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Nirmala Kallagadda
Research Scholar, Department of Zoology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

Dr. V Venkatarathnamma
Assistant Professor, Department of Zoology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

Correspondence
Nirmala Kallagadda
Research Scholar, Department of Zoology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

Effect of an organophosphate Phosalone (35% EC) on respiratory metabolism of *Ctenopharyngodon idella*

Nirmala Kallagadda and Dr. V Venkatarathnamma

Abstract
Background: In aquatic media, over stimulation or depression of respiratory activity is one the most important manifestations of toxicity of chemical pollutants. Steady and progressive decline in ventilator pattern and decline in oxygen consumption. Majority of the pesticides have tendency to accumulate in tissues of aquatic animals and excess pesticides in the environment cause damage to the behaviour and physiology of living organisms especially the respiratory physiology. The assessment of these effects of insecticides to non-target aquatic organisms is very difficult.
Aim & Objective: To study the effect of an organophosphate Phosalone (35% EC) on respiratory metabolism of *Ctenopharyngodon idella*
Results: In present investigation phosalone pesticide was used to study its effect on respiratory metabolism of *Ctenopharyngodon idella*. The result showed significant decrease in oxygen consumption rate. In sub-lethal concentrations of Phosalone (35% EC) commercial grade; it was observed that fish *Ctenopharyngodon idella* showed tendency of increase in oxygen consumption during the initial time of exposures i.e. 1 to 6 hours and a gradual decrease was observed during the subsequent period of study. The presence of sub-lethal concentration of toxicants is inevitable. In such a case, the fish *Ctenopharyngodon idella* was more sensitive to toxicant. The toxicant stress in oxygen consumption along with depletion in oxygen in aquacultures practices make them less fit and reduction in growth due to lack of proper metabolism. The fish was in more stress during first hour and later they are showing signs of recovery. That recovery is evident as the toxicant exposure is increased in time, during 24 hrs experiment.
Conclusion: The present study stressful behaviour of respiratory impairment due to the toxic effect of Phosalone on the *Ctenopharyngodon idella* gills. It is presumed that the toxicant directly or indirectly affects the respiration of fish. The rate of oxygen consumed by the affected fish was very low. Once the respiration of the fish is affected, in turns all the biological activities of the fish will also be reduced.

Keywords: Ctenopharyngodon idella, Phosalone, respiratory mechanism, oxygen

Introduction
Water is one of the precious liquid of the natural resources available, Aquatic animals have to pass large quantities of water over their respiratory surface and are subjected to relatively greater risk of exposure to the toxic substances (Shelke and Wani, 2005) [1]. The aquatic environment is also polluted by pesticides and it leads to many changes in organism physiology. The pesticide enters into the aquatic ecosystem through various routes affecting adversely to the aquatic biota (Magari, 1992) [2].

The necessity of determining the toxicity of substances to commercially aquatic forms at the lower level of the food chain has been useful and accepted for the water quality management.

In aquatic media, over stimulation or depression of respiratory activity is one the most important manifestations of toxicity of chemical pollutants. Steady and progressive decline in ventilator pattern and decline in oxygen consumption are noticeable with few exceptions difficult to generalize (Veeraiah and Durga Prasad, 2001) [3]. Pesticides are introduced into natural aquatic systems by various means incidentally dewing manufacture, dewing their application and through surface water runoff from agricultural land after application (Sujad *et al.*, 2014) [4].

Respiration is one of the most important characteristics of life; it provides energy to perform various activities of body like movement, metabolic reactions, growth and development, muscular contraction, reproduction etc. A steady supply of oxygen is essential for the maintenance of life and the supplied oxygen oxidizes food materials to release the energy that in turn powers various life processes.

The activity of animal can be measured in terms of the oxygen uptake. Aquatic animals have to pass large quantities of water over their respiratory surface and are subjected to relatively greater risk of exposure to the toxic substance (Shelke and Wani, 2005) [1].

Aquatic organisms like prawns, fish, bivalve, crab respire through gills, Fisheries and aquaculture is a source not just of health but also of wealth (FAO, 2014) [5]. Respiration plays important role in studying aquatic toxicology. Such respiratory surfaces may leads to the alteration in the respiratory area which causes reduction in oxygen consumption and physiological imbalance in the organism (Mukke and Chinte, 2012) [6]. Fish is a good indicator of aquatic contamination because its biochemical stress responses are quite similar to those found in mammals. Oxygen (O_2) in its molecular state is essential for many metabolic processes that are vital to aerobic life. Life all aerobic organisms, fish are susceptible to the effects of reactive oxygen and have inherent and effective of different biotic and abiotic factors on antioxidant defenses in fish (Martinez-Alvarez *et al.*, 2005) [7].

Total oxygen consumption is one of the indicators of the healthy status of a fish and it may also 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. Studies on oxygen consumption form a suitable tool in the assessment of stress due to toxicants on the aquatic organisms and give an index of energy expenditure mechanisms for environmental variations (Franklin *et al.*, 2010) [8].

Once Phosalone an organophosphate is introduced into environment usually from spraying on crops or in wide urban or residential areas, it may cause problems to the aquatic organisms including fishes. Most of the fish breaths in the water, in which they live, changes in the chemical properties of water due to pesticide toxication reflected in the animal's ventilator activity, particularly affect respiratory gas exchange. Organophosphorus insecticides including phosalone are widely used in crop protection. Indiscriminate application of these insecticides affects non-target organisms including economically important fresh water fish *Ctenopharyngodon idella.* A change in respiration rate is common physiological responses to toxicants. It is an easily detectable through changes in oxygen consumption rate, which frequently used to evaluate the changes in metabolism under environment deterioration.

Majority of the pesticides have tendency to accumulate in tissues of aquatic animals and excess pesticides in the environment cause damage to the behavior and physiology of living organisms especially the respiratory physiology. The assessment of these effects of insecticides to non-target aquatic organisms is very difficult. As a result of the pollutants from industrial areas and agricultural runoff into the environment pollute water bodies (Tyagi *et al.*, 2000) and their chemical persistence; many freshwater ecosystems are facing the spatial or temporal alarming high levels of xenobiotic chemicals (Brack *et al.*, 2002). Considerable attention has been paid towards crustacean and molluscan animals chiefly because of their important role on aquatic food chain. Depletion in oxygen content occurs in the medium when pesticides, chemicals, sewage and other effluents containing organic matter are discharged into the water bodies.

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. To the action of pollutant initial response given by an organism on oxygen uptake, which reflects bioenergetical process and metabolism is a good analyzer of the physiological state of an organisms. The most important part of this aspect is the reduced oxygen consumption which would create physiological imbalance to the organism. Heavy metal pollution and pesticides cause alterations in the oxygen consumption in freshwater animals (Pawar and Katdare, 1984). Shaikh *et al.*, (2010) [9] studied the effect of mercuric and cadmium chloride on oxygen consumption of freshwater crab, *Barytelphusa cunicularis*.

Material and Methods

The experiment on the oxygen consumption of the fish *Ctenopharyngodon idella* was carried out in a respiratory apparatus developed by Job (1955). The fish was brought from a local fish farm and acclimatized to the laboratory conditions in well aerated water for 15 days. The water used for acclimatization and experimentation was the same as used in the toxicity experiments (Table). During this period, the fish were regularly fed, but the feeding was stopped for two days prior to the experiment. The fish measuring 6 to 7 cm in length and 6 to 8 gm in weight, all the precautions laid down on recommendations of the toxicity tests to aquatic organisms are followed. For finding 96 hrs static bioassay experiments were set by using the toxicant Phosalone insecticide.

Description of respiratory chamber

The apparatus used for the measurement of whole animal oxygen consumption is a wide mouthed bottle which is called a respiratory chamber (RC). Its mouth was fitted with a four holed rubber stopper(S) and through one of the holes a thermometer (T) was passed to know the temperature of the medium in the respiratory chamber. The respiratory chamber was coated black to avoid photochemical reactions and to keep the animal activity at normal during the experiment.

Setting up of the Apparatus

Only one fish was introduced into each respiratory chamber and was filled with water drawn through T_1 from the reservoir. After checking the air tightness pinch lock P_2 was closed and pinch lock P_3 was opened slightly so that a very gentle and even flow of water was maintained through the respiratory chamber. This was continued for 15 minutes to facilitate the animal in returning to a state of normality from the state of excitement, if any, due to the handling and also to allow the animal to adjust to the darkness in the chamber (acclimatization).

Collection of the initial and final samples

After allowing the animal to settle in the chamber, the initial sample was collected from the respiratory chamber through T_3. After the collection of initial sample, the respiratory chamber was closed by closing P_3 first and then P_1 after one hour. The next sample was collected from the respiratory chamber. Likewise, other samples were also collected at the end of each hour for a total of 24 hours period of the experiment. Along with the experimental fish chamber, one respiratory chamber without toxicant was maintained as control. The control serves to estimate initial amount of oxygen, at each experimental period i.e 2hrs. The experiment was conducted with sub-lethal and lethal concentrations of Phosalone to fish *Ctenopharyngodon idella*. The amount of dissolved oxygen consumption was calculated per gram body weight per hour.

$$\text{O}_2 \text{ consumed by fish/ Gram body weight/hour} = \frac{\alpha - \beta \times \text{N of hypo} \times 8 \times 1000}{\text{Vol. of the sample taken} \times \text{Correction factor} \times \text{Wt. of the fish} \times \text{Time interval for each sample}}$$

Where:
α = hypo rundown before exposure
β = hypo rundown after exposure

Result and Discussion

The comparative data on the whole animal oxygen consumption of control and experimental fish, calculated per gram body weight in sub-lethal and lethal concentrations of Phosalone (35% EC) fish *Ctenopharyngodon idella* was given in the Table.1. The results of the experiments and control values are graphically represented by taking time on X-axis and the amount of oxygen consumed per gram body weight on Y-axis, Fig.1.

In present investigation phosalone pesticide was used to study its effect on respiratory metabolism of *Ctenopharyngodon idella*. The result showed significant decrease in oxygen consumption rate. In sub-lethal concentrations of Phosalone (35% EC) commercial grade; it was observed that fish *Ctenopharyngodon idella* showed tendency of increase in oxygen consumption during the initial time of exposures i.e. 1 to 6 hours and a gradual decrease was observed during the subsequent period of study. The presence of sub-lethal concentration of toxicants is inevitable. In such a case, the fish *Ctenopharyngodon idella* was more sensitive to toxicant. The toxicant stress in oxygen consumption along with depletion in oxygen in aquacultures practices make them less fit and reduction in growth due to lack of proper metabolism. The data as per Table: .1. The fish was in more stress during first hour and later they are showing signs of recovery. That recovery is evident as the toxicant exposure is increased in time, during 24 hrs experiment.

The most widely used class of Organophosphates insecticides have become in the world replacing persistent problematic organochloride compounds. Exposure of aquatic ecosystem to these insecticides is difficult to assess because of their short persistent in water column due to low solubility and rapid degradation hence monitoring of these insecticides is important (Chebbi and David, 2010) [10]. It is widely used organophosphate insecticide because of its relatively low toxicity to mammals and high selectivity for insects compared with other organophosphate insecticides. There are many earlier finding that clearly warned of the genotoxic potential of technical-grade Malathion in wide range of organisms including fish (Kushwaha *et al.*, 2000) [11].

Throughout the experimental period the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased mucous secretion, higher ventilation volume and decrease in oxygen uptake efficiency, labored breathing and engulfing of air through the mouth when exposed to Phosalone.

Similarly when a comparison is made between the effects of sub-lethal concentrations of Phosalone (35% EC) on *Ctenopharyngodon idella* decreased in oxygen consumption was observed. Under lethal concentration of Phosalone (35% EC) a significant increase is found in the initial stages of exposure i.e., 1-6 hrs in *Ctenopharyngodon idella*. Hence, it has sensitivity on toxic stress as a result of more oxygen consumption.

Table 1: The amount of oxygen consumed in mg/g body weight/hour of the fish *Ctenopharyngodon idella* exposed to sub-lethal and lethal concentration of Phosalone (35%EC):

Hours of Exposure	Control	Sublethal	% change	Lethal	% change
0	0.794± 0.004	0.781± 0.002	-1.637	0.789±0.004	-0.62
2	0.787±0.003	1.374± 0.026	74.58	0.779± 0.004	-1.01
4	0.769±0.002	1.412± 0.009	83.61	0.759±0.005	-1.30
6	0.754±0.002	0.745±0.003	-1.193	0.734±0.003	-2.65
8	0.733±0.003	0.694±0.002	-5.320	0.602±0.006	-17.87
10	0.704±0.003	0.645±0.009	-8.380	0.596±0.006	-15.34
12	0.672±0.005	0.591±0.004	-12.05	0.512±0.005	-23.81
14	0.662±0.006	0.531±0.005	-19.78	0.491±0.004	-25.83
16	0.646±0.004	0.463±0.007	-28.32	0.411±0.006	-36.37
18	0.621±0.007	0.386±0.003	-37.84	0.352±0.003	-43.31
20	0.592±0.005	0.332±0.004	-43.91	0.303±0.005	-48.81
22	0.569±0.005	0.291±0.004	-48.85	0.243±0.003	-57.29
24	0.525±0.004	0.265±0.013	-49.52	0.224±0.006	-57.33

Values are the mean of five observations: *Standard Deviation is indicated as (±).*, Value are significant at $p < 0.05$

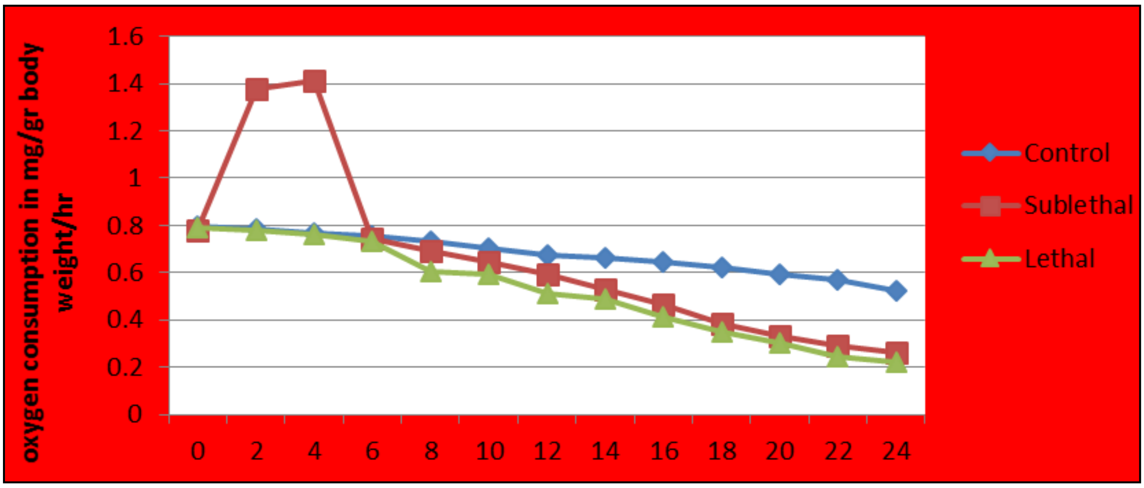

Fig 1: The amount of oxygen consumed in mg/g body weight/hr to the fish Ctenopharyngodon idella exposed to sublethal and lethal concentration of Phosalone 35%EC:

The effect of pesticide on the respiratory metabolism of different organinsm varies with the dose of pesticide and duration of exposure of pesticide. Mukke and Chinte (2012) [6] observed decrease in oxygen level with increase in concentration. The pesticide causes a physiological stress to fresh water organisms. Pesticides alter the metabolic rate and affects oxygen consumption in different animals due to deposition or accumulation of pesticides in the body of animals.The decline in oxygen consumption was greater in higher concentration which might be the result of an reduced state of metabolism owing to toxicant stress (Lomte and Massaeat, 1993) [12].

Although many biological early warning systems monitor abnormal opercular movement as an indicator of respiratory stress, a more direct measurement of stress in this sense necessities the quantification of oxygen consumed by the fish. Determination of oxygen consumption by the fish is useful for the assessment of lethal effects and is one of the important indicators which reflect physiological state of animal. In aquatic body toxicants present above the normal level i.e., at lethal concentrations bring about mortality of fish and also increase the rate of oxygen consumption in survived fish (Tilak *et al.*, 2007) [13]. The recent development of biomarkers based on the study of the response of organisms to toxic chemicals has provided essential tools for the implementation of environment contamination monitoring programmes (Korami *et al.*, 2000) [14].

Fish bioassay experiments, the trophic level connection in aqua systems are indices to determine the acute toxicity and possible effect on oxygen consumption due to the toxicant stress (Subrahmanyam, 2004) [15]. A large amount of the pesticides used, never reaches the intended targets and they enter in the aquatic environment which is currently under threat of the indiscriminate use of pesticides.

Respiratory activity of a fish is often the first physiological response to be affected by the presence of contaminants in the aquatic environment. A more direct measurement of stress in this sense necessitates the quantification of the oxygen consumed by the fish. Although many biological early warning systems monitor abnormal opercular movement as an indicator of respiratory stress, the oxygen consumption is not often used as a bio indicator of pollution associated stress in biological early warning systems. Respiratory responses were found to be less sensitive, but also could be successfully used in bioassay testing of treated industrial and municipal effluents before they are discharged into receiving waters. Among different classes of pesticide, organophosphorous insecticides represent one of the most widely used classes of pesticide with high potential for human exposure in both rural and residential environments (Ngoula *et al*, 2007) [16].

Vineetkumar *et al.*, (2008) [17] reported that the disturbance in oxidative metabolism leads alteration in whole animal oxygen consumption in different species of fish exposed to pesticides. The present investigation is in confirmation with the trend observed in earlier investigations. Dharmalata and Namitha Joshi (2002) [18] reported that the respiration is a vital phenomenon of the life and the rate of oxygen consumption in turn controls the metabolic activities and changes in respiratory rates have been used as the indicator of the stress in pollutant exposed organisms. In the present study, the oxygen consumption was gradually decreasing with increasing exposure periods as observed.

In the present study the variation in oxygen consumption is an indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is evident from the studies that the phosalone affected. Oxygen consumption of *Ctenopharyngodon idella* under lethal and sublethal concentrations compared with control.Many researchers investigated that pesticide toxicity induced respiratory distress in fishes. David (2003) [19] showed disturbances in oxidative metabolism in *Tilapia mossambica* under cypermethrin toxicity. Nataranjan (1981) [20] showed reduction in oxygen consumption in *Channa punctatus* exposed to organophosphate pesticide. Janardhan Rao (1991) [21] studied oxygen consumption rate of *Macronunssalki* exposed to endosulfan.

Malla Reddy (1988) [22] reported effect of fenvalerate and cypermethrin on the oxygen consumption of fish, *Cyprinuscaprpio* showed significant drop in rate of oxygen consumption. 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. It can be inferred from the results that, during sub lethal exposure to toxicant, the fish may be adapting to augment the physiological adjustment for elimination of the chemical stress.

Present study, exposed to *Ctenopharyngodon idella* in lethal and sublethal concentrations of Phosalone findings are in accordance with findings of various reports in which increased oxygen consumption was observed Veeraiah (2002) [23] in *Labeorohita* exposed to cypermethrin, Shereena *et al.*, (2009) [24] in *Tilapia mossambica* exposed to dimethoate. The analysis of data from the present investigation indicates a considerable effect of Phosalone (35% EC) on oxygen consumption in selected fish in lethal as well as sub-lethal concentrations. The present study revealed alterations in the oxygen consumption of *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of organophosphate insecticide. Variation in the oxygen consumption in Phosalone (35% EC) exposed fish was probably due to impaired oxidative metabolism and pesticide induced stress.

The increase in activity might be to boost up oxidative metabolism for an increased supply of energy to combat the toxic stress (David *et al.*, 2003) [19]. A variation in respiration rate was an indicator of stress and is frequently used to evaluate the changes in metabolism under environmental deterioration. Pesticides are indicated to cause respiratory distress or even failure by affecting respiratory centers of the brain or the tissues involved in breathing. Numerous studies such as *Cirrhinus mrigala* (Mushigeri and David, 2003) [25], *Labeo rohita* (Patil and David, 2008), *Oreochromis mossambicus* (Logaswamy and Remia, 2009) [26], *Ctenopharyngodon idealla* (Tilak and SwarnaKumari, 2009) [27]; *Oreochromis niloticus* reported either increase or decrease their respiration rate in response to variety of pesticides. The fluctuated response in respiration may be attributed to respiratory distress as a consequence of the impairment in oxidative metabolism. Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in *Tilapia mossambica* (David *et al.*, 2003) [19]. If gills would be destroyed due to xenobiotic chemicals (Griniwis *et al.*, 1998) [28] the membrane functions are disturbed by a changed permeability (Hartl *et al.*, 2001) [29], oxygen uptake rate would even rapidly decreased. On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. Kalavathy *et al.* (2000) [30] reported that the dimethoate is efficiently absorbed across the gill and diffuse into the blood stream resulting toxic to fish *Sarotherodon mossambicus*.

Conclusion

Further, the metabolic rate in relation to respiration of fish could be increased under chemical stress. The present study stressful behavior of respiratory impairment due to the toxic effect of Phosalone on the *Ctenopharyngodon idella* gills were similar with the report. It is presumed that the toxicant directly or indirectly affects the respiration of fish. The rate of oxygen consumed by the affected fish was very low. Once the respiration of the fish is affected, in turns all the biological activities of the fish will also be reduced. The decrease in whole animal oxygen consumption might be due to the damage in the structural integrity of the cells of respiratory organs.

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Conflict of Interest

None

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