Histopathological Impact of Dimethoate Induced Toxicity on Gills for Oxygen Consumption in an Air Breathing Fish *Channa Gachua*

Qaisur Rahman[1]* and Shamim Akhter Choudhary[2]

[1]University Department of Zoology, Vinoba Bhave University, Hazaribag-825301 Jharkhand, India
[2]Department of Zoology, Government College for Women, Gandhi Nagar, Jammu-180004, J & K, India
*** Corresponding author: qaisur.rahman@gmail.com; drshamimch05@gmail.com**

Abstract
Behavioral alterations like uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, drowning and change in body pigmentation became more apparent with increase in duration of exposure at all test concentration. The results of the water quality of the tap water used in the bioassay are in the normal range and suggest that parameters of the test water were not the cause of fish mortality. However, temperature, hardness, pH, alkalinity and biological factors such as sex, age, health, weight and physiological status are reported to have profound effects on the acute toxicity of pesticides in *Channa gachua*. Toxicity of dimethoate is relatively lower when compared to other air breathing fishes. In the present investigation the histopathological effects of dimethoate in *Channa gachua* were exposed to sublethal concentration of i.e. 1/10th of 96 hour LC_{50} (0.599 ppm) for 30 days for study of histopathology and oxygen consumption. The histopathological studies revealed pathological changes in the gills. The rate of oxygen consumption was also found to be increased initially up to 48 hours then decreased up to end of experiment. The details will be discussed in this paper.
Keywords: Histopathology, dimethoate, oxygen consumption, *Channa gachua*

Introduction

While liberal use of chemical fertilizers and synthetic pesticides helped in ensuring food security to rising population, it inflicted severe injury to the environment especially to the health of soil and aquatic ecosystem. Most pesticides used in agriculture and in hygiene programs are non selective, more or less persistent and bio accumulate in the food chain and pose great danger to the health of non target organism in fresh water. Although mostly pesticides occur at low concentrations in ponds and other water bodies they create serious problems for non target aquatic biota especially the fishes due to their extensive range of biological activity affinity and stability. Fishes are one of the most susceptible animals to pesticide pollution because of their anatomy and physiology. Fishes live in intimate contact with surrounding water through their gills and branchial surface comprises over half the surface area of the body. Only a few microns thick delicate gill epithelium separates the internal environment of fish from external aquatic environment which makes the fish very susceptible to aquatic pollutants respectively. Therefore, contamination of water bodies by pesticides causes acute and chronic poisoning of fish and results in severe damage to vital organs (Singh *et. al.,* 2009). Dimethoate is a broad spectrum systemic organophosphate insecticide active against acaridae, aphididae, aleyrodidae, coccodidea, coleopteran, collembola, diptera, Lepidoptera, pseudococcidae and thyanoptera in cotton, cereals, fruits, vegetables, tea, coffee, tobacco and pastures (Aysal *et. al.,* 2004). Like other organophosphates, dimethoate is an inhibitor of acetyl cholinesterase and causes accumulation of acetylcholine in nerve tissue and effecter organs with the principal site of action being the peripheral nervous system. The accumulation of acetylcholine results in a prolonged stimulation of the cholinergic receptors downstream leading to intense activation of autonomic nervous system, which depending upon the

severity of acetyl cholinesterase inhibition results in tremors, convulsion, respiratory arrest and death. Though the organophosphate pesticide may disappear rapidly from the body either by hydrolysis or elimination, long term and repeated exposure to these pesticides have cumulative effect on fishes respectively. In the aquatic environment the pesticides pollute the ecosystem and find their way into the body of aquatic animals by means of gills, digestive tract and general body surface. Some pesticides accumulate in different tissues of body and produce toxic effects. In fishes it is observed that the organs are affected due to foreign bodies or toxic materials causing loss of equilibrium, irregular movements, and increase in opercular movements, imbalance and finally leading to death. Histopathology deals with the study of pathological changes induced in the microscopical structure of body tissue. Any peculiar alteration of cells may indicate the presence of disease or the effect of toxic substance. In fishes, it is observed that the external organ get affected due to toxic chemical causing irregular movement, loss of equilibrium, increased opercular movement, shedding of scales, lesion on head and gills, finally leading to death.

Materials and Methods

Channa gachua is also known as an air breathing murrel fish belonging to the family channidae of the order channiformes. It is found in estuaries and freshwaters of India. It has a very good flavour and is popular as food. This fish has dual mode gas exchange mechanism as it extracts oxygen from water through gills and from air by accessory respiratory organs (Figure 1). The accessory respiratory organs comprise one pair of supra branchial chambers. The gills of *Channa gachua* had the same basic structure as those seen in most teleost fish four gill arches, each bearing two rows of filaments. Lamellae, the gas exchange units of the gills, projected from both sides of the filaments. The lamellae consisted of the pillar cell system covered by basement membrane and two or three epithelial cell layers. In general, the cells of the inner most epithelial cell layer were flat and those of the outermost cell layer were cuboidal. Mucous and chloride cells were distributed throughout the filament epithelium, which was stratified and contained in 5–7 cell layers as reported earlier by Rahman and Sadhu (2014).

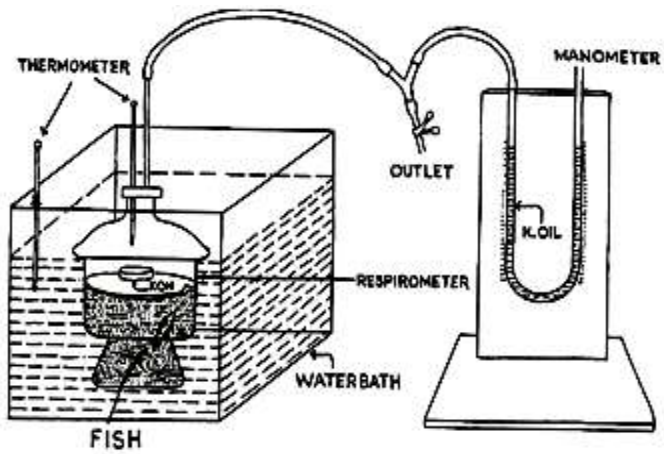

Figure 1: Experimental set up for the measurements of dual mode of oxygen uptake in *Channa gachua.*

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25º 59'N and Longitude 85º 22'E) and maintained in large glass aquaria size (90 x 60 x 60cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 15 days in the laboratory. Routine oxygen consumption from air and still water was measured in a closed glass respirometer containing 3 litres of water (initial O_2 content = 6.5 mg O_2/Litre, pH = 7.2) and 0.51 ML of air. The fish had free access to air through a small semi circular hole (10 cm diameter) in a disc float. Carbosorb (B.D.H) or KOH in a petridish placed on the float absorbed CO_2. Thus the fish could exchange gases with water by way of its gills as

well as with the air using the suprabranchial chamber. The air phase of respirometer was connected to a differential manometer. Movement of the manometer fluid follow uptake of oxygen when the CO_2 is absorbed by "Carbosorb" (KOH). The fish were acclimatized to the respirometers for at least 12 hours before the readings were taken. The concentration of dissolved oxygen in the water was estimated by Winkler's volumetric method (Welch, 1948).The oxygen uptake through gills was calculated from the difference between the oxygen levels of the ambient water in the respirometer before and after the experiment and the reading of volume of water in the respirometer. The oxygen uptake from air was measured and calculated from the reading of volume change in the manometer and by the use of the combined gas law equations and vapour pressure. Mean values of oxygen consumption in a series of observations, on each fish at standard temperature pressure dry and standard errors were calculated. The experiments were conducted at 29.0 ± 1.5°C. The pH of the ambient water was measured by an electronic pH meter (Systronics). The respiratory chambers were thermostated by immersion in a temperature controlled water bath. However, sexually mature fishes of almost same weight group (40-50g) were used respectively. For the study of histopathology and oxygen consumption the live test fish were cleaned by using 0.1% $KMnO_4$ to avoid the dermal infection. These fishes are acclimatized in the laboratory for two week prior to the experimentation. Fishes showing normal activity were selected for each test. The test fish, *Channa gachua* were exposed to sub lethal concentration of dimethoate 1/10th of 96 hrs LC_{50} (0.599 ppm) for 60 days. Simultaneously a control set was also maintained. At the end of exposure period the survived fishes were decapitated and immediately the tissues like gills removed and fixed in aqueous Bouin's fluid for 24 hours. These tissues were dehydrated in different grade of alcohol and blocks were prepared in paraffin wax (60-620C). The sections of 5-6 thickness were cut and stained with hematoxyline and Eosin and then mounted in DPX. At the same time rate of oxygen consumption was measured at 7, 15, 30, 45 and 60th days of exposure. Each experiment repeated three times.

Results and Discussion

In the present investigation, the histopathological and oxygen consumption alterations induced by treatment of dimethoate in tissues like gills. The gills of the fish exposed to dimethoate exhibited marked histopathological changes. The main features observed in gills exposed to sublethal concentration of dimethoate were partial degeneration of epithelium of secondary gill lamellae. In some place adjacent secondary gill lamellae appeared to adhere each other. Fusion of secondary gill lamellae resulting in reduction of respiratory surface and vacuolization was also recorded. No change was observed in primary gill lamellae. The effect of dimethoate on gill to different exposure period is shown in plate. The effect of dimethoate on the rate of oxygen consumption in 1, 7, 15, 30, 45 and 60 days exposure period is 0.6242, 0.5815, 0.6566, 0.5812, 0.6347 and 0.6514 in 0.0 ppm but in 0.599 ppm it was 0.5214, 0.7783, 0.8148, 0.6540, 0.5165 and 0.3847 mg/lit/g weight of fish/hour respectively. The similar results were reported by various workers (Ali, 1985; Choudhary and Pandy, 1987), while studied on toxicity of various pesticides on fresh water fish. Srivastava and Srivastva (1980) studied effect of sub lethal concentration of malthion chloride on the histopathology of the gills of *Channa gachua* and observed hyperplasia, hypertrophy vacillation in fish (Figure 2). Kumari and Kumar (1995) and Kumari and Kumar (1997) reported several histopathological changes in kidney, liver, gills, intestine and ovary due to impact of industrial effluents in the fish, *Channa punctatus* and *Hetropneustes fossils*.

Necrosis and destruction of secondary lamellae was noticed after four weeks of exposure. Tilak *et. al.,* (2005) reported that the effect of butachlor technical and machete 50% EC has induced marked pathological changes in fish gills. The changes included the bulging of tips of primary gill filaments, secondary filaments lost their original shape and cutting of secondary gill filaments, pillar cell nucleus showed necrosis and developed vacuoles in the secondary gill epithelium (Figure 3). Malla (1987) studied effect of fenvalerate and cypermethrin on the oxygen consumption of fish, *Cyprinus carpio* and reported that the significant drop in rate of oxygen consumption. Fall in the rate of oxygen consumption in this case was more at higher 3.7 mg/L concentration. Maximum reduction in oxygen consumption was noted at 48 hour and this is because of reduction in physiological activity and damage caused to the gills. Saxena and Chauhan (2003) reported that the decrease in dissolved oxygen caused a stress and resulted in an increase in the rate of oxygen

consumption by the fish while working on oxygen consumption in fish *Labeo rohita* (Ham) caused by distillery effluent. They stated that the inorganic and organic salts might have interfered with respiration in *Labeo rohita* by coagulation of gill mucous and caused asphyxiation as well as inhibition of enzyme system at mitochondrial level. This resulted in decreased in oxygen consumption. Prashant *et. al.,* (2003) studied effect of cypermethrin on toxicity and oxygen consumption in the freshwater fish *Cirrhinus mrigala*, and reported that the decreased in level of oxygen consumption exposed to lethal concentration for 1, 2, 3 and 4 days and also in sublethal concentration of 1, 7, 14 and 21 days. It is may be due to the respiratory dystress as a consequence of the impairment of oxidative metabolism. Aruna and Nagrajan (2007) worked on effects of different ratio of oxygen and water on the survival of gold fish *Carassius auratus* and reported that dissolved oxygen 7.2 mg/ h recorded in all the treatments during the start of the experimental period and latter these parameters gradually decreased at the end of the experimental period. On above literature on the rate of oxygen consumption and histopathology shows that rate of oxygen consumption decreased as concentration of toxicant and time of exposure period increased. It may be due to reduction in respiratory potential of gill tissues probably caused by tissues damage under pesticide tress or it may be due to suppression of metabolic activity of fish at lethal concentrations, dimethoate toxicity like other organophosphate is rapidly reflected in behavioral alterations of exposed fishes. Decrease in opercular rate appears to be an effort of exposed fish to reduce contact of gill epithelium with the poison. To compensate for the loss of oxygen uptake from water fish frequently swims to the surface to gulp air. Increased mucous secretion probably helps in countering irritating effect of dimethoate in skin and mucous membrane. Excitement, hyperactivity and abnormal jerky swimming observed in exposed fishes may be caused by accumulation of neurotransmitter in neuromuscular junction. Loss of balance and drowning reflect the progression towards death as fish succumbs to the continued high exposure of dimethoate. Similar alterations in behavior of dimethoate exposed fish have been reported earlier in *Heteropneustes fossilis* (Pandey *et. al.,* 2009) and *Cyprinus carpio* by Singh *et. al.,* (2009); Rahman and Shamim (2013) in *Channa gachua* reported that gills are vital respiratory organs and cellular damage induced by the metal might impair the respiratory function of the fish by reducing the respiratory surface area. It is concluded that dimethoate is highly toxic to fish which is swiftly reflected in behavioral alterations culminating in death. Further studies on toxicity of dimethoate and its combinations with other pesticides in laboratory and field may help in deciding judicious use of pesticides. Rahman *et. al.,* (2014) in *Channa gachua* reported that the histopathological changes found in gills of the examined fresh water fish are typical for the clinical finding in polluted with heavy metals water of habitat. Influence of water pollution is not only devastating to human being, animals, insects but also aquatic organisms. The more polluted industrial water destroys the aquatic ecosystem and reduced its biodiversity. The decrease in the rate of oxygen consumption after exposure to monocrotophos in *Channa gachua* due to the sluggishness of the fish, as a result of the pesticide stress and also the secretion of excessive mucous, which formed a thin film over the gill there by preventing absorption of oxygen during the process of gaseous exchange. The present study also suggests that, the dimethoate pesticide is very harmful to the aquatic life especially to the fishes, and its urgent need to control this water pollution.

Figure 2: Showing the gills structure with dimethoate in *Channa gachua*.

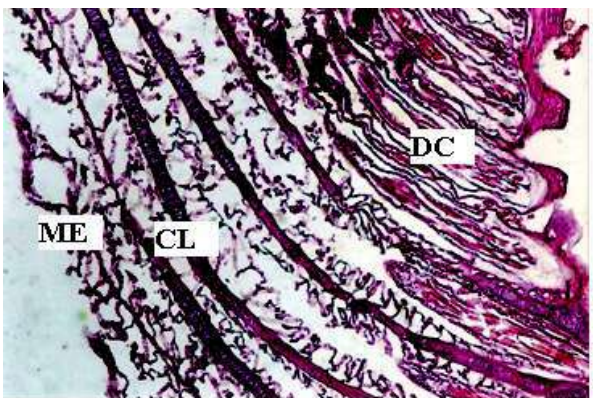

Figure 3: Showing the gills lamella and epithelium in *Channa gachua.*

Acknowledgement

The authors express their sincere thanks to Professor Dr. Azra Nahid Kamili, Ex. Director, Centre of Research for Development, Head, Department of Environmental Science, University of Kashmir, Srinagar for encouragement and valuable suggestions during the present investigation.

References

Ali, S. M., Ilyas R and Mokashi, N. V., 1985. Oxygen consumption of fish *Channa gachua* (Hamilton) after exposure to dimacron and aldicrab. ***Geobios.*** **2:** 44-48.

Arun K. J. 2007. Effects of different ratio of oxygen and water on the survival of gold fish (*Carassius auratus)* . ***J. Ecotoxicol. Environ. Monit.*** **17(2):** 197-199.

Aysal P., Tiryaki, O. and Tuncbilek, A. S., 2004. Dimethoate residues in tomato and tomato products. ***Bull. Environ. Contam. Toxicol***. **73:** 351-357.

Choudhay M. S. and Pandey, A. K. 1987. Histopathological changes in the gills of *Punctius ticto.* ***J. Environ. Biol.*** **24:** 67-71.

Kumari, A and Kumar S. N. 1995. Histopathological lesions caused by industrial effluents in kidney, liver and gill of fish, *Hetropneustes fossilis* in Hussain sagar lake, Hydrabad, India. ***Bull. Pure. App. Sci***. **14(2):** 57-64.

Kumari, A and Kumar S. R. 1997: Effect of polluted water on histochemical localization of carbohydrate in a freshwater teleost, *Channa punctatus* (Bloch) from Hussian sagar lake, Hydrabad, Andhra Pradesh. ***Poll. Res*****. 16(3):** 197-200.

Malla R. P. 1988. Effect of fenvalerate and cypermethrin on the oxygen consumption of fish, *Cyprirus carpio* ***J. Mendel***. 4: 209-211.

Pandey R. K., Singh R. N., Singh, S., Singh, N. N., and Das, V. K.. 2009: Acute toxicity bioassay of dimethoate on freshwater air breathing catfish *Heteropneustes fossilis* (Bloch). ***J. Environ. Biol*****. 30:** 437-440.

Prashanth, M.S., David, M. and Kuri, R. C. 2003. Effect of cypermethrin on toxicity and oxygen consumption in the freshwater fish, *Cirrhinus mrigala*. ***J. Ecotoxicol. Environ. Monitorin.*** **13**: 271–277.

Rahman, Q., and Shamim A. C., 2013: Effect of zinc cyanide on the behaviour and oxygen consumption in air breathing fish *Channa gachua***.** ***J. Res. Dev.*** **13:** 67-79.

Rahman, Q., Sadhu D. N., and Shamim A. C. 2014: Effect of monocrotophos on histopathological changes in gills of an air breathing fish *Channa gachua* (Ham.). ***J. Res. Dev.*** **14:** 9-13.

Rahman, Q. and Sadhu, D. N. 2014: Morphometrics partioning of the respiratory surface area and diffusion capacity of gills in an air breathing fish *Channa gachua*. ***J. Res. Dev.*** **14:** 66-74.

Saxena K K and Chauhan R. R. S. 2003. Oxygen consumption in fish, *Labeo rohita* (HAM.) caused by distillery effluent. ***Ecol. Environ. Conserv.*** 9:357–360.

Singh, R. N., Pandey R. K., Singh N. N. and Das V. K., 2009: Acute toxicity and behavioural responses of common carp *cyprinus carpio* (Linn.) to an organophosphate (Dimethoate). ***World. J. Zool.*** **4:** 70-75.

Srivastava, A. K. and Singh, N. N. 1980. Observation of hyper glycemia in the murrel *Channa punctatus* after acute exposure to methyl parathion. ***Comp. Physiol. Ecol.*** **5:** 100-107.

Tilak, K. S., Veeraiah, K. and Rao, D. K.. 2005. Histopathological changes in the gill, liver, brain and kidney of the Indian major carp Cirrhinus mrigala (Hamilton) exposed to chloropyrifos. ***Pollut. Res.,*** **24:** 101-111.

Welch, P. S., 1948. ***Limnological Methods.*** The Balkiston Company, Philadelphia. 381 pp.