchial epithelium disorganization were evident. Furthermore, the epithelial layer detached completely from the central portion of each lamella (Plate 5).

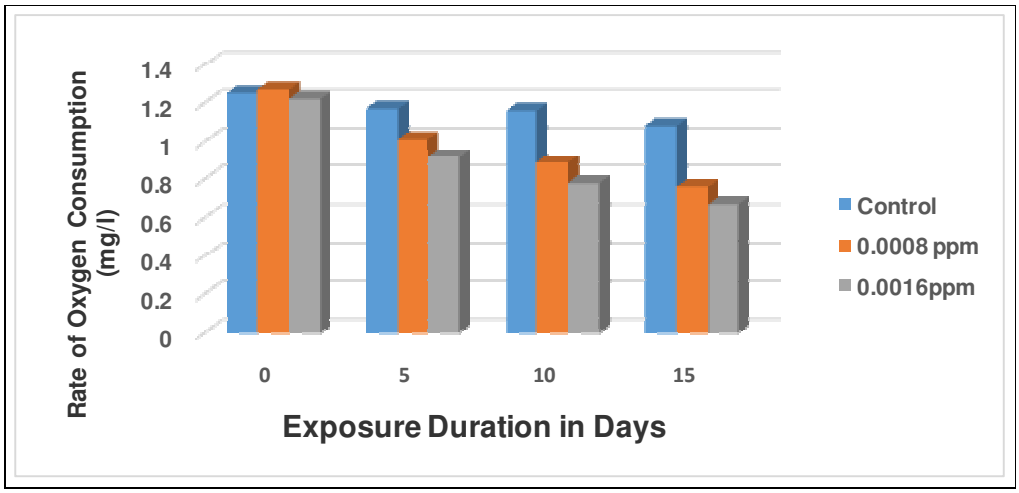

Figure 1. Effect of profenofos on the rate of oxygen consumption in Indian major carp, *Catla catla.*

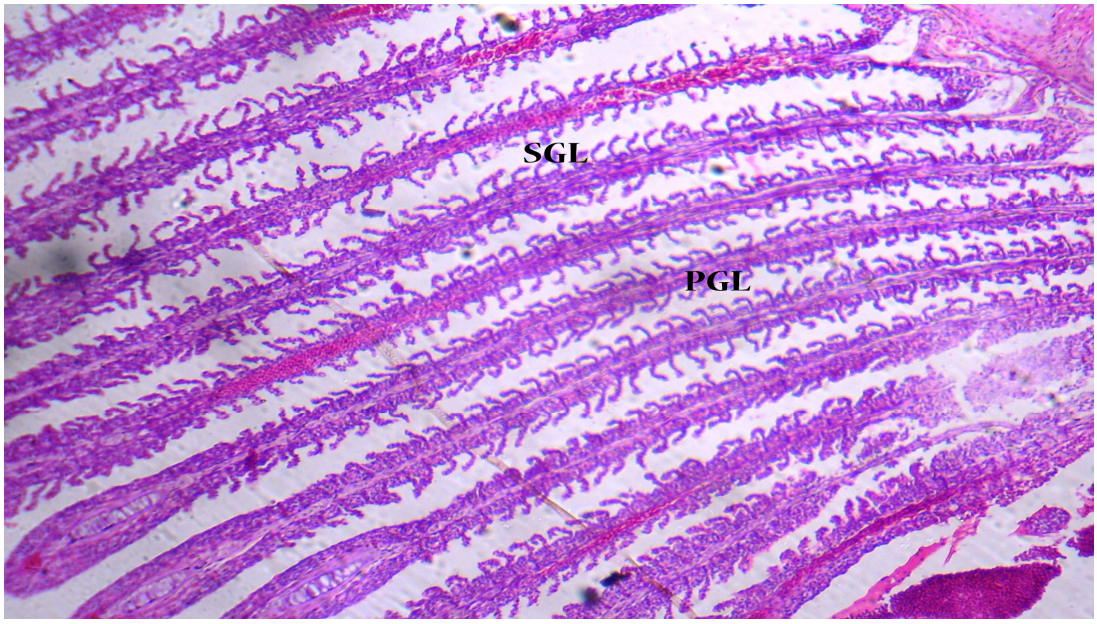

Plate 1. Photomicrograph showing the T.S of control gill in *Catla catla* (Abbreviations: PGL- Primary Gill lamellae, SGL- Secondary Gill lamellae).

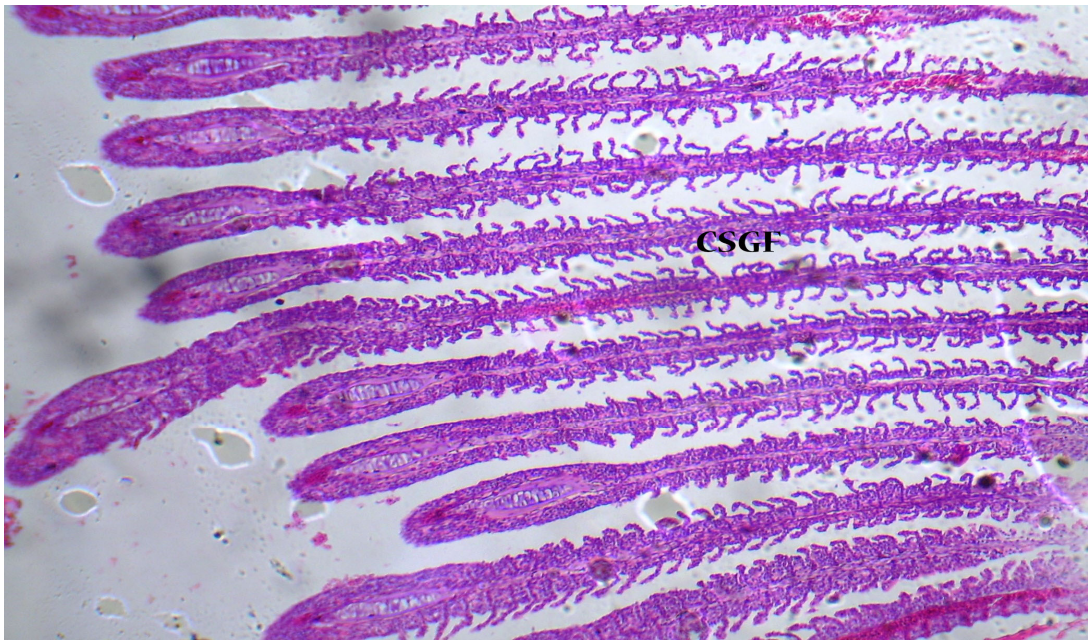

Plate 2. Photomicrograph showing the T.S of control gill in *Catla catla* treated with 0.0008 ppm concentration of profenofos after 5th days of exposure (Abbreviations: CSGL- Curved Secondary Gill Filaments).

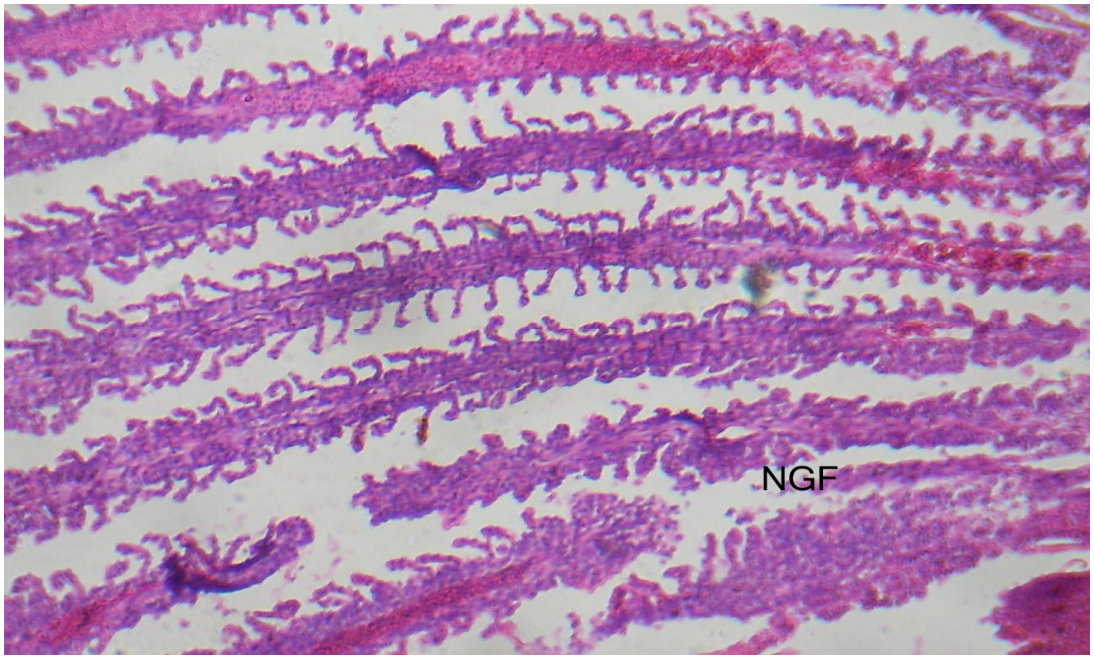

Plate 3. Photomicrograph showing the T.S of control gill in *Catla catla* treated with 0.0008 ppm concentration of profenofos after 15th days of exposure (Abbreviations: NGF- Necrosis of Gill Filaments).

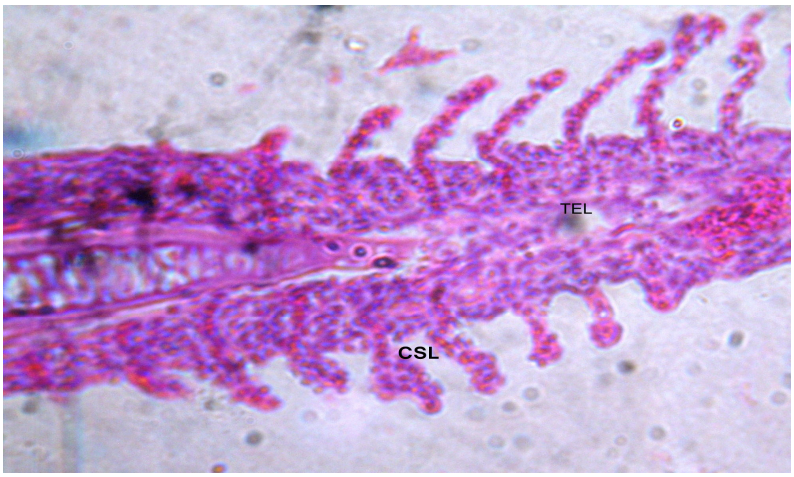

Plate 4. Photomicrograph showing the T.S of control gill in *Catla catla* treated with 0.0016 ppm concentration of profenofos after 5th days of exposure (Abbreviations: TEL – Telangiectasis, CSL – Conjestion of Secondary Lamellae).

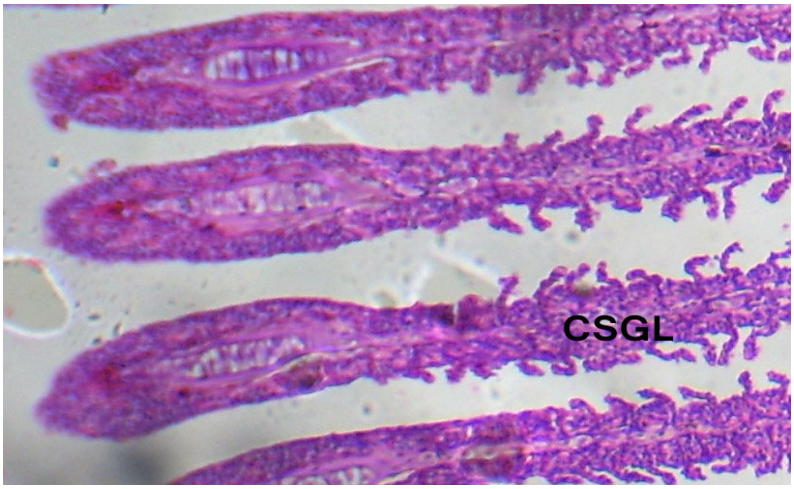

Plate 5. Photomicrograph showing the T.S of control gill in *Catla catla* treated with 0.0016 ppm concentration of profenofos after 15th days of exposure (Abbreviations: CSGL – Conjestion of Secondary Gill Lamellae).

DISCUSSION

During this study severe respiratory distress, rapid opercular movements, leading to the higher amount of toxicant uptake, increased mucus secretion, higher ventilation volume, and decrease in the oxygen uptake efficiency, laboured breathing and engulfing of air through the mouth were observed in all the three major carps exposed to both the toxicants. However, the above said changes in the fish were more pronounced Profenofos. The increased oxygen consumption in *Labeo rohita* and *C. catla* under sub lethal concentrations of both the toxicants is in colloboration with the increased consumption of oxygen in trout exposed to permethrin (Haya, 1989). The decrease in oxygen consumption at sub lethal concentrations of the toxicant indicates lowered energy requirements which in turn indicate pronounced haematological changes (Tilak and Satyavardhan, 2002).

Histopathological changes observed were hemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells, distortion of the secondary lamellae, and disruption of the secondary lamellar, disruption of epithelial cells from pillar cells. Shorter gill lamellar, fusion, complete destruction of lamella, increased vacuolation, and irregular appearance of gill lamellae were observed in *C. catla* exposed to profenofos.

Histopathological results indicated that gill was the primary target tissue affected by profenofos. Gills are generally considered good indicator of water quality (Rankin *et al.,* 1982) since the gills are the primary route for the entry of pesticide. In fish, gills are critical organs for their respiratory, osmoregulatory and excretory functions. Many investigators have reported the histopathological changes in gills of different fish species exposed to pesticides. Mucus extrusion, lamellar swelling, fused and reduced microridges, were observed in blud gill sunfish, *Lepomis macrochirus* to different sublethal concentrations of monocrotophos on the gills of *Anabas testudineus* was reported by Santhakumar *et al*. (2001). In the present study accordant that uncontrolled regeneration of the primary lamellae and secondary lamellae, hypertrophy, hyperplasia, necrosis of the epithelial cells, epithelial lifting, dilation of the blood sinuses of the secondary lamellae, lamellar aneurism, hemorrhages in the gill of fish exposed to profenofos.

Changes in the gill surfaces and increased mucus production are consistent with observed histological effects such as gyperplasia, necrosis and lamellar aneurysms in all the three fish exposed to sub lethal concentration of profenofos. Kumaraguru *et al.,* (1982) reported that the gill is the target organ for synthetic pyrethroid toxicity in fish. The technical as well as commercial formulations will pass through the gills and interfere in the gill movements which are directly proportional to the respiratory activity of the fish, primarily affecting the oxygen uptake. The respiratory metabolism was impaired and damage was also observed in the gill of fish exposed to pesticides (Ramamurthy, 1988). In the freshwater fish, *Ctenbopharyngodon diella* exposed to Nuvan an organophosphate, the depletion of the oxygen consumption is due to the disorganization of the respiratory action caused by rupture in the respiratory epithelium of the gill tissue. The experimental data reveals that oxygen consumption decreases with the time of exposure to toxicant (Tilak and Swarna kumari, 2009).

Under toxic conditions, the oxygen supply becomes deficient and a number of poisons become more toxic increasing the amount of poison being exposed to the animal. The fish breathe more rapidly and the amplitude of respiratory movements will increase. Lloyd (1961) reported that the toxicity of several poisons to rainbow trout increased in direct proportion to decrease in oxygen concentration water. This reduces the rate of passage of blood through the gills, so allowing a longer period of time for uptake of oxygen, and also conserves oxygen by reducing muscular work. The zone of resistance is reached when the oxygen tension in the water is so low that homeostatic mechanisms of the fish are no longer able to maintain the oxygen tension in the afferent blood and the standard metabolism begins to fall.

Conclusions

Changes in the architecture of gill under profenofos stress would alter diffusing capacity of gill with consequent hypoxic/anoxic conditions and thus respiration may become problematic task for the fish. The results of the present study suggest that the altered rates of respiration of fresh water fish may serve as a rapid biological monitor of the pesticide exposure to important components of fresh water community.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal and the HOD of Zoology, Khadir Mohideen College for the facilities provided to carry out this work and encouragements.

REFERENCES

APHA, 1985. Standard methods for the examination of water and waste water APHA, AWWA and WPCF, New York.

Bhadhade, B.J., Sarnaik, S.S. and Kanekar, P.P. 2002. Bioremediation of an industrial effluent containing monocrotophos. *Cur. Microbiol*., **45**: 346-349.

Bradbury. S.P.M., Coats, J.R. and McKim, J.M. 1986. Toxicokinetics of in rainbow trout *Salmo gaideri*. *Environ.Toxicol.Chem*., **5**: 567-576.

Haya, K. 1989. Toxicity of pyrethroid insecticides to fish. *Environ. Toxicol. Chem*., **8**: 381-391.

Holden, A.V. 1973. Environmental Pollution by pesticides (Ed), CA Edwards, Plenum Press, Chapter 6, 542.

Humason, G.L. 1972. Animal tissue Techniques, 3rd Ed., W.H. Freeman and Company, San Francisco.

Jyothi, B. and Narayan, G. 1999. Certain pesticide- induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus* (Linn.). *Food. Chem. Toxicol*., **37**: 417- 421.

Kumaraguru, A.K, Beamish, F.W.H. and Ferguson, H.W. 1982. Direct and circulatory paths of permethrin causing histopathological changes in the gills of rainbow trout. *J. Fish Biol.,* **20**(1): 87-91.

Llyod, R. 1961. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout, *Salmo gairdneri*. *J. Exp. Biol*., **38**: 447-455.

Mishra, R. and Shukla, S.P. 2003. Endosulfan effects on muscle malate dehydrogenase of the fresh water catfish *Claria batrachus. Ecotox. Environ Safe*., 425-433.

Naqvi, S.M. and Vaishnavi, C. 1993. Bioaccumulative potential and toxicity of Endosulfan insecticide to non-target animals. *Comp. Biochem. Phys.*, **105**: 347-361.

Premdas, F.H. and Anderson, J.M. 1963. The uptake and distribution of 14 C labelled DDT in Atlantic salmon, *Salma saleni*. *J. Fish Res. Board. Can.,* **20**: 827.

Prosser, C.L. and Brown, F. A. 1977. Comparative animal Physiology. 3rd Edition, W.B. Saunder Co., Philadelphia.

Rankin, J.C., Atagg, R.M. and Bolis, L. 1982. Effects of pollutants on gills. In: Gills, eds., D.F. Houlihan, J.C. Rankin, T.J. Shuttleworth. Cambridge University Press, pp. 207-220.

Ramamurthy, K. 1988. Impact of Hepatochloron on haematological, histological and selected biochemical parameters in the fresh water edible fish *Channa punctatus*. Ph.D. Thesis, S.V. University, Tirupathi, Andhra Pradesh, India.

Santhakumar, M., Balaji, M. and Ramudu, K. 2001. Gill lesions in the perch *Anabus testudineus*, exposed to monocrotrophos. *J. Environ. Biol.,* **22**: 87-90.

Skidmore, J.F. 1970. Respiration and osmoregulation in rainbow trout with gills damaged by Zinc sulphate. *J. Exp. Biol.,* **52**: 484-494.

Tilak, K.S. and Satyavardhan, K. 2002. Effect of fenevalerate on oxygen consumption and haematological parameters in the fish *Channa punctatus* (Bloch). *J. Aquatic Biol.,* **17**: 81-86.

Tilak, K.S. and Swarnakumari, R. 2009. Acute toxicity of Nuvan, an organophosphate to freshwater fish *Idella ctenopharyngodon* and its effect on oxygen consumption. *J. Environ. Biol*., **30** (6): 1031-1033.

Welch,T.S. 1952. Limnology, 2nd Ed.Mc.Graw Hill Book Co., New York, 538.

Cite this article as:
Maharajan, A., Usha, R., Paru Ruckmani, P.S., Vijaykumar, B.S., Ganapiriya, V. and Kumarasamy, P. 2013. Sublethal effect of profenofos on oxygen consumption and gill histopathology of the Indian major carp, *Catla catla* (Hamilton). *Int. J. Pure Appl. Zool.,* **1**(2): 196-204.