EFFECT OF CHLORPYRIFOS TECHNICAL GRADE AND 20% EC ON THE OXYGEN CONSUMPTION IN THE FISH *CHANNA PUNCTATA*

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Abstract

A physiological process of respiration, which involves both inspiration and expiration, had/have/has an impact on the fish due to the toxic action of the pesticides, chlorpyrifos, an organophosphate in the laboratory study of the fish *Channa punctata* both in lethal and sub-lethal concentrations, exposed to the technical grade as well as 20% EC. The toxicants resulted an alterations/changes in the consumption of the oxygen while during the process of the inspiration. The results of the increase initially and decrease during the later periods at both the concentrations and toxicants, resulted due to the reason of the toxicant stress actions only, because the entry point of the toxicants and diffusion of gases is one and the same, that is the gills. They, all, are going to be discussed with the literature of the earlier work reports, in the context of the defilement act due to their indiscriminate use and are transported to the aquatic bodies that are nearby and effect the non-target organisms, especially, the fishes which are all going to be reviewed.

Keywords: *Channa punctata*, Chlorpyrifos, Technical grade, 20% EC, Oxygen consumption, lethal and sub-lethal concentrations.

INTRODUCTION

The intense use at one end in agricultural operations, of course, the chemical, pesticides and at the other end the indiscrimination in their usage cause the contamination by the transportation only by any means. That act of defilement, effect the organisms termed as non-target ones, and especially the fish, in water are going to have more risk of health and growth. This can be quite possible if the organisms are not in a position to take the regular quantum of oxygen that take in the dissolved state of water only which will be useful for the metabolism for survival which is consequetnly the growth limiting factor only.

The effect of the pesticides on oxygen consumption received much attention in fact 'aspread' and reports of such studies were mentioned in review articles of Sunanda *et al*. (2016) and Deb and Das (2013) for chlorpyrifos and also for other pesticides by Ullah and Zorriezahara (2015) and Murthy *et al*. (2013).The reports for organophosphates, as individual studies by Balques (2018), Lokhande (2017). Illavaznahan *et al*. (2017 & 2010), Padmanabhan *et al.* (2015), Muttappa *et al*. (2014) Jothinarendran (2012), Logoswamy and Reemia (2009) Joshi and Kulkarni(2007) and Mushigiri and David (2005) for the different fish of the different pesticides as toxicants were few to mention which are at present more informative. Even for the fourth generation group, the synthetic pyrethroids as the toxicants and such studies reported by Satyanarayana *et al*. (2020), Srinivasa Rao *et al.* (2018) and Balakrishna Naik (2017), and Neelima *et al*. (2016) and also the ones mentioned in the review articles of Sana Ullah *et al*. (2019) are also handy.

Styonova *et al*. (2020), while using a system of the biomarker assessment in common carp, apart from enzymes as one of them, the anhydrase which is important for exchange of oxygen and carbon-dioxide through chloride shift play an important role and its inactiveness, or lower in activity can reduce the oxygen uptake.

Nnamdi Henry Amazee *et al.* (2020), reported on the toxicity of the African cat fish Clarias gariepinus, a comparative study hinted up that the chlorpyrifos toxic action, the consequences of oxygen uptake as effects had an imperative bearing on Haematology of the fish and in terms of toxic effect the toxicant is ranked 6^{th} place out of Ten (10) chemicals that were aimed.
Akter *et al*. (2020) while on the report of the toxic effect of organophosphate pesticide envoy 50% EC in the fish *Heteroneustes fossils*, due to the inhibition of enzyme actyl cholinesterase (AChE), the fish had a lack of the swimming activity in its normal nature, and do not have also the normal quantum of oxygen uptake as a consequence the metabolism is impaired. Sharmila and Kavitha (2019) study of the fish, *Cyprinus carpio* due to the exposure of the monocrotophos, an organophosphate pesticide too opined on the similar lines that the toxic action of the chemical resulted in the haematological alterations due to the falling of the oxygen intake only.

According to Natalia et al (2019) study report in the fish *Astayanax aeneus*, the neurotoxic action caused by the organophosphates as the toxicant resulted a respiratory stress and as a consequence the depletion in the oxygen uptake only.

Pallavi Srivatsava *et al.* (2016), Somayyah *et al*. (2017), Sunanda *et al*. (2016) and Deb and Das (2013), in their review articles of the organophosphates and chlorpyrifos, especially, mentioned that the research work related to the toxicant action of oxygen uptake only as one of the main effects which is affected by the chemicals.

Hence the present study is attempted to know the effect on the oxygen consumption while in the inspiration of respiration that resulted by the exposure to the technical grade as well as 20% EC of chlorpyrifos, an organophosphate as toxicants in the fish *Channa punctata*.

MATERIALS AND METHODS

Experiments on the oxygen consumption of the fish *Channa punctata* were carried out in a respiratory apparatus developed by Job (1955). The fish were brought from a local fish market, Guntur (Andhra Pradesh). They were acclimatized to the laboratory conditions in well aerated water for 10 days. The water used for fish acclimatization and experimentation was the same that was used in the toxicity experiments. It has the following physical and chemical characteristics; Turbidity – 8 silica units, Electrical conductivity at 28^{o}C–8.16 Micro ohms/cm, pH at 28^{o}C-8.2.

Alkalinity: Phenolphthalein-Nil, Methyl orange as $CaCO_3$– *472, Total Hardness-*320, Calcium Hardness-*80, Magnesium Hardness-*40, Nitrite nitrogen (as N) – Nil, Sulphate (as SO_4) – Trace, Chloride (as Cl^-) - *40, Fluoride (as F^-) – I.S., Iron (as Fe)-Nil,(*All these values are as micrograms/liter), Dissolved Oxygen – 8-10 ppm, Temperature - 28±2^{o}C. During the experimentation period, the fish were regularly fed, but the feeding was stopped for about two days prior to the experimentation. The fish measuring 8 to 10 cm in length and 8 to 10 g in weight were used in the experiment. All the precautions mentioned by APHA (1998, 2005 & 2012) are followed, for maintaining the fish. The fish were exposed to 96h LC_{50} lethal 2.6 µg/L and also sub-lethal (1/10th of 96h LC_{50}) as 0.26 µg/L of technical grade and also 20% EC lethal 1.4 µg/l and 0.14 µg/l EC as sub-lethal (1/10th of 96h LC_{50}) respectively in the respiratory chamber.

The technical grade chlorpyrifos was supplied by M/S Hindustan aggrotech industries, Ahamadabad, India and the 20% EC was purchased from the local market of Guntur, AP India.

The samples for the estimation were taken from the respiratory chamber, at every two hours intervals for a total period of 24 hours apart from the control (total 13 samples of each test determination and five of each to have averages).

Description of the respiratory chamber

The chamber used for the measurement of the 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 placed to know the temperature of the medium in the respiratory chamber. From the remaining three holes three glass tubes were passed whose outer ends were fitted with the rubber tubes. These three tubes serve as delivery tubes and are designated as T_1, T_2 and T_3 respectively. They were fitted with pinch cocks P_1, P_2 and P_3. T_1 was connected with the reservoir ('R') and through this water could be drawn (inlet) into the respiratory chamber. T_2 was the atmospheric tube useful for testing the air tightness of the respiratory chamber which is taken into account as the fish is having the bi-model respiration hence extra care not to allow any air. Through the T_3 tube (outlet) samples from the respiratory chamber were taken for the estimation of the dissolved oxygen. The respiratory chamber was coated black to avoid any photo chemical reactions and to keep the animal activity at normal, during the entire period of the experiment.

Setting up of the Apparatus

Only one fish was introduced into each of the respiratory chamber that was with water drawn through T_1 from the reservoir. After checking the air tightness pinch cock P_2 was closed, to avoid any air to enter checked twice and the pinch cock 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 normal from the state of experiment, if any, difficulty due to the handling and also to allow the animal to adjust to 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 collection of initial sample, the respiratory chamber was closed by closing P_3 first and then P_1 after two hours,untill the next sample was collected from the respiratory chamber. Likewise, other samples also were collected at the end of each two hours for a period of 24hrs.To calculate the amount of oxygen present in the water, the method followed is popularly known as the modified method of Winklers that was given by Golterman and Clymo (1969).

Along with the experimental fish chamber, one respiratory chamber without the fish (control) was maintained. The control serves to estimate the initial amount of oxygen that was consumed by the fish. The experiments were conducted in sub-lethal as well as in lethal concentrations of both the technical grade and 20% EC of the chlorpyrifos that were used as the toxicants.

$$O_2 \text{ consumed by fish / gram body weight/hour} = \frac{\alpha - \beta \text{ x N of hypo x 8 x 1000}}{\text{Vol. of the sample x Correction factor x Wt. of the fish x Time interval for each sample.}}$$

α = Hypo rundown before exposure
β = Hypo rundown after exposure

Student t-test was employed to calculate the significance of the differences between control and experimental means. P-values of 0.05 or less were considered statistically significant (Fisher, 1950).

RESULTS

Comparative data on the whole animal oxygen consumption of control and experimental fish calculated per gram body weight in lethal and sub-lethal concentration of the technical grade and 20% EC for *Channa punctata* and their percent variations all are graphically represented as figures 1A, 1B and 2A, 2B. By taking time on X-axis and the amount of O_2 consumed per gram body weight on the Y-axis and both the line and bar modes are shown.

The results indicate that the toxicant contaminated water during the experimental period, continuously in the flow through, due to the immediate contact point only being the gills and also, the entry point had a stress on it. Not only that the fish not able to revert the sudden defilement act to cope up the situation it consumed more oxygen during the initial period and try to recover only at the later period.

The consumption of the more quantity of the oxygen, recovery and try to be stable in that situation as a sort of resistance and this effect is more precluded in 20% EC than to the technical as per the figures.

DISCUSSION

Balquees (2018), reported in the freshwater fish due to toxic stress of chlorofate, at four different concentrations of the toxicant. The fish that were tested includes *Gambusia affinis, Cyprinus carpio* and *Ctenopharyngodon idella* by taking LC_{50} values of 24 h only. Histopathological damage of the gill, the mucus deposition over the gills and over all failure of the harmony of biochemical imbalance combinely/cumulatively responsible for the change in the respiration particularly the oxygen in take, the physiological phenomenon which had a devasting consequences. As per the studies of the present fish the same is true even in the present case.

Lokhande (2017) while studying in the fish, *Rasbora daniconius* exposed to the toxicant, dimethoate, organo-phosphate, reported on the oxygen consumption a pattern similar to the result of the present study. The behaviour pattern itself and a change in operculam movement was responsible for the result they got.

Illavazhahan *et al.* (2010) reported the synergistic concept of the impairment of oxygen intake in the fish *Labeo rohita.* The mucus along with bacterial accumulation too interfere in the diffusion of gas oxygen while during inspiration.

Sathick *et al*. (2017) in the fish *Mugil cephalus* while exposed to 10% of the lethal concentrations of 96h LC_{50} value as sub-lethal, for 10, 20 and 30 days and reported the decrement of the oxygen uptake. The reasons for such effect which they explained were: (1) due to microcytic anaemia of the gills, (2) as a protective measure to cope the situation of lowering the intake of the toxic substance and (3) also, due to the respiratory inhibitory action of the enzymes that are being responsible for such actions. The initial period the increase and later a decline as in the present study can be taken into justification of the present result. The present study resulted even in the 20% EC formulation also which by the action of the additive toxicity resulted much variation almost similar to the technical grade.

Ravindra and Patel (2016) while studying an organochlorine, endosulphan in the fish *Channa punctata* exposed to sub-lethal concentrations. The pH of the water, temperature at which experiments were conducted and the sub-lethal concentration value as consideration being different (static in the case of their study and continuous flow through system as in the present study) and measurement of quantity of oxygen at end of 24, 48, 72, 96 h not as in the present study of the 2 h interval period all these aspects cannot be a good comparison of the their study, yet the reasons they mentioned that the architectural damage of the gill which they focused as the reason for the less uptake as consumption which is in agreement of the present study but during initial period, the perturbation of the toxic molecules caused them suffocation and showed hyperactive aspect to intake more gas and later then was a decrement.

Similarly, Kharat *et al*. (2016) in the fish *Rasbora daniconius* while studying on the effect of glyphosate herbiside, by the static bioassay, only considered $1/10^{th}$ of the 96 h LC_{50} value not similar in the present study (but in the present study the test is not by the static method determined value that is considered but $1/10^{th}$ of Continuous flow through test of 96 h method) reported a decreased respiratory rate of the toxicant effect. Due to the toxic stress during the initial period the fish consumed more oxygen to cope the toxic stress and that required more extra energy to tackle the toxic situation.

Neelima *et al*. (2016) using the synthetic pyrethroid, cypermethrin as a toxicant in the fish *Cyprinus carpio* reported a result of the increase of the oxygen intake initially, to cope the demand for the energy and as a consequence the slow or high rate of the movement of the gills along with the mucus accumulation and the gill lesions, all, were the probable reasons of the changes in oxygen consumption.The fish showed that in the initial period not at all comfortable in the toxic chemical environmenthence consumed more oxygen and the later periods tried to adjust and recover in sub-lethal whereas in lethal succumbed to death as in the present study of the toxic evaluation.

While studying fenvalerate as a toxicant, in the fish, *Heteroneusteus fossilis* Siddiqua *et al*. (2016), in the Indian major carps, *Labeo rohita, Catla catla* and *Cirrhinus mrigala* by Anitha *et al* (2010), Tilak and Satyavardhan (2002) in the fish *Channa punctata*, all, the above findings mentioned that the damage to gill architecture, toxic stress and biochemical alteration of the total proteins, all, are responsible for the alterations of the oxygen intake, which even in the present study,resulted the same. Padmanabhan *et al*.

(2015) reported on the oxygen consumption due to chlorpyrifos toxic action where the chemical is one and the same which opined in the similar lines for the same result as in the present study in the fish *Oreochromis niloticus.*

Kulkarni and Bhilave (2015), reported for the fish *Labeo rohita* due to exposure to diazinon, an organo-phosphate.The method of LC_{50} value taken into consideration was static only, different from the present continuous flow through test method, $1/10^{th}$, 96 h LC_{50} value apart from the differing in the physical and chemical parameters that were different of the medium of the experimentation. The accumulation of the mucus interfered in the gases (Oxygen/Carbon-di-oxide) exchange by the phenomenon of the diffusion. The operculum movement that covers the gills, decreased and as a consequence less amount of the gas in its consumption.

Ram Narayan Singh (2014) reported in the common carp (*Cyprinus carpio*) due to dimethoate (EC 30%) toxic action an electrolyte imbalance that existed was mainly responsible for the oxygen intake in lesser quantity as the time progress which is different at the initial period.

Priyanka and Ansari (2014) made a study of comparative account by using three toxicants endosulphan, chlorpyrifos and permethrin in Zebra fish *Danio rerio*. Each toxicant belong to the three different class of compounds one is chlorinated hydrocarbon, second one is organophosphate and the other one is the synthetic pyrethroid.While mentioning the toxic variations of the endosulphan that was more toxic followed by permethrin and least by the chlorpyrifos to the fish, studied, the mortality effect was due to respiratory distress, 'gasping for an' the possible way to explain the cause in the fish studied.

Sapna Devi and Gupta (2014) too while studying the growth effects of the fish *Anabas testudineus* due to the toxic impact of the permethrin as a toxicant, opined that the import growth parameter related to metabolism which in-turn related to the oxygen intake only due to the heterotrophic nature. The present studied fish *Channa punctata* is the highest palatable fish for the consumption and the size of it as such and consumption will be a factor of it only. Which when not grown fully may not be preferred in eating. The same result of the similar lines was also reported by Muttappa *et al*. (2014) while using Quinol-phos, an organophosphate in the fish *Cyprinus carpio*.

Madura Mukundam and Kulkarni (2014) even studied an esturine clam *Katelysia opima* (Gmalin) due to the toxic action of cypermethrin had an impact on the oxygen consumption. Jispa *et al*. (2014) in the fish *Tilapia mossambica*, Manjula and Veeraiah (2014) in the fish *Cirrhinus mrigala* and Paritha Bhaun and Deepa (2014) in the fish *Oreochromis niloticus*, all, the studies only used cypermethrin as the toxicant and the reasons of such results, mentioned in the respective studies were as: (1) consequence of disturbance of anabolism and catabolism processes, (2) the architectural damage of the gill, and (3) toxic stress only. The same aspects are not an exception to the present study result.

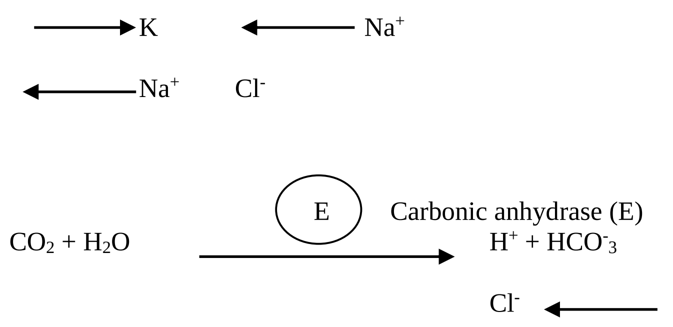
Maharajan *et al*. (2013) reported that the profenofos an organophosphate toxic action in the fish, *Catla catla* which had a profound bearing on the consumption of the oxygen only. By taking into consideration of only 24 h LC_{50} value of the static tests the oxygen consumption effect was aimed and the result of decrement in the consumption was due to the architectural damage of the gill, increased mucus secretion and also higher 'ventilation volume' which all resulted a cumulative aspect of the oxygen in take efficiency and all the reasons are even true even in the present study. Even Rao *et al*. (2013) in the fish *Oreochromis niloticus* exposed to chlorpyrifos, the present tested toxicant reported in the similar lines of the present study.

Jothinarendran (2012) also reported in the fish *Channa punctata* by using dimethoate another organophosphate as toxicant. The fish were kept in the different concentrations ranging from 0.15 ppm to 0.6 ppm, up to 96 h duration.The rate of the consumption except in the initial period of the exposure is the concentration dependant only, initially elevated and the stability reached at 72 hrs. The 'defection' of the normal gill surface area reduced to extent to such that which can result a decrease in the oxygen diffusion possible. The oxidative, acceleration towards the enhanced metabolism in the initial period that resulted an increase of the uptake of oxygen in the toxic stress.The results of this can also reiterate the results of the present study. Even in the fresh water bivalve mollusk, *Lamellidens corsianus* exposed to an organochlorine, thiodan, which was reported by Kumar et al (2012) and they mentioned that the stress factor as a cause and as a consequence of it the metabolism enhanced to cope the energy demand that finally resulted the variations in the oxygen consumption.

Maria Christiana *et al*. (2010), for many species of fish using Talsar a permethrin synthetic pyrethroid formulation, Chebbi and David (2010) in the fish *Cyprinus carpio* using Quinolphos as the toxicant, Sameer and David 2014 in the same fish and with the same toxicant, Shareena *et al*. (2009) in the fish, *Tilapia mossambica* using Dimetheate as the toxicant, Marigounder *et al*. (2009) using cypermethrin in the fish *Labeo rohita*, Tilak and Swarna Kumari (2009) in the fish *Ctenopharyngodon idella* with Nuvan (an organophosphate) as the toxicant, Vineeth Kumar and David (2008) in the fish Labeo rohita using Malathion, an organophosphate as the toxicant, Tilak and Koteswara Rao (2003) using chlorpyrifos in the three major carps, all, as the cause that reported in their respective studies emphasized only the stress factor resulted changes in the oxygen consumption and used this as a study of the biomarker for assessing the toxicant action. They all, also mentioned mainly the accumulation of the mucus on the gills and the architectural damage of the branchial filaments, the primary and secondary lamellae to be the valid reasons for such alterations in the oxygen consumption due to the stress and is same even for the present study also.

Evans (2005 & 1987) explained the mechanism of the gas exchange while during respiration. The toxic stress leads to the damage of the gill epithelium and as a consequence the epithelial transport of the ions is also affected. There are cells called chloride cells found in the lamellar epithelium. Their role in the ion transport, the afferent artery brings deoxygenated blood at its place where efferent one also at that same point, normal process takes place if anything happens the diffusion got impaired. There is a branchial epithelium that is sand-witched between serosal and mucosal blood. There are cat-ions K^+, Na with Cl^- ions. When Na^+ goes out CI ions are more inside and externally Na^+ will be more. Positivity in the serosal blood, as-ion in the afferent artery. Later, CO_2

when diffused inside with water forms H^+ and HCO_3^- after the dissociation of the weak carbonic acid. When HCO_3^- goes out one Cl^- ion gets inside. The osmo-regulatory, acid base and blood dynamic actions if it not affected by the environment pollutants. This can be explained as follows:



ATPase enzymes, Carbonic anhydrase (E) and Na^+, K^+ ions as in-flux and out-flux will be disturbed. The mechanism of actions is clearly explained in the article reported by Evans (2005 & 1987) and the above diagrammatic representation explains and if it is altered the O_2 diffusion is curtailed. Such similar mechanism is in operation even in the present work.

CONCLUSION

Oxygen is a parameter of life for its sustenance in living organisms and for the heterotrophs (animals-Fish) was a must for their metabolism. All such anabolism and catabolism reactive activities depend on the purification of the blood via the elimination of the metabolites, including gaseous carbon-dioxide. Gill is the entry point of the toxicant and is again at the same point, the exchange by diffusion of respiratory takes place. The reasons of the alteration of the consumption of the oxygen, at one end can be explained by the architectural damage of the respiring organ apart from the excretory organ, too, and at the other and the biochemical impediments of the blood, due to the failure of which only, not, to have the normal quantity of the oxygen to be carried to all cells/tissues/organs and that can be a good reason to be explained. All is well for the fish but it not, the health condition, disease prone, toxic stress even in sub-lethal concentrations have made them to suffer. It is a study of the biomarker in ecotoxicology and is the indices of the toxic action.

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Figure 1A. The amount of oxygen consumed in mg/gr body weight/hr of the fish *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos technical grade (Formulation)

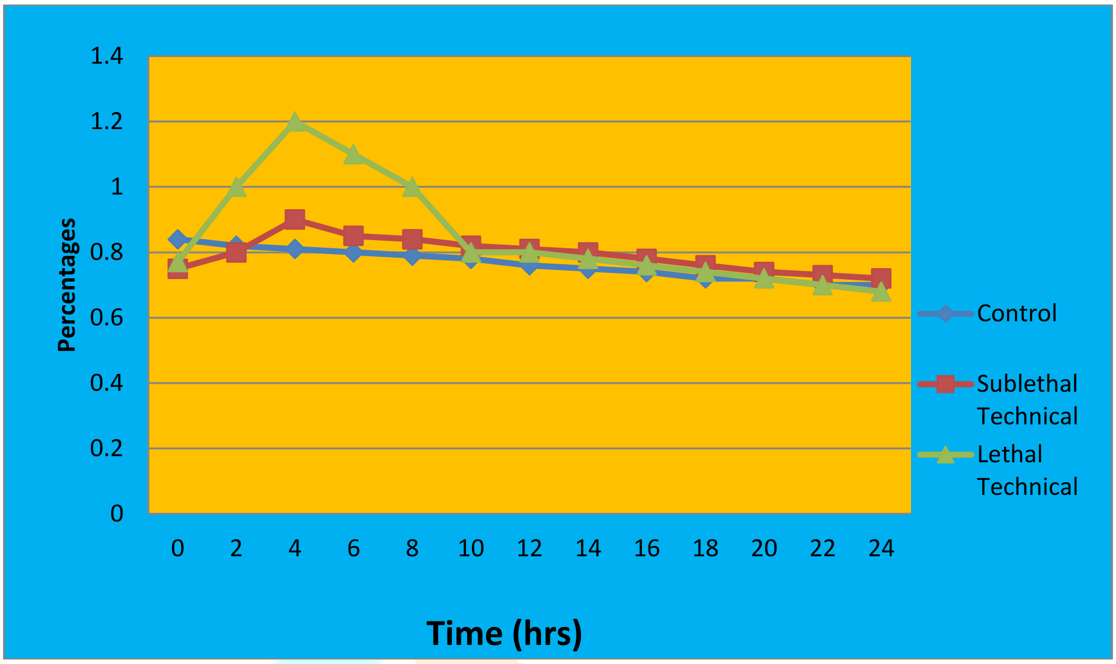

Figure 1B. The amount of oxygen consumed in mg/gr body weight/hr of the fish *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos technical grade (Formulation)

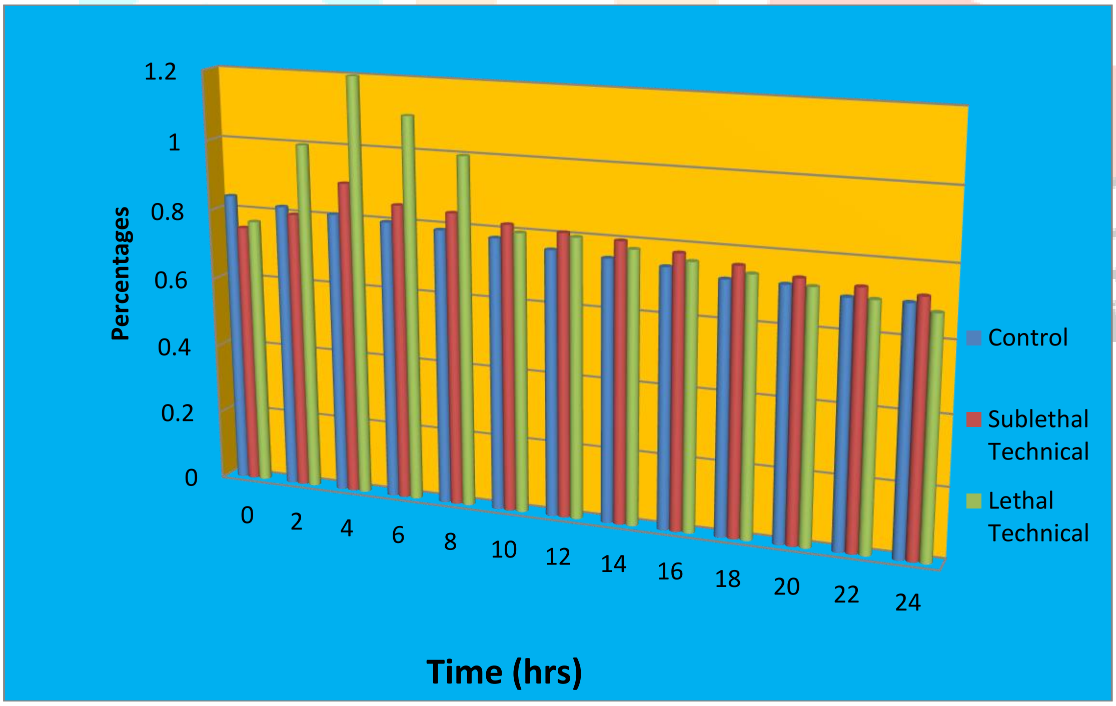

Figure 2A. The amount of oxygen consumed in mg/gr body weight/hr of the fish *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos 20% EC (Formulation)

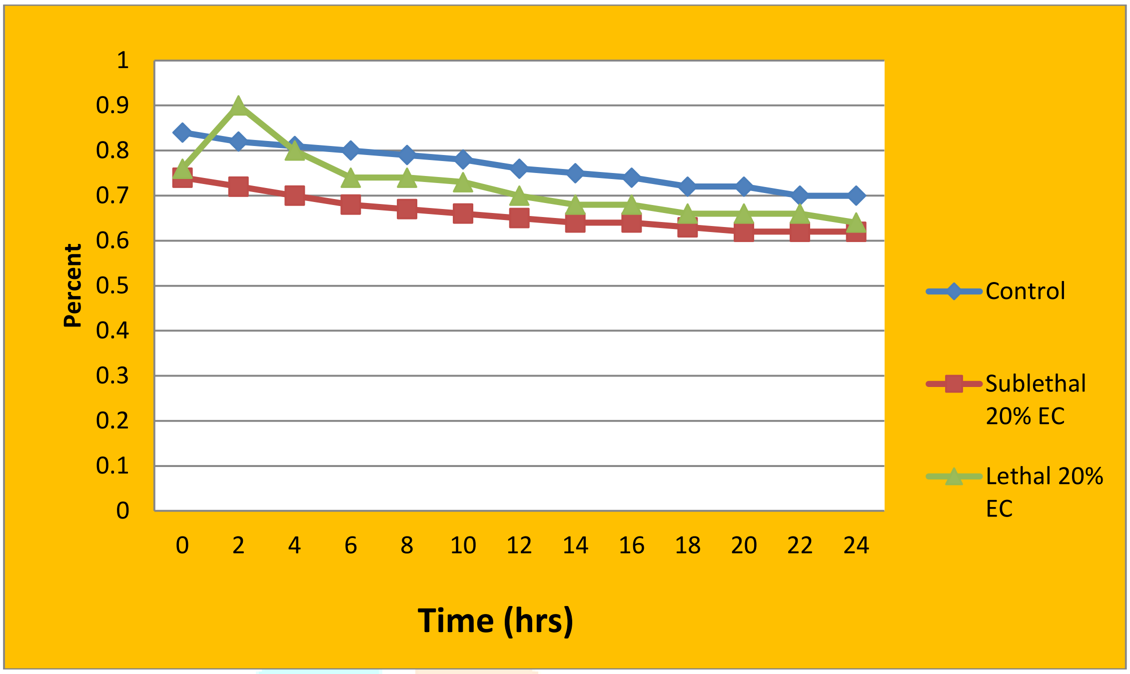

Figure 2B. The amount of oxygen consumed in mg/gr body weight/hr of the fish *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos 20% EC (Formulation)