A STUDY ON ACUTE TOXICITY, OXYGEN CONSUMPTION AND BEHAVIOURAL CHANGES IN THE FRESH WATER FISH *CATLA CATLA* (HAMILTON) EXPOSED TO SODIUM FLUORIDE

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Abstract

Acute toxicity experiments were conducted using static renewal bioassay 24 h, 48 h and 96 hours on the fresh water fish *Catla catla* (Hamilton) fry and fingerlings. LC50 values for 24 h - 380.18 mg / L, 48 h LC50 value - 337.28 mg/L and 96 h LC 50 value - 328.01 mg/L for fry. 24 h LC50 value for the fingerling stage 297.85 mg/L. The fingerling stage is found to be more sensitive than fry stage. For further studies only fingerling stage is selected. The toxicant exposed fish showed anomalous behaviour like surfacing phenomenon, rapid opercular movements and gulping, loss of equilibrium etc. The fish was exposed to $1/10^{th}$ 24hr $LC_{50,}$ 24 hr LC_{50} and > 24 hr LC_{50} concentrations of toxicant sodium flouride to study the oxygen consumption rate for 12 hours and the concentrations were 29.683mg/ lit, 296.83mg/lit and 301.22 mg/lit respectively. The oxygen consumption was recorded at the end of each hour. During experimentation severe respiratory distress, rapid opercular movements leading to the higher amount of toxicant uptake increased mucous secretion decrease in the oxygen uptake efficiency and labored breathing was observed. It resulted in decreased oxygen consumption of the fish which indicates the fish is under toxicant stress. It also resulted behaviour demand for more energy which leads to the depletion of energy sources.

Key words: Behaviour, *Catla catla*, Toxicity, Sodium Flouride, Oxygen consumption.

Introduction

Aquatic environment can be polluted by a variety of pollutants that originate from natural and anthropogenic sources which are toxic to the aquatic organisms including fish. The aquatic environment is severely affected by different types of chemicals which are toxic to the inhabiting organisms (Kopeca et al. 2006). Long term exposure to these pollutants causes countless abnormalities and reduces the life span of organisms (Hussain et al. 2011, Khan et al. 2012). Pollution of aquatic ecosystem by domestic and untreated or partially treated industrial effluent greatly contributes to massive kill of fish and other important aquatic biota (Shaikh 2013). Fluoride is one such pollutant. Fluoride not only enters into the aquatic media through various human activities but also from sediment rocks. The main source of fluoride in ground water is fluoride bearing rocks such as cryolite, fluorite, fluorspar, fluorapatite and hydroxylapatite (Meenakshi, et al.2004). It is also released through phosphate fertilizer production. Fluoride is the 17th most abundant element and is highly electronegative element never encountered in nature as element. High fluoride concentration in the ground water and the surface water in many parts of the world are of great concern.

Among the water quality parameters fluoride ion exhibits unique properties i.e. below 1.0 to 1.5 ppm in drinking water is advantageous to health and in case of exceeding concentration above the permissible levels effects the health (Venkat et al. 1995). A review by Barbier et. al. (2010) has sketched a number of cellular processes in which fluoride can have negative effects. Fluoride exists as fluoride compounds which are constituents of materials in rocks and soil (Dhar et al. 2009). In Andhra Pradesh in most of the districts high concentration of fluoride (> 1.5 mg/l) have been reported. The toxic effects of elevated fluoride on various aquatic species has been reported by Camargo (2003). Sodium fluoride is the most common inorganic fluoride toxic to aquatic organisms reported by Sanders and Cope (1966).

The present investigation was undertaken to study the toxic effect of fluoride on fish *Catla catla* as it is important edible fish which occupies the highest trophic level in food pyramid. Fish can be considered as indicator organisms of environmental pollution (Met calfe - Smith et al. (2003), Bhatnagar et al. (2006). Long term exposure to these pollutants causes countless abnormalities and reduces the lifespan of organisms (Hussain et al. (2011) & Khan et al. (2012).

Materials and Methods: The fresh water *Catla catla* (Hamilton) fry of both sexes, length 1.5 to 2.5 cm and weight 45 to 70 mg, fingerlings 4-6 cm weighing 2500-3500 mg have been used as the test organisms for the present investigation. Healthy and active fish were obtained at Nandivelugu, Guntur District, A.P., India.

The fish were acclimatized to the laboratory conditions in large plastic water tanks for two weeks at room temperature 28+1°C and 12h dark and light cycle. Water was renewed every day during the period of acclimatization, the fish were fed (at libitum) with groundnut oil cake on rice bran. Feeding was stopped one day prior to acute toxicity test. All the precautions recommended by APHA toxicity test to aquatic organisms APHA (1998, 2005 and 2012) were followed. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

Physical and Chemical properties of water used for the present experiments are (in mg/l). Turbidity-8 silica units, Electrical conductivity at 28°C-816 micro ohms/cm, pH at 28°C-8.1, Alkalinity, Phenolpthalene - Nil, Methyl orange as CaCo3232 Non-carbonate hardness as (MgCo3) - Nil, Nitrate nitrogen as (N) - Nil, Sulphate (As So4) Trace chloride (As Cl) - 40 Fluoride (as F) 1.8, Iron as (Fe) - Nil, Dissolved oxygen 8-10 ppm, Temparature 28+2°C.

Sodium fluoride reagent grade was used as a toxicant supplied by LOBA chemical company, Bombay. The test solution of Sodium fluoride, was prepared by using water. The water used for acclimatization of the fish and for conducting experiments was the same.

Acute Toxicity Test: Experiments were conducted to determine acute toxicity of Fluoride to *Catla catla* fry and fingerlings for 24 hours, 48 hours and 96 hours in static system. First pilot tests were conducted to choose the concentrations at which the mortality of fishes were observed. Five replicates were taken for each concentration, along with the control group. The concentrations of the test chemical used in short term definitive tests were in between lowest concentration at which there was no mortality and the highest concentration at which 100% mortality resulted. Ten fish were introduced in each test chamber having ten liter of test solutions. The numbers of dead fishes at each concentration are noted but precaution was taken to remove the dead fish immediately. The data observed from these tests were recorded from time to time. Toxicity experiments were conducted to choose the mortality rate from 10% to 90% for 24 h, 48 h and 96 h in the static system.

Finney's Probit analysis (Finney 1971) as recorded by Roberts and Boyce (1972) was followed to calculate LC50 values. The respective Probit values were taken from Fisher and Yates (1938) and the data was analysed by Probit analysis and computer generated output is taken which has given 24 h LC 50, lower and upper limits, regression equation, slope and R^2 values (Finney 1952) and is represented graphically.

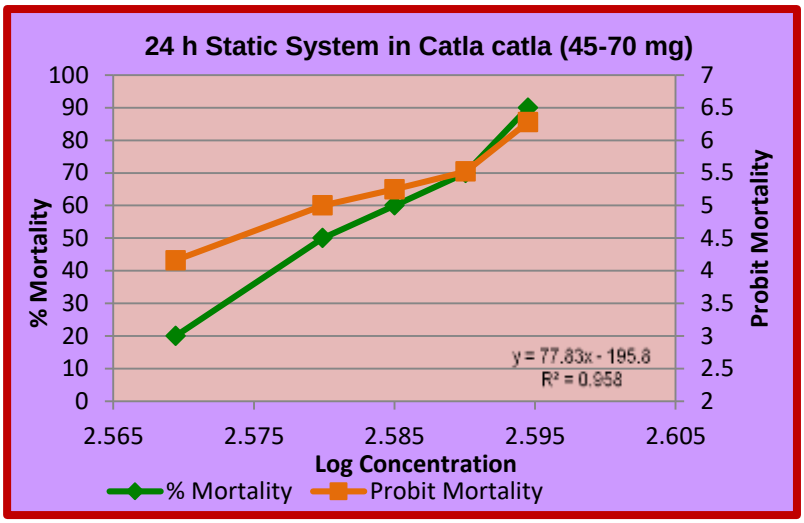
Determination of Oxygen: Experiment on the oxygen consumption of the fish *Catla catla* (Hamilton) was carried out in respiratory apparatus developed by Job 1955. The fish were brought from a local fish form at Nandivelugu, Guntur District. They were acclimatized for 15 days in the laboratory conditions in well aerated water. All the precautions laid down by APHA (1998, 2005 and 2012) were followed for the water quality and experiment. The fish measuring 6 to 8 cm in length 2500-3500 mg weight were used. The fish was exposed to $1/10^{th}$ 24hr $LC_{50,}$ 24 hr LC_{50} and > 24 hr LC_{50} concentrations of toxicant sodium flouride to study the oxygen consumption rate for 12 hours were 29.683mg/ lit, 296.83mg/lit and 301.22 mg/lit respectively. The samples for estimation were taken from the respiratory chamber at the interval of 1 hour period upto 12 hours. The amount of dissolved oxygen consumed was calculated per gram body weight / hour.

$$\text{Oxygen consumed by fishgm body wt.} = \frac{\text{Hyporun down} \times \text{No.of Hypo} \times 8 \times 1000}{\text{Volume of sample} \times \text{correction factor} \times \text{wt.of the fish} \times \text{time interval for sample}}$$

Student t-tests was employed to calculate the significance of the differences between control and experimental means 'P' values 0.05 or less were considered statistically significant.

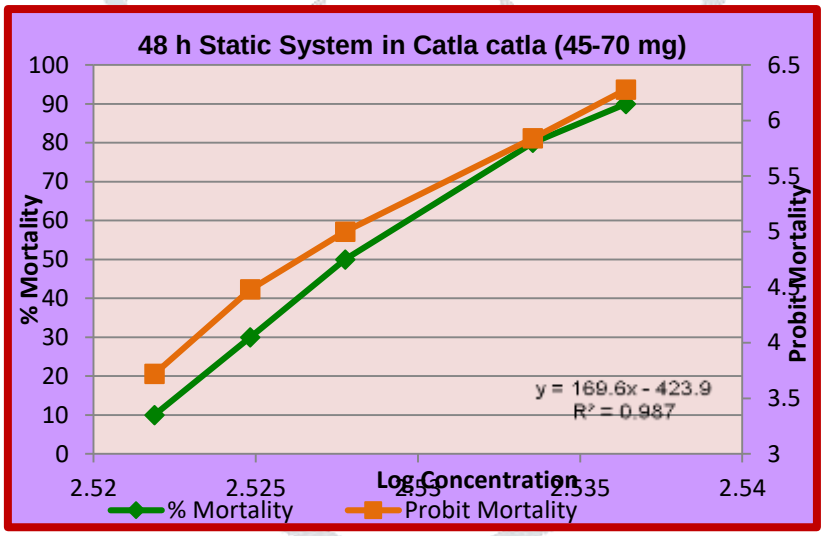
Results and Discussion: *Catla catla* fry in more resistant than fingerlings when exposed to toxicant. In case of *Catla catla* fry with increase in duration of exposure to toxicity decreased shown in figures 1,2, 3 i.e. LC50 values of 24 h duration is at high concentration and followed by 48 h LC50value and 96 h LC50value 380.18 mg / L, 337.28 mg/L and 328.01 mg/L for fry respectively. LC50 value for the fingerling stage 297.85 mg/L shown in figure 4. The fingerlings are more sensitive than *Catla catla* fry. The fish was exposed to $1/10^{th}$ 24hr $LC_{50,}$ 24 hr LC_{50} and > 24 hr LC_{50} concentrations of toxicant sodium flouride to study the oxygen consumption rate for 12 hours were shown in figures 5 and 6, the concentrations were 29.683mg/ lit, 296.83mg/lit and 301.22 mg/lit respectively. In the early hours of exposure the fish timed to overcome the toxicant effect by consuming more oxygen but due to rapid ventilation more toxicant entered the body resulting decreased oxygen consumption due to toxicant stress.

Fig 1: Graphical representation of 24h LC50 value in static system for Sodium fluoride in *Catla catla*



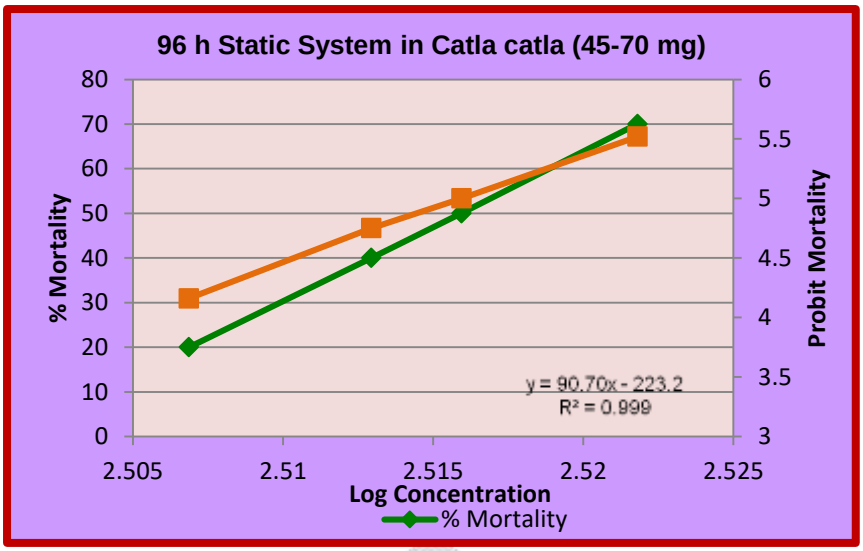
LC50=380.18, 95% confidential limits: 373.46 -387.13

Fig 2: Graphical representation of 48h LC50 value in static system for Sodium fluoride in *Catla catla*



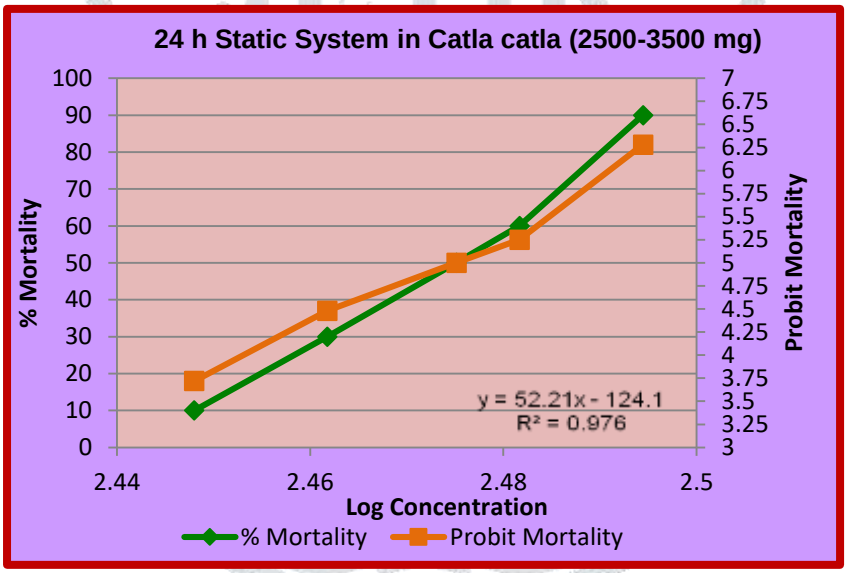
LC50= 337.28, 95% confidential limits: 330.76 -343.83

Fig 3: Graphical representation of 96h LC50 value in static system for Sodium fluoride in *Catla catla*



LC50= 328.019, 95% confidential limits: 321.54 -334.48

Fig 4: Graphical representation of 24h LC50 value in static system for Sodium fluoride in *Catla catla*



LC50= 297.85, 95% confidential limits: 290.76 -304.83

Figure – 5 Amount of Oxygen consumed in mg/Kg body weight of the fish *Catla catla (Hamilton)* exposed to Sodium Fluoride

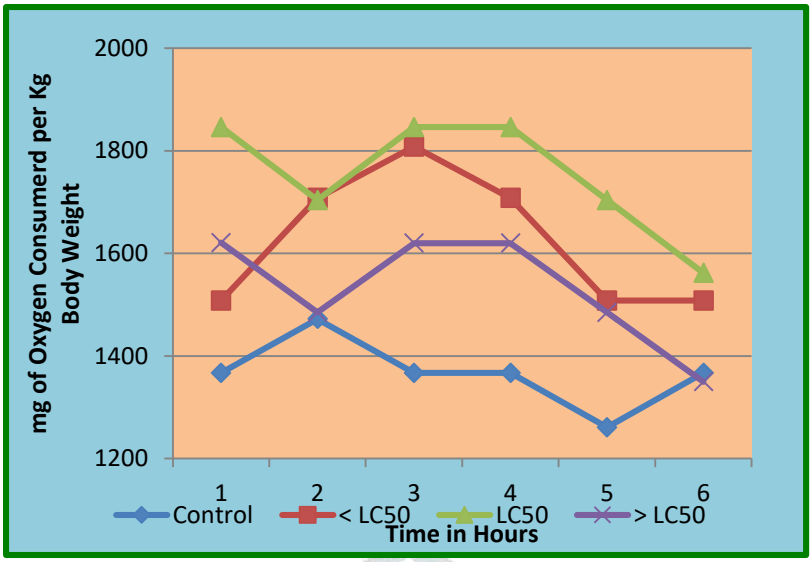
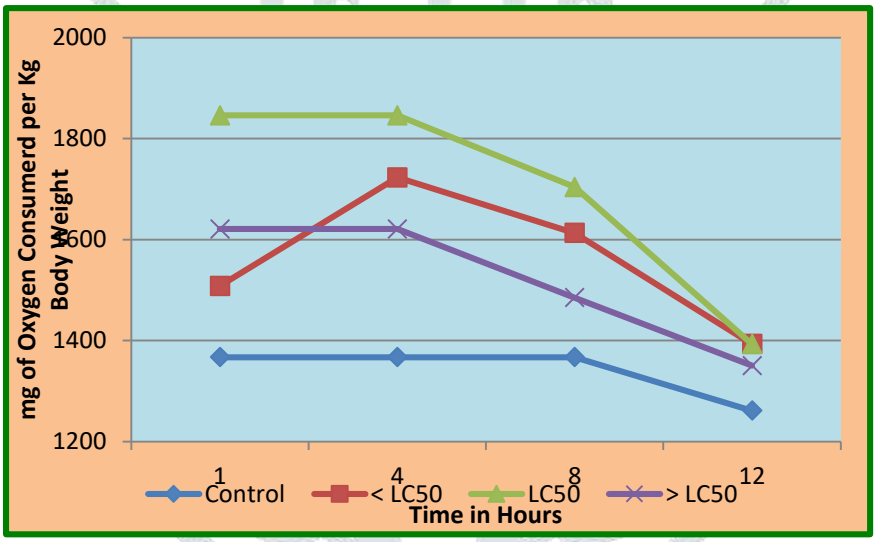


Figure – 6 Amount of Oxygen consumed in mg/Kg body weight of the fish *Catla catla (Hamilton)* exposed to Sodium Fluoride



Herbert and Shurben (1964) recorded the mortality in yearling rainbow trout at 8.5 mg/l fluoride (Hemens and Warwick 1972) have shown that some estuarine species may also be susceptible to comparatively low fluoride concentration. Wright (1977) reported the Probit percentage kill against log time indicating mortality rates of fluoride levels below 29 mg/l in brown trout (S. trutta). According to Wright 1977 at fluoride concentration below 20 mg/litre, there are points where the*re is distinct fall in mortality rate occurs. Herbert and Shurben (1964) recorded no mortality in yearling trout after 21 days in water containing 75 mg/l fluoride, having hardness level of 45 mg/l CaCo3.

Wright (1977) reported that *Salmo trutta* are more sensitive to the fluoride than *S.gairdnerii* egg's and fry despite the fact that the water used in considerably harder and further reported that difference in response of species varied due to difference in species sensitivity. Acute toxicity of fluoride effluent in *Catla catla* fry was reported by Pillai and Mane (1985). Catla catla fry died within one hour at different concentrations of effluent i.e. 100%, 90%, 80%, 100% and 92% mortality took place within 12 hours and 16 hours respectively in fry exposed to 30% effluent dilution. They reported that 16 h LC50 was 28.7 ppm. Besides high fluoride context in these dilutions the low pH also played an important role in killing the fry. 96 h LC50 was reported as 4.8 ppm, F in dilutions having the same pH as in control. LC 50 concentration of Fluoride for *Channa Punctatus* (blotch) was reported as 10 ppm (Chitra 1979). LC50 concentration using mixture of NaF and HgCl2 at different concentrations (Chitra et al. 1983) were reported. The lethal concentrations were found to range from 3 ppm HgCl2 +10 ppm of NaF, to 5 ppm of HgCl2 +20 ppm of NaF, 5 ppm of NaF+1.25 ppm mercuric chloride for 96 h of acclimation. 2.5 ppm of NaF+0.63 ppm HgCl2 for 168 h of acclimation 1.25 ppm NaF+0.32 ppm of HgCl2 for 240 hours of acclimation.

Pillai and Mane 1987 reported that *Catla catla* eggs exposed to fluoride effluent (16.68 ppm) of fluoride showed delayed hatching. The weight of eggs exposed to fluoride in both the media i.e. effluent and Sodium fluoride decreased considerably with the increase in fluoride and exposure time. Water hardness influence the toxicity of fluoride to rainbow trout. (Salmo gaird nerii) and 96 h medial lethal concentration values increased from 51 to 193 mg/l as water hardness level rose from 17 to 385 mg/l

CaCo3. The toxic nature of fluoride ion in the extuarine fish and crustacea containing 5 mg/l was reported. Mortality and physical deterioration was not observed in *Mugil cephalus*, *Penacus indicus* and *Tylodipax* even after 113 days of exposure. The result is different from the present report as there is difference in physical parameters of toxicant medium.

It was shown that comparatively the mullet is not highly sensitive and 96 h exposure to a 100 mg/l concentration has no effect on juvenile mullet *Mugil cephalus*. A 72 h exposure to 52 mg/l concentration has resulted in 3 deaths in exposed animals (Hemens and Warwick 1972). It was also reported that the Sodium fluoride showed no toxic effects on Mullet (*Mugil cephalus*) and two small fish *Ambassis safgha* and *Terapon jarbua* during 96 hours of exposure at concentration < = 100 mg F/l (Hamens and Warwick, 1972). They reported exposure to 52 mg F/l and 20 salinity in our door estuary models without food supply which resulted in physical deterioration and increased mortality in mullet and the Crab *Tylodepax blephariskias* and adversely affected the reproduction in the shrimp *Palaemon Pacificus*. Agriwanstil et al. (2014) reported statistically significant effect of different doses of fluoride on gonadosomatic index and weight gain in *Clarias batrachus* and *Heteropneustes fossilis*. The effect of fluoride on chromatophores was reported by Tripathi et al. (2005); Bajapai et al. (2012) in *Heteropneustis fossilis* as the fish change its color to black as a result of toxic effect. It agrees with the present study of morphological behaviour.

The toxicity can be quantified in terms of mortality, growth suppression and tissue damage. Environmental factors such as temperature, hardness and alkalinity of water play a major role and reduce or enhance the toxicity respectively. Not much work has been done on the toxicity of *Catla catla* the fresh water fish at different environmental conditions. The LC50 values of Catla catla were represented graphically as Fig. 1, 2, 3 & 4 for 24 h, 48 h and 96 h & 24 hours respectively. The LC 50 values obtained in the present study are much higher than natural water fluoride levels. Though the fluoride concentrations are always lethal even in very low fluoride waters are capable of accumulating fluoride gradually for prolonged period result in chronic fluorosis. Hemens et al. (1975) reported the accumulation of fluoride in the estuarine fish and crustacea containing 5 mg/l in the medium. Tripathi et al. (2005) found that the higher concentration of fluoride inhibits the growth of fishes such as weight, length, in the fingerlings of *Heteropneustis fossils.*

The toxicant exposed fish *Catla catla* showed anamalous behaviour like surfacing phenomenon, irregular erratic and darting, swimming movements and hyper exitability, loss of equilibrium, bittering to the walls of test tank before finally sinking to the bottom changing its body colour to black just before death. The present study cognisize with the reports made by Newhold and Singler (1960) as biomolecules get reduced in the body tissue has marked effect on fish, with the symptom of acute fluoride intoxication in carp and rainbow trout include lethargy and anorexia followed by erratic movements. Similar reports were made by Aziz et al. (2014), Narwaria et al. (2012); Rout et al. (2013) stated the toxicant affect the behaviour and ultimately resulted in death. Agniwanshi et al. (2014) reported statistically significant effect of different doses of fluoride on gonadosomatic index and weight gain in both species *Clarias batrachus* and *Heteropneustis fossils*. Tripathi et al. (2005); Bajapai et al. (2012) observed in *Heteropneustis fossils* the change in body color to black as a result of toxic effect. It agrees with the present study the fish when exposed to pesticides / chemicals changes were induced in respiratory behaviour and matabolic rate. Since the respiratory distress is recognised as one of the symptoms of pesticide toxicity Butchiram et al. (2013) the observed decrease in the rate of oxygen consumption should be due to the respiratory distress as a consequence of the impairement of oxidative metabolism or it may be due to reduction in the uptake by gills probably due to the tissue damage in the said organ Bajpai et al. (2012). The reduction in the oxygen consumption of the fish exposed to sublethal concentration of the toxicant is probably due to prolonged exposure leads tissue damage of gill by degenerative changes Anitha susan et al. (2012). The results of the present study i.e. oxygen consumption of *Catla catla* at different fluoride concentrations were represented graphically as Fig. 5 and 6. There is decrease in the oxygen consumption as a result of toxicant stress. Fresh water edible fish *Rita rita* when exposed to LC10 (30 mg/l), LC10 conc. of Sodium fluoride resulted in decreased RBC and Hb consequent decrease in oxygen consumption Somnath et al. (2016). Similar observations in decline of oxygen uptake due to gill damage was reported Srinivasa Rao et al. (2018). The oxygen uptake of fish is said to be intimately connected with the damage of gill, changes in the architecture of the gill Butchiram et al. (2013) would lower the oxygen diffusing capacity of gill with consequent hypoxic / anoxic conditions and thus respiration may be come problematic task for the fish. Laboured breathing was observed in the fish with concomitant gill damage respiring through mouth as an indication of respiratory distress and / or hypoxic condition in and around the fish Anitha susan et al. (2010).

The decrease in oxygen uptake may also be due to the interference of the toxicant with Hb, lowering its transport efficiency. The reduction in Hb may also be responsible for decreased transport as reported by Bajpai et al. (2012), Kumar et al. (2007) in *Clarias batrachus*. In the present study the decrease in uptake capacity of fluoride exposed *Catla catla* was supported by previous studies. The fish *Catla catla* when exposed to three different concentrations of NaF i.e. < LC 50 (29.683 mg/l), LC 50 (296.83 mg/l) and > LC 50 (301.220 mg/l) the oxygen consumption was reduced significantly in the high concentration after six hours of exposure. After 12 hours of exposure irrespective of toxicant concentration there is reduction in oxygen uptake of fish. Fluoride ion acts as enzymatic poison inhibiting the enzyme activity and ultimately interrupting the metabolic processes such as glycolysis and synthesis of proteins. (Usharani et al. 2014). The decrease in protein synthesis results in decreased Hb production (Yadav et al. 2014). Bajpai et al. (2012), Butchiram et al. (2007) reported histological alterations in gills, kidneys of fresh water fish *Heteropneustes fossils* (bloch).

When the fish was exposed to NaF, the toxicant which is biologically reactive will effect the different parts of body, the gills are the doors through which toxicant finds its way into target organism starts effecting its oxygen consumption leading to depleation of energy the root cause of various biological activities resulting in anomalous behaviour like surfacing phenomenon and suppression of growth.

Conclusion: Aquaculture is of paramount importance for healthy food production through culture of major carps. In the coastal districts of Andhra Pradesh aquaculture is the source of income for the farmers, simultaneously improves the state economy. *Catla catla* the preferred edible species to be grown in culture ponds must be protected from agricultural runoff, industrial effluents into fresh water resources to prevent fluoride pollutant into natural waters and culture medium.

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