International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 2, Issue 2, pp: 63-67, 2017
http://www.ijzab.com
https://doi.org/10.5281/zenodo.1311627

Research Article

STUDIES ON OXYGEN CONSUMPTION OF THE FISH *LABEO ROHITA* (HAMILTON) AS INFLUENCED BY TOXIC SYNERGISM

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Article History: Received 25th December 2017; Accepted 23th February 2017; Published 13th March 2017

ABSTRACT

An investigation was carried out to assess the impact of pathogens (*Pseudomonas putida*), pesticide (Methyl parathion) and metal (Ferrous sulphate) on the oxygen consumption of the fish *Labeo rohita* (Ham.). The effect of oxygen consumption was studied by exposing the fish *Labeo rohita* to the toxicant. The change in the oxygen increment was noticed with increasing exposure time.

Keywords: *Pseudomonas putida*, Methyl parathion, Ferrous sulphate, *Labeo rohita.*

INTRODUCTION

Pollution is an undesirable change in the physical, chemical or biological characteristics of our land, air or water that may or will harmfully affects human life or that of desirable species. Freshwater ecosystem consists of a large number of fauna and flora in them. These aquatic organisms are very sensitive to even a slight alteration in the environment. The causes for pollution are mostly man-made and therefore the prevention and control measures must be taken as much as possible. Freshwater is a prime necessity of life. Disposal of industrial and domestic waste into lakes and rivers pollute the freshwater and makes it unpotable. The pollutants usually consist of wastes from industries, pesticides, herbicides and fertilizers which run off from agricultural lands and the domestic waste caused due to urbanization. Green Revolution was a result of compulsion on the part of the Nation to meet the demands of ever increasing human population. It has fulfilled the Nation's priority of increasing food production by domesticating and altering the plant life, but has ended up with severe outbreak of pests. This has compelled the farmer to go in for pest control measures using pesticides. Pesticides are a mixed blessing: they improve agricultural productivity and public health through the control of disease carrying pests, but can adversely affect people and the non-target organisms such as fish and wildlife and the environment. Application of pesticides has become a necessary evil of the agro technology practices. Inadvertent and calendar-based application of pesticides has lead to resistance among the pests and eventually resurgence of pests that necessitated the use of more toxic variants of pesticides. As a result, toxic residue posed severe threat to the ecosystem. Considerable quantities of pesticides enter the aquifer due to seepage of residue and the aquatic system in the vicinity of agricultural fields receives considerable amounts of toxicants due to air drift and runoff.

Bacterial diseases are a constant threat to fish farming, and because of the rapid course and severity of manifestation, they represent a significant part in fish pathology and also have a great economic importance (Jeremic *et al.*, 2005). Harmful effect of bacterial diseases on fishes are increased morbidity and Mortality rate, decreased feed conversion efficiency, decreased growth rates, weakening of reproductive capability.

Pesticides are mainly used against pests of crop and disease causing vectors, but their improper use in agricultural practice has posed a serious threat to human life and his environment. Highly effective pesticides are used extensively and indiscriminately, which on entering the aquatic environment bring multiple changes in organisms by altering the growth rate, nutritional value, behavioral pattern.

In nature, aquatic animals are constantly exposed to toxicants including metallic ions. Concentrations of metals in water are determined by geochemical processes and

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large scale release into the aquatic environment by human activities. Intensive activity in industrial and agricultural sectors has inevitably increased the levels of heavy metals in natural waters (Jordao *et al.,* 2002). Heavy metals play a major role among pollutants of environmental concern (Singer *et al.,* 2005).

Although, trace metals are essential for normal physiological process, abnormally high concentrations can be toxic to aquatic organisms (Javed, 2003). Heavy metals being non-biodegradable primarily necessitate knowledge on their uptake, distribution and persistence in tissues of organisms (Lewis *et al.,* 2004).

Toxicity of a substance is known by its capacity to cause adverse effects on the living organisms. Toxic impact may bring about physiological, biochemical or pathological alterations in the organisms; the signs of toxicity may reveal symptoms of illness varying from simple local effects - structural and behavioral (Shivakumar *et al*., 2005) to complex disorders resulting in mortality. The intoxication includes a sequence of events that start with the exposure of a substance to an organism. Subsequently, the toxic substances are absorbed into the viscera by various routes causing an internal exposure (Tilak *et al*., 2005). Within the body of an organism, the substances are converted into metabolites which may either be more toxic or less toxic. The sequence of events and interaction of toxic substances with target molecules of organism depend on various factors such as the nature of the toxicant, duration of exposure, physiological state of the organism, biotic and abiotic factors of environment (Subramaniam, 2004).

Any variation in the environment acts as a stress on the organisms. When a pesticide or any pollutant reaches the aquatic ecosystem, the fish are exposed to severe stress and as a natural instinct; the fish tend to adapt themselves by reacting suitably to overcome the stress. When two toxicants act together, the stress shall be more and severe and cause irreparable damage. In an aquatic environment the pollutants reach easily as runoff from pesticide residues and heavy metals like Iron, copper, mercury, nickel, lead and zinc pollute the aquatic system through industrial and municipal wastes. In such a scenario, the metal compounds react with the chemical pesticides to form complexes that may have entirely different properties than the original one. Such complexes formed may be more toxic than the individual toxicant or may even be less toxic. When the fish are exposed to such complexes the reaction to stress by the fish may be different.

Hence an attempt has been made in the present study to know the effects of a pathogen, pesticide and a heavy metal compound acting independently and combined. For this study, a pathogen, *pseudomonas putida*, a pesticide, Methyl parathion and a heavy metal compound, Copper sulphate ($CuSO_4$) were chosen as the toxicants and one of the commercially important Indian major carp, *Labeo rohita* chosen as the target animal to test the toxic effects on oxygen consumption.

MATERIAL AND METHODS

Fingerlings of *L. rohita* of relatively same size ranging from (8 to10 cm) and weight about (5-8 gm) were collected from culture ponds of Bharath Fish Farm, Poondi, Thiruvallur district, Tamil Nadu, India. The fish, after conditioning, were oxygen packed in tins and brought to the lab. They were slowly released into cement tanks; half filled with bore water and seasoned overnight. These were maintained in stocking tanks, where the fish were quarantined and acclimatized for two days.

The fish feed was prepared with rice bran, groundnut oil cake, tapioca powder and mineral mixture (Ramaiah, 1982).

The fish were fed daily with pelleted feed at 5% body weight in two split doses, in the morning and evening. Feeding was started one day after the fish were stocked and stopped 24 hr prior to experiment.

All the fish were maintained in the glass aquaria of the size 1'L x 2'B x 1'H throughout the period of experimentation. The tanks were disinfected with potassium permanganate thoroughly, before and after use. These tanks were filled with known volume of water per fish (30 litres for 10 fish) and covered with nylon mesh to prevent the mosquitoes breeding in the tanks and also to restrain the fish jumping out.

Healthy fish without any observable pathological symptoms were chosen for the experiments. Fish were divided into groups of ten each and exposed to the different toxicants *viz.*Bacteria, Methylparathion and Copper sulphate independently and synergistically. In the synergistic toxicity experiments, the fish were exposed to two toxicants together.

Fingerlings of *L. rohita* were treated with Pathogen, pesticide and heavy metal individually and synergistically to determine 96hr LC_{50} values for the test toxicants. Groups of fish were maintained in separate tanks and considered as experimental groups which were categorized as follows:

Independent toxicity

1. Bacterial Pathogen
2. Methyl Parathion
3. Copper Sulphate

Combined synergism

1. Bacteria + Methyl parathion
2. Bacteria + Copper sulphate
3. Copper sulphate + Methyl parathion

Suitable controls were maintained in normal bore well water for all the experimental groups, without dissolving the chemicals.

Groups of fish were exposed to the toxicants at SLC and sacrificed on 4, and 7, days post exposure to study of their physiology.

Rate of Oxygen consumption were analyzed on zero day control and on 4th and 7th day after exposing the fish with the toxicants in the experimental groups of independent toxic exposure and combined synergistic toxic exposure.

Rate of oxygen consumption was studied in the control and experimental groups by following the modified Winkler's method. The dissolved oxygen content of the water in the respiratory chamber was estimated, before the fish was released. After one hour, water from the respiratory chamber was carefully siphoned out avoiding air bubbles, and the dissolved oxygen content was estimated. The fish were weighed accurately to the nearest milligram after the fish were carefully wiped on a blotting paper. From this data, the unit oxygen consumption was calculated for each fish and expressed as ml/gm/hr. The dissolved oxygen in water was calculated using the formula.

$$\frac{K \times 200 \times 0.698 \times \text{Titre Value}}{\text{Volume of Sample}}$$

$$\text{Rate of Oxygen consumption} = \frac{\text{Oxygen Consumed}}{\text{Wt. of fish}} = \text{ml/gm/hr.}$$

For all the parameters analysed, ten estimations were made and subjected to standard statistical methods to arrive at Mean, Standard Deviation and Test of Significance. The results were tabulated and the data represented in suitable tables and histograms.

RESULTS AND DISCUSSION

Oxygen consumption by the fish was estimated both in the control as well as treated groups exposed to bacterial pathogen (*Pseudomonas putida*), pesticide (Methyl parathion) and a heavy metal (Copper sulphate) individually and combined as bacteria + $CuSO_4$, bacteria + methyl parathion and $CuSO_4$ + methyl parathion synergistically. The experimental analyses were done on 4th day and 7th day post exposure to toxicants. The experimental groups which were exposed to bacteria, pesticide and heavy metal independently recorded a higher rate of oxygen consumption on 4th day and 7th day compared to control groups with 0.19 and 0.16 ml/gm/hr respectively.

In the experiments of combined synergism, where the test fish were exposed to two toxicants together *viz.*, bacteria + $CuSO_4$, bacteria + methyl parathion and $CuSO_4$ + methyl parathion also, similar observations were recorded on 4th day and 7th day of experimentation with a two fold increase.

Several workers take up oxygen consumption as an important study to know the effects of toxicant. Prashanth *et al.* (2003) observed that the rate of oxygen consumption increased when exposed to pesticide, cypermethrin. Tripathi *et al*. (2003) studied the effect of bacterial pathogen on freshwater fish *L. rohita* and observed increased opercular movements under the influence of bacterial pathogen.

Metals like cadmium compounds also brought down the oxygen consumption in the freshwater fish *Ctenopharyngodon idella* (Espina *et al.,* 2000). The decline in the consumption of dissolved oxygen may be due to gill damage that reduces the efficiency of oxygen uptake. Alam and Mangalam (1995) observed that the lead has also been reported to accumulate in the body of fishes from the surrounding water and detritus and there by produce its adverse physiological effects through biological mechanism.

The rate of oxygen consumption in *Catla catla* treated with DTC, CuSO4 and ZnSO4 showed an increasing trend in all the experimental groups as the duration of exposure increased (Sujatha, 2006). The test organism exposed to sub lethal concentrations of pathogen, pesticide and a heavy metal independently showed that rate of oxygen consumption was increasing in all the experimental groups as the duration of the exposure increased. The differences observe in the group were highly significant, when compared to the control fish.

Oxygen consumption can yield important information as an index of the metabolic rate. The alteration or failure of respiratory metabolism is one of the early symptoms of pesticide poisoning in any organism. Decrease in the rate of respiration with increasing concentration to toxicants was recorded in fishes.

In an aquatic environment, one of the most important manifestations of the chemical toxicant is over stimulation or depression of respiratory activity (Murheed Thomson, 1977). Tilak *et al.* (2002) stated that the rate of oxygen consumption decreased with an increase in the duration of exposure resulting in absorbance of more toxicant through gill. At higher concentrations of metals, the dissolved oxygen contents of the test medium decreased significantly. This shows that high concentrations of metallic ions induced stress in the fish that resulted in significantly more oxygen consumption and thus, dissolved oxygen concentrations of the test medium declined. Environmental conditions such as oxygen concentration, temperature, hardness, salinity and presence of other metals may modify metal toxicity to the fish.

Toxicants in the environment mainly enter into fish by means of their respiratory system. A mechanism of toxicant uptake through gill probably occurs through pores simple diffusion and is then observed through gill membranes (Sampoorani *et al*., 2001). From the results obtained, it is clearly evident that the toxicants affect the oxygen consumption of *Labeo rohita* under all exposed concentrations. The observed increased in oxygen consumption by the whole animal may be due to respiratory distress as a consequence of the impairment of oxidative metabolism.

The elevation in the oxygen consumption by 50% in the experimental group of fish treated with pathogen independently revealed the nature of toxic effect and its impact on the test organism. These results corroborate with the observations of behavior like increased opercular beats, increase in surface behavior, increase in secretion of mucus, irregular swimming activity, rapid and darting movement. Moreover, the higher rate of oxygen consumption may be due to increased metabolic rates in the experimental fish as an adaptation to overcome stress induced by the toxicants.

Sampoorani *et al.* (2001) studied in fresh water fish *Labeo rohita*, an increased rate of oxygen consumption due to textile effluent treatment, while treatment with dairy effluent brought down the rate of oxygen consumption. Sujatha (2006) reported that the disturbance in oxidative metabolism leads alteration in whole animal oxygen consumption in different species of fish exposed to pesticides. The alternative reason for the elevation of oxygen consumption would be due to the internal action of toxicants. The results of the present study confirm the earlier reports on oxygen consumption by fish in toxicants mixed water.

Table 1. Effect of independent toxicity on oxygen consumption of *Labeo rohita.*

Experimental condition	Oxygen consumption (ml / l / hr)					
		Zero day control	4th Day control	4th Day treated	7th Day control	7th Day treated
Exposed to bacteria	Mean	0.19	0.19	0.28	0.16	0.32
	S.D.	0.012	0.005	0.032	0.031	0.015
	P <		0.001		0.001	
Exposed to $CuSO_4$	Mean	0.19	0.19	0.47	0.16	0.39
	S.D.	0.012	0.005	0.049	0.031	0.035
	P <		0.001		0.001	
Exposed to parathion	Mean	0.19	0.19	0.33	0.16	0.34
	S.D.	0.012	0.005	0.014	0.031	0.024
	P <		0.001		0.001	

Table 2. Effect of combined synergistic toxicity on oxygen consumption of *Labeo rohita.*

Experimental condition	Oxygen consumption (ml / l / hr)					
		Zero day control	4th Day control	4th Day treated	7th Day control	7th Day treated
Exposed to bacteria + $CuSO_4$	Mean	0.19	0.19	0.34	0.16	0.39
	S.D.	0.012	0.005	0.024	0.031	0.013
	P <		0.001		0.001	
Exposed to bacteria + parathion	Mean	0.19	0.19	0.35	0.16	0.41
	S.D.	0.012	0.005	0.032	0.031	0.045
	P <		0.001		0.001	
Exposed to $CuSO_4$ + parathion	Mean	0.19	0.19	0.20	0.16	0.31

ACKNOWLEDGMENT

The authors express sincere thanks to the Principal and HOD of Advanced Zoology and Biotechnology, Sir Thyagaraya College for the facilities provided to carry out this research work.

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