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## RESEARCH ARTICLE

### CYFLUTHRIN TOXICANT STRESS ON THE OXYGEN CONSUMPTION IN THE FISH *CATLA CATLA*, A BIOMARKER STUDY

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#### ABSTRACT

One of the synthetic pyrethroid, Cyfluthrin technical grade and 10% WP is selected for study the stress as impact on oxygen consumption in the fish *Catla catla* which is a biomarker study. The fish is exposed at lethal and sublethal concentrations (1/10<sup>th</sup> of 96 hrs LC<sub>50</sub> value) of both technical grade 2.2 µg/L and 0.22 µg/L respectively and for 10% WP 1.4 µg/L and 0.14 µg/L respectively. Due to the inhibition of the enzyme AChE, the respiratory stress leads to variations in the oxygen consumption, of inspiration of respiration. Any change in the consumption of the gas leads to impairment of metabolism which is detrimental for growth. This is important in aquacultural practices because it will be having an impact on the venture of the culture. Hence, the levels of the concentrations are to be monitored and uncontaminated water only should be used as the medium of water for culture.

## INTRODUCTION

The use of pest controlling chemicals, the pesticides is inevitable not only in developing countries but also in the developed countries. When such usage in there the ecological consequences imparted by them, sometimes indiscriminately are much worried when the global ecosystem is taken into consideration. According to Tudi *et al* (2021) the application is on a large scale to control-wise variety of pests damage that substantially reduced the agricultural yield. According to Sinha *et al* (2022), these spraying of different chemicals apart from significant and association which is inseparable due to advancement of agricultural operations as achievements in our country, like India. According to Devi *et al* (2017), our country is biggest in Asia, 12<sup>th</sup> in the world and occupies at 4<sup>th</sup> position for export, production and consumption. Even Shefali *et al* (2021) reiterated the point that the urbanization as well as industrialization, made the loss of natural resources apart from several anthropogenic activities, all resulted an increase of the pollution. Among the pollutants, the toxicants caused not only death in acute concentration but even in chronic concentrations when present in the aquatic water bodies had an impact on the oxygen carrying capacity. Water is the ultimate sink for all pollutants/toxicants due to runoff to nearby aquatic bodies (lotic/leutic) and all the ambient organisms, had absorption/adsorption of the chemicals directly as a primary poisoning. In other aspect, eating the pesticide contaminated prey, which is a secondary

poisoning also all had an impact on the fish, nektonic, poikilothermic organisms (Shefali *et al* 2021). Zeeshan and Parveen (2022), the agrochemicals that are sprayed left in the environment without any change and quantitatively it is about 90%. These with such long duration of persistence can cause deleterious effects on non-target organisms especially fish. Hence, they recommended certain biomarker studies of oxidative stress nature to monitor the pesticidal pollution. Pradip *et al* (2019) cautioned that pesticide sprayings are legally responsible for the contamination of downstream fish mortality resulted finally. While the concentrations are not acute, the chronic levels pose the problem of fish inspiration and finally metabolisms, the growth is effected. Defilamented water when used the culture of the fish, the growth can be a hindrance and as such the very purpose of 'blue revolution' is a failure. Hence, the chronic levels are really lethal in the long run. According to Kaushlendra Kumar (2019), the very sensitive physiological process that got effected is none other than oxygen consumption and any modification of the respiratory activity is a biomarker study of stress. According to Prusty *et al* (2015), pyrethroids, the new generation class of compounds and can be also called, IV generation ones and they are highly effective in their use. They are more toxic to lower level vertebrates (Fishes and Amphibians) whereas less toxic to birds and mammals, the homeotherms. The toxic variation is due to absence of the enzyme hydrolase in fishes and Amphibians the poikilotherms. They form an important link in the food chain, connected to the

terrestrial environment. According to Sana Ullah *et al* (2019a), the pyrethroids when ecotoxicological studies can be of several biomarker studies and oxygen/respiration study is one among them. As the earth's subdivision the hydrosphere (largest), when transported into non-point source, the effects are many fold and the quantum of them use is appended in table 1. Even Kaviraja and Gupta (2014) too mentioned in the review article, the studies pertaining to respiration can be a biomarker study, apart from others. It serves as the indices of metabolism effect because all animals are heterotrophic organisms only. The presence of the toxicants as residues occur both in lethal (acute) as well as in sublethal concentration (chronic), while in chronic levels it had a bearing on the oxygen consumption and such studies proved that pollutants of all kinds need not be toxicants but reverse is true. The fish, heterotrophic organism, metabolism depends on the respiring gas and is also a growth factor. Hence among the cultivable major carps, one among them (*Catla catla*) is the organism that was tested as a biomarker study. Both in lethal as well as sublethal concentrations, the experimentation is made to know how much quantity of intake had an impact of Cyfluthrin technical grade and the commercial formulation that was marketed as 10% WP.

## MATERIALS AND METHODS

Experiments on the oxygen consumption of the fish *Catla catla* were carried out in a respiratory apparatus developed by Job (1955). The fish were brought from local fish farms, Nandivelugu, Guntur (dt.), A.P., India and stored in the laboratory conditions in well aerated water for 10 days. They were acclimatized fish and are used for the laboratory condition and such acclimatized fish for experimentation. The water that was used in the toxicity experiments and for acclimatization was same. It has the following physical and chemical characteristics; Turbidity – 8 silica units, Electrical conductivity at 28°C-8.16 Micro ohms/cm, pH at 28°C-8.2.

**Alkalinity:** Phenolphthalein-Nil, Methyl orange as  $\text{CaCO}_3$ -\*472, Total Hardness-\*320, Calcium Hardness-\*80, Magnesium Hardness-\*40, Nitrite nitrogen (as N) - Nil, Sulphate (as  $\text{SO}_4$ ) - 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 -  $28 \pm 2^\circ\text{C}$ . 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 10g in weight were used in the experiment. All the precautions mentioned by APHA (1998, 2005 & 2012), OECD (2019) are followed, for maintaining the fish. The fish were exposed to 96h  $\text{LC}_{50}$  lethal 2.2  $\mu\text{g/L}$  and also sub-lethal (1/10th of 96h  $\text{LC}_{50}$ ) as 0.22  $\mu\text{g/L}$  of technical grade and for 10% WB lethal 1.4  $\mu\text{g/l}$  and 0.14  $\mu\text{g/l}$  EC as sub-lethal (1/10th of 96h  $\text{LC}_{50}$ ) respectively in the respiratory chamber. The pure (almost was earlier obtained from a reputed company, the manufacturers of the Cyfluthrin, from a Agro Chemical multinational Company by name M/s. Bayer India Ltd., (Bombay, Maharashtra-400 079, India) and the formulation was purchased from the available pesticides shops, local of Guntur, Andhra Pradesh, 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, until 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 24 hrs. 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.

$$\text{O}_2 \text{ consumed by fish/} = \frac{\alpha - \beta \times \text{N of hypo} \times 8 \times 1000}{\text{gram body weight/hr} \quad \text{Volume of the sample} \times \text{Correction factor} \times \text{Wt. of the fish} \times \text{time interval for each sample}}$$

$\alpha$  - hypo rundown before exposure

$\beta$  - 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 10% WP for *Catla catla* and their percent variations are graphically represented as figures 1A & 1B and 2A & 2B. By taking time on X-axis and the amount of  $\text{O}_2$  consumed per gram body weight on the Y-axis and both the line and bar modes are shown.

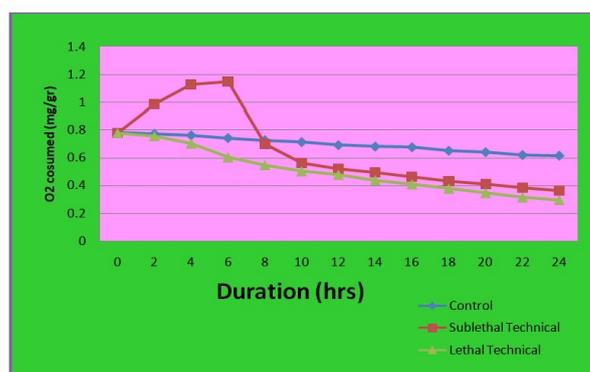
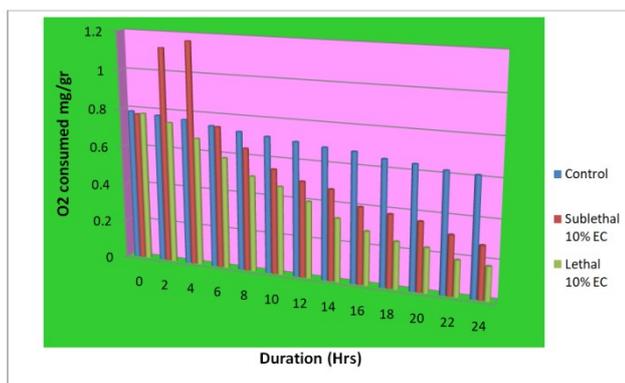
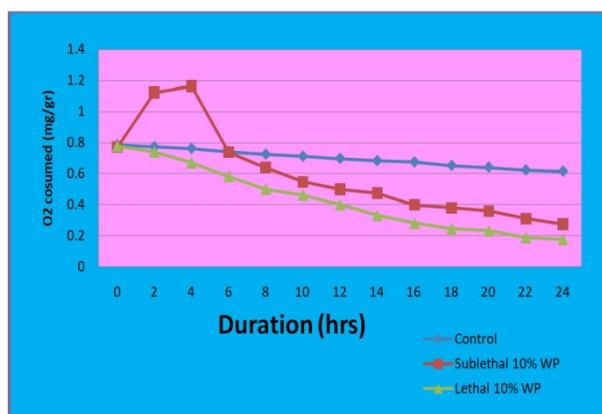


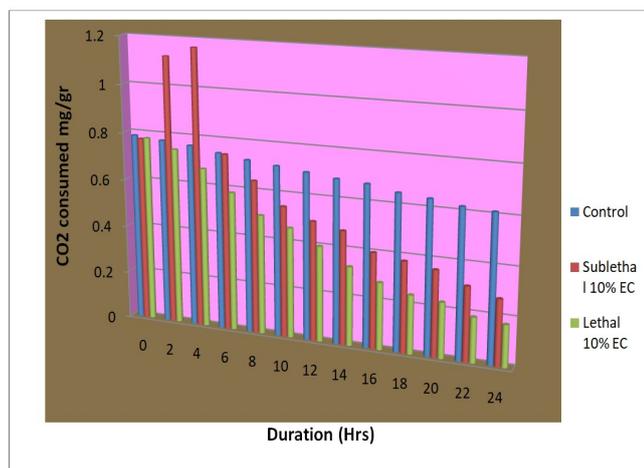
Figure 1A. The amount of oxygen consumed in mg/gr body weight/hr of the fish *Catla catla* exposed to sublethal and lethal concentrations of Cyfluthrin, technical grade



**Figure 1B.** The amount of oxygen consumed in mg/gr body weight/hr of the fish *Catla catla* exposed to sublethal and lethal concentrations of Cyfluthrin, technical grade



**Figure 2A.** The amount of oxygen consumed in mg/gr body weight of the fish *Catla catla* exposed to sublethal and lethal concentration of Cyfluthrin 10% wetttable powder (WP)



**Figure 2B.** The amount of oxygen consumed in mg/gr body weight of the fish *Catla catla* exposed to sublethal and lethal concentration of Cyfluthrin 10% wetttable powder (WP)

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 more quantity of the oxygen recovery and try to be stable in that toxic aspect of situation a positive sign of resistance and their effect is more precluded in 10% WP than to the technical grade is evident as per the figures.

## DISCUSSION WITH SYNTHETIC PYRETHROIDS

Practically, the research studies on the oxygen consumption variations due to the toxicant Cyfluthrin are nil inspite of using it throughout the globe are nil. Sana Ullah *et al* (2019a), while in their review article, the studies of the biomarkers of pyrethroid toxic action as toxicity to fish mentioned that the inhibition of the enzyme AChE, the neurotransmitter that resulted a change in opercular movement apart from coming to the surface for more gulping of the gas. The oxidative stress, that lead to ROS & LPO reactions which had an impact on the antioxidant enzymes (Catalase, peroxidase superoxide dismutase and glutathione reductase) activity increased (Sana Ullah *et al* 2019b). Similar such aspect was also viewed by the study of Parlak (218), VIEIRA and dos Rais Martinez (2018), Classen *et al* (2018), Kutluyer *et al* (2016), Ullah (2015), Abdel Daius *et al* (2015); Ullah *et al* (2014), Guardiola *et al* (2014) and Ensibi *et al* (2013), in different fish with Deltamethrin, Cypermethrin and alfa Cyhalothrin, the synthetic pyrethroids of type II. The metabolites that are formed as aldalydes and cyanides are responsible for the production of the Reactive Oxygen Species (ROS) that lead to Lipid Peroxidation (LPO) increase all culminate the toxic action. The fishes and Amphibians lack the enzyme hydrolase hence no detoxification mechanism however the birds and mammals had the enzyme for detoxification hence not toxic to them. Hasibur Rahman *et al* (2014) in the review article of deltamethrin, mentioned that it is highly toxic to the fish and due to the resultant of gill arches flaring as well as differences in osmomolarity all cumulatively brought change in oxygen uptake. Saumya Biswas *et al.* (2019) in the review article while referring the reports of Jispa *et al.* 2014, Logoswamy and Rewia (2009) and Marigoudar *et al.* (2009), the synthetic pyrethroids of group II has alterations in the oxygen consumption of the fish, *Tilapia mossambica*, *Tilapia mossambicus* and *Labeo rohita* respectively.

The impact will be more in sublethal concentrations and has delayed and extended effect. Qihong *et al.* (2019) referring to deltamethrin toxicity, which was due to oxidative stress referring to all vertebrates of neurotoxic action. Srinivasa Rao *et al* (2018) reported in the fish *Ctenopharyngodon idella*, deltamethrin had a severe impact on the oxygen consumption due to the toxic stress and concluded that it was really the sublethal concentrations are lethal. Balakrishna Naik *et al.* (2018) in the fish *Cyprinus carpio* reported that due to exposure to synthetic pyrethroid of type I permethrin due to architectural damage of the gill that resulted alterations in the oxygen uptake. The same was the case in the present study. Similarly, Balquees (2018) too opined of the similar lines of the above working with the permethrin as well as an organophosphate pesticide chlorofete, in the fresh water fishes *Gambusia affinis*, *Cyprinus carpio* and *Ctenopharyngodon idella*. Lenin Suvetha *et al.* (2015) while reporting on the deltamethrin toxicity to the fish *Labeo rohita*, the toxicity effect was due to hormonal and enzymological effects, particularly the cholinesterase that disturbed the oxygen metabolism. Guardiola *et al.* (2014) opined also in the fish *Sparius aurela* L., deltamethrin as a toxicant effected the fish due to oxidative stress.

In the study of the fish *Anabas testudineus* by Sapana Devi and Abhik Gupta (2014) using deltamethrin as well as permethrin as toxicants. The work even though on different orientation, wherein the result proved that Deltamethrin is more toxic than permethrin, had a profound impact on food consumption and growth resulted by the chemical stress where it cannot met the energy demand due to the alterations in oxygen consumption, of respiration. Huynh *et al.* (2012) reported the deltamethrin toxicity, wherein they emphasized that in black tiger Shrimp (*Penacus monodon*) due to environment factors of temperature and salinity that interacted resulting oxidative stress lead to changes in respiration particularly in oxygen consumption. Velisek *et al.* (2011, 2007 & 2006) while working on deltamethrin toxicity to fish too opined that alterations in the oxygen uptake were possible due to toxic stress. Neelima *et al.*, (2016a&b) using cypermethrin 25% EC as toxicant which also belong to type II synthetic pyrethroid to which the present toxicant of evaluation belongs to the fish *Cyprinus carpio* exposed to both lethal and sublethal concentrations.

The result reported that there was a demand for more oxygen consumption which is more in lethal concentration than in sub-lethal concentration. The changes are due to respiratory distress as a consequence of impaired oxidative metabolism. The LC<sub>50</sub> value calculation method is not continuous flowthrough system that is employed and is only static renewal and the experimentation method was not with technical grade cypermethrin. However, the toxicant belongs to the same class with cyanogroup similar to the present study toxicant but experimentation includes technical grade Cyfluthrin also, but the fish is not same, but the concentrations of the toxicants are different and the stress factor influenced to take more oxygen and can be considered as a general principle of demand for more oxygen. Madhura and Kulkarni (2014) reported that the acute toxicity of cypermethrin, a synthetic pyrethroid to Estuarine clam *Katelysiaopima* (Gmelin) and its effect on oxygen consumption. The study reported on the rate of oxygen consumption which fluctuated with an increase in the exposure period of the toxicant. The decrease they observed is attributed to variation in the volume of water ventilated through the gills caused by the intermittent closure and opening of the shell valves.

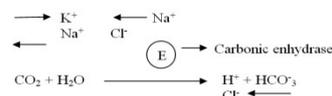
They reported that the main reason/factor responsible for decreased oxygen uptake was accumulation of mucus on gills due to cypermethrin exposure. It caused reduction in the effective transfer of oxygen to internal tissues which adversely affected the absorption of oxygen from the ambient medium. The bivalves usually try to avoid toxicants and in doing so they can minimize the metabolic activity due to stress of the drug which resulted a decrease in oxygen consumption. This was also confirmed by histopathological structure of the gills of Crustaceans, where in the organ showed inefficiency. Thus, mucus secretion, stress, impaired oxidative metabolism and architectural changes in the gills resulting pathological condition there by reduced efficiency of diffusion of gases. The present study which also included the same concepts even through the quantities of the variations of oxygen are different as well as organisms tested. The main entry, that is the first entry and the first damaged organ are none other than the gills and as a consequence, oxygen consumption is not being normal and which might be altered and even in the present study where was a damage of the gills. Jispa *et al* (2014) reported on the impact of a cypermethrin insecticide on oxygen consumption and certain biochemical constituents of the fish *Tilapia mossambica*. The study objective also includes to observe the impact of sublethal concentration of cypermethrin on the rate of oxygen consumption of the fish, *Tilapia*.

The values obtained in the tested media of water, in the control and exposed to 24, 48, 72 and 96 h LC<sub>50</sub> values also showed marked changes while demanding more oxygen due to stress. They reported all the parameters of biochemical nature except glucose are decreased. The increased values of glucose are due to failure of metabolism where the substrate is not subjected to anabolism due to failure of oxygen intake. Manjula and Veeraiah (2014) too reported cypermethrin effect on oxygen consumption of freshwater fish *Cirrhinus mrigala* (Hamilton) exposed to sublethal concentration 1/10 of 96 hour LC<sub>50</sub> value observed that one of the earliest symptoms of acute pesticide poisoning as the respiratory stress. The *Cirrhinus* is the bottom feeder, acclimatized to live in low oxygen concentration hence showing least indices in pesticide toxicity. On the contrary, the present study fish *Cyprinus carpio* the silver carp, an omnivorous require more oxygen and can be a good indices of toxicity. In both cases of studies severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased mucus secretion higher ventilation volume decreases in oxygen uptake efficiency labored breathing and engulfing air through mouth when exposed to the toxicant.

Paritha Bhanu and Deepak (2014) reported the toxicity of cypermethrin influenced by pH and temperature on fresh water fish *Oreochromis mossambicus*. They opined pH and temperature influence the toxicity and dissolved oxygen is dependent on temperature which not only effect the toxicity and decreased oxygen consumption that impaired the respiratory activities.

The present study of Cyfluthrin, was conducted at low temperature and accordingly LC<sub>50</sub> values are calculated that too in continuous flowthrough system where methodology is different that too in sublethal concentration. Hence, the contradictory results are obtained. The elevation of pH will lead to acidosis in fishes which would decrease the oxygen carrying capacity of blood. The present study at the specific pH of water a decreasing trend is reported (as per the physico-chemical conditions of water) and the hydrographical conditions are such that no situation of acidosis like the work of above which resulted an enhanced oxygen uptake in stress due to the toxicant exposure. Anita Susan *et al.*, (2012 & 2010) reported a study on acute toxicity, oxygen consumption and behavioural changes in the three major carps, *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) *Cirrhinus mrigala* (Hamilton) exposed to Fenvalerate another type II synthetic pyrethroid with cyanogroup. The study revealed that, 20% EC was found to be more toxic than technical grade and at sublethal exposure had profound effect not only on the behaviour but also in the oxygen consumption.

During experimentation due to severe respiratory distress and rapid opercular movements that resulted a change in respiration in the fish experimented. Finally, the report of the work concluded that due to the effect of the toxicant on respiratory centers of the brain or on the tissues involved in breathing had change in oxygen consumption. The total oxygen consumption is one of indicators of the general healthy and active fish. The damage inflicted on the animal in the gill epithelium could either increase or decrease the oxygen uptake. Pesticides in general were observed to cause respiratory distress or even failure by affecting the respiratory centres and the effect of the brain or the tissues involved in breathing. The respiratory potential and oxygen consumption of an animal are the important physiological parameters to assess the toxic stress because it is a valuable indicator of energy expenditure and metabolism in general (Proser and Brown 1977). The fenvalerate is type II synthetic pyrethroid and the fish is the same that had employed like in the present study. The lack of oxygen increases the ventilation volume of the fish and the cardiac output is reduced. This reduces the rate of passage of blood through gills 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 slow 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 (Jones 1973; Bradbury *et al.*, 1987a&b). A significant drop in the rate of oxygen consumption in *Cyprinus carpio* exposed to both fenvalerate and cypermethrin was observed by Malla Reddy (1987). The actual mechanisms while in the exchange of the respiratory gases (O<sub>2</sub>/CO<sub>2</sub>) are explained by Evans (2005 and 1987, the transport of the ions K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, HCO<sub>3</sub><sup>-</sup> across the lining of the gill squamous epithelium. There are actually both afferent artery that is supposed to carry deoxygenated blood coming in contact with efferent at one point. In the normal situation, it is a smooth affair. If any change is resulted the diffusion of the physical process goes out wrongly. It can be like the following:



At the meeting/junction point the above things will happen. The positive Na<sup>+</sup> ions goes the Cl<sup>-</sup> ions of negativity increases like that ionic imbalance happens, resulting a situation of CO<sub>2</sub> combining with water result in K<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> dissociation and Cl<sup>-</sup> ion plays its role. Thus such Osmo regulatory, acid-base in the blood having some dynamic actions prevail until environmental toxicants results an imbalance. The Na<sup>+</sup> and K<sup>+</sup> ions influx and outflux, (E), carbonic anhydrase along with at par activity all in coordination if that coordination locked then the exchange of O<sub>2</sub>/CO<sub>2</sub> got impaired. Such similar aspects can be visualised in the present study. Respiration is the vital process for living organisms, wherein cellular oxidation in heterotrophic animals energy demand to be met. All the chemical characters of water if normal the sustenance is possible.

But due to contamination of the natural waters, xenobiotic compounds result alterations resulting a change in respiration particularly heterotrophic cold blooded animals the gill respire. The concentration may not be such that, to kill them immediately but in low level termed sublethal for long duration of exposure make them hard to lead life normally, hence the decrement in oxygen uptake. The decrease is not only for the present toxicant but also any toxicant of pesticide of different classes. The fish, *Cyprinus carpio* cultured fish along with *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* in freshwater, if the concentrations are sublethal the cellular oxidation is at low ebb and is going to be hindrance to growth. The present studied *Catla catla* fish lives in surface waters hence any changes it cannot resist.

## CONCLUSION

The toxicant and the fish study are first of its kind in the methodology when the type II synthetic pyrethroid Cyfluthrin is studied and that gives information about the candidate species for culture to have natural care. Hence, all the toxicants have to be tested for all the fish to monitor the pollution in environmental policy and planning and the present study can be inferred that sublethal are really lethal as in the case of failure of normal oxygen intake while in respiration.

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