ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.3.L12-16

International Journal of Life science and Pharma Research

Research Article

Toxicology for safe therapy

Respiratory Stress of Sub-Lethal Concentration of Chlorine on *Oreochromis niloticus*

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Abstract: In aquatic environment, one of the most significant manifestations of the toxic stress on aquatic organisms, especially fishes are the over stimulation or depression of respiratory activities. These variations in respiratory activities have been used as in indicator of environmental stress. This study was aimed to assess the sub-lethal toxicity of chlorine through respiratory stress on *Oreochromis niloticus*. It was noticed that in all dosage experiments as the concentration increased rate of gill movement decreased. The rate of gill movement during the first minute after the dosage in control was 128 per minute. Serial dilution method employed in this study helped to assess the LC_{50} of chlorine and the value noted was 2 ppm. The minimum number of gill movement noted was in 20ppm dosage and the value was 98 per minute whereas the maximum noted was in 5ppm dosage and the value was 101 per minute. In 60th minutes after dosage also the control showed a value of 126 per minute and 20 ppm dosage showed a very low value such as 32 per minute whereas 5ppm dosage of chlorine showed a reasonably good value of 61 per minute. It was noted that in the case of average gill movement, control showed a very high value such as 127 per minute, whereas 5ppm dosage of chlorine showed a reasonably good value such as 84 per minute, and 20 ppm dosage showed a low value 72 per minute. When a comparison was made between control and differently dosed fishes, it was observed that in all dosed cases the average gill movement was very low when compared with control. A significant difference exists between gill movement in the dosed group and control ($P<0.05$). Consumption of oxygen increased with an increase in dosage and decreased with increased period of exposure. In control the average oxygen consumption was 0.012 mg/ml/gm body weight, in 5ppm it was 0.014 mg/ml/gm body weight, in 10 ppm it was 0.0155 mg/ml/gm body weight, in 15 ppm it was 0.021 mg/ml/gm body weight and in 20ppm it was0.022 mg/ml/gm body weight.

Keywords: Chlorine, LC_{50}, Gill movement, *Oreochromis niloticus*

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Recieved On 07 August 2020
Revised On 07 November 2020
Accepted On 11 November 2020
Published On 04 May 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Asna Salam, A.U.Arun* and Shalu Soman , Respiratory Stress of Sub-Lethal Concentration of Chlorine on Oreochromis niloticus.(2021).Int. J. Life Sci. Pharma Res.11(3), L12-16 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.3.L12-16

1. INTRODUCTION

Life on earth originated in water and is unsustainable without clean water, so water is the most important and precious resource of nature, especially for aquatic organisms like fishes. It is a major physico-chemical abiotic factor or component on earth, influencing the life of organisms. Any alteration in fish physiology indicates the deterioration of water quality parameters since fishes are one of the biological indicators of water quality[1]. 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[2,3]. For aquatic organisms the quality of water becomes important. Pesticides in sublethal concentrations present in the aquatic environment are too low to cause rapid death directly but may affect the functioning of the organisms, disrupt normal behavior, and reduce food consumption[4]. Studies on oxygen consumption form a suitable tool in the assessment of stress due to toxicants on aquatic organisms and give an index of energy expenditure mechanisms for environmental variations [5-9]. Chlorine is one of the most commonly manufactured and extremely reactive chemical elements. In water, chlorine reacts to form hypochlorous acid and hypochlorites, when pH decreases chlorine becomes more effective and chlorine can be lethal. At high concentrations n, chlorine is extremely dangerous and poisonous for all living organisms. Fish bioassay experiments are indices to determine the acute toxicity and possible effect on oxygen consumption due to pollutant stress.[10] Chlorine is used in drinking water, swimming pools, freshwater sources to kill harmful bacteria.[11]The chlorination process used as part of the sanitation process for industrial waste sewage and it is valuable for the protection of public health but when it released into water people may be exposed by touching or drinking water that contains chlorine[12]. It is also harmful to the living organisms in the freshwater r habitat like ponds, lakes, rivers. Although many biological early warning systems monitor abnormal opercular movement as an indicator of respiratory stress in fishes, a more direct measurement of stress in this sense necessitates the quantification of oxygen consumed by the fish.[13] In aquatic body toxicants present above the normal level i.e., at lethal concentrations bring about the mortality of fish and also increase the rate of oxygen consumption in survived fish.[14] Toxicity of chlorine varies with fish species, duration of exposure, temperature, pH, dissolved oxygen, acclimatization to concentrations of chlorine, and other water quality conditions[15]. Exposure to chlorine showed that the gill tissues of affected fish were congested severely and excessive mucus secretion on the body surface. The toxin will attack fish's gill, preventing them from breathing[16]. The chlorine in water reacts with living tissues and organic matter causing acute necrosis in fish and gill necrosis can lead to respiratory difficulty and asphyxiation. Chlorine poisoned fish appear much stressed and quickly get sick and die. Smaller fishes are more susceptible than large fish. In these circumstances, this study aimed to evaluate the respiratory stress of chlorine on *O.niloticus*. Normally chlorine is used as a disinfectant in the aquatic environment. In Kerala, when any aquatic body is cleaned, normal use of chlorine takes place, these chlorine leaches out into other aquatic bodies and finally affects aquatic organisms especially fishes. In order to understand the impact of chlorine on aquatic organisms, this work was undertaken.

2. MATERIALS AND METHODS

Experimental fishes were collected from a fish farm located at Keezhillam, Ernakulam (Dist), Kerala. Moderate size fishes were selected for the experiment having weight 11+/-4 gm. The collected fishes were acclimatized in the laboratory. By mixing chlorine with the normal water, four different concentrations (5ppm, 10ppm, 15ppm, 20ppm) of chlorine water was prepared. Transferred the fishes into four glass chambers with the said concentrations of chlorine water and it's breathing rate was measured intermittently for one minute each for the 1st minute,5th minute,15th minute, 30th minute and 60th minute (1 hour). Fishes were introduced into a glass chamber containing 2000ml of water, with 0 ppm concentration of chlorine was taken as control. Dosed fishes were fed regularly and aeration was provided throughout the experiment. Modified Winkler Method (Micro Winkler method) [17] was used for the estimation of Oxygen consumption. To determine the dissolved oxygen Winkler A and B were used for fixation and Sodium thiosulphate was used for titration with starch solution as indicator. Four healthy fishes were introduced into a 2000 ml measured volume of freshwater. An even layer of liquid paraffin was poured over the water to prevent further dissolution of atmospheric oxygen to it. At fixed time intervals, samples were taken for the estimation of oxygen. The difference between first and second (one sample – preceding or following sample) give the oxygen consumption of that organism during that time interval. Each experiment was repeated for six times. The experiment was carried out under standard environmental conditions (Temperature - 30 +/- 2, Ph-7 +/- 0.2).

3. STATISTICAL ANALYSIS

The experimental data obtained were analyzed using Excel and SPSS. Student's (paired) "t" test was used for comparison. The data were presented in graphical mode with standard deviation (SD). Probability value (P) of less than 0.05 was considered statistically significant.

4. RESULTS AND DISCUSSION

In control the gill movement was steady throughout the period (128 in 1st minute to 126 in 60th minute), whereas in the dosed group there was a gradual decrease in rate of gill movement (Fig.1). In 5ppm it was varying from 101(1st minute) to 61(60th minute), in 10 ppm it was between 100(1st minute) to 77 (60th minute), in 15ppmit was between 99(1st Minute) to 52(60th Minute) and in 20ppm it varied from 98(1st minute) to 32(60th minute). A decrease in the respiratory rate in both the lethal and sub-lethal concentrations due to toxicant induced stress, avoidance and biotransformation.[18]If gills or membrane functions are destroyed due to xenobiotic chemicals or the membrane functions are disturbed by a change in permeability the oxygen uptake rate would rapidly decrease.[19,20]

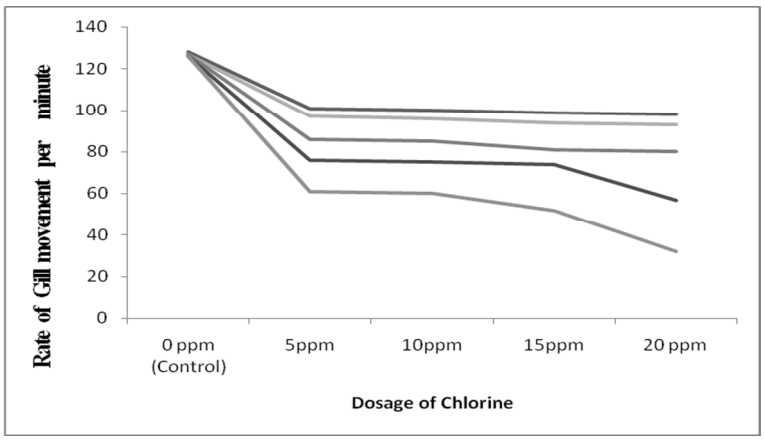


Fig 1. Rate of gill movement during different dosage

During experimentation the control fish demanded a constant uptake of oxygen, hence the gill movement became steady, while the exposed fish demanded more oxygen, hence in the dosed category the gill movement in the initial period was high and later it got decreased (Table 1). Similar observation was noted in *Cyprinus carpio*[21] and Oreochromis Sp[22]. The gill movement is high in the initial phase and gradually decreases and it is presumed that the toxicant directly or indirectly affects the respiration of fish[23]. The normal respiratory rate of gills may alter due to intimate contact with the water which would affect the diffusing capacity of the gill.

Table.1. Consumption of oxygen (mg/ml/gm body weight) by fishes during different time interval and dosage

Time	control	5ppm	10ppm	15ppm	20 ppm
1st	0.0125 +/- 0.001	0.021+/- 0.002	0.022 +/- 0.001	0.028 +/- 0.002	0.031+/- 0.002
5th	0.0119 +/- 0.001	0.016 +/- 0.001	0.018 +/- 0.001	0.025 +/- 0.001	0.03 +/- 0.001
15th	0.0118 +/- 0.002	0.015 +/- 0.002	0.019 +/- 0.002	0.024 +/- 0.002	0.026 +/- 0.002
30th	0.0118 +/- 0.001	0.0093 +/- 0.001	0.0094 +/- 0.001	0.017 +/- 0.001	0.017 +/- 0.001
60th	0.0117 +/- 0.001	0.0092 +/- 0.001	0.0093 +/- 0.001	0.015 +/- 0.001	0.01+/- 0.001

Values are mean +/- SD ;(n=6), P< 0.05 when compared with control

In the case of average gill movement, control showed a very high value such as 127, whereas 5ppm showed a reasonably good value 84 and 20 ppm showed a low value such as 72 and other concentrations showed a value in between them. The ventilation rate of bluegill (*Lepomismacrochirus*) exposed to chlorine decrease gradually and reaches the minimum in higher concentration of chlorine.[24]

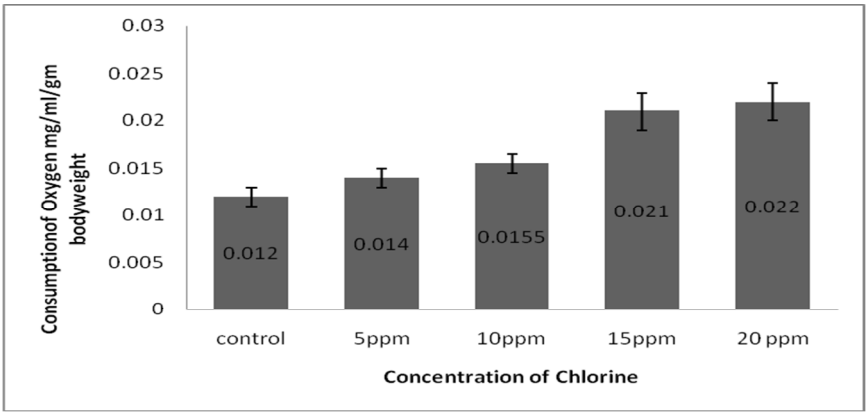
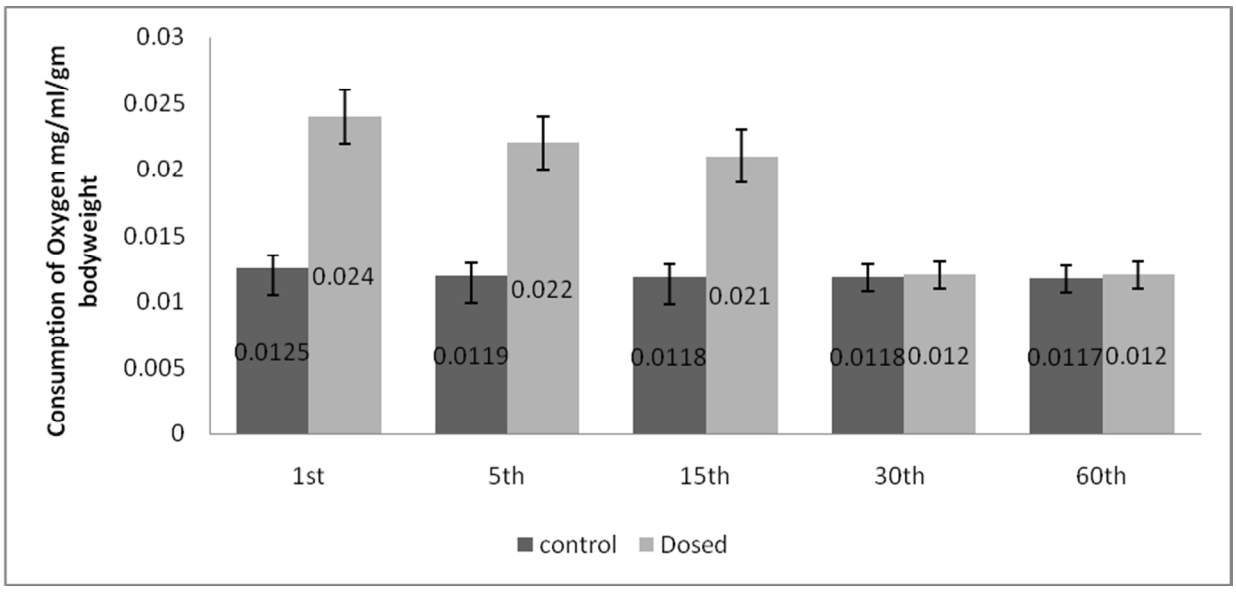


Fig 2. Consumption of oxygen (mg/ml/gm body weight) by fishes dosed to different concentrations.

The average oxygen consumption increases gradually from control to 20 ppm dosage (Fig 2). In control the average oxygen consumption noted was 0.012 mg/ml/gm body weight, in 5ppm it was 0.014 mg/ml/gm body weight, in 10ppm it was 0.0155 mg/ml/gm body weight, in 15 ppm it was 0.021 mg/ml/gm body weight and in 20ppm it was 0.022 mg/ml/gm body weight. It was observed that the rate of oxygen consumption was higher in dosed fishes than the control. The metabolic rate in relation to respiration of fish could be increased under chemical stress.[25]The increase in activity might be to boost up oxidative metabolism for an increased supply of energy to combat the toxic stress. This probably accounts for an elevation in oxygen consumption than control.[26] 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.[27] The oxygen consumption of both sexes in the control groups showed a uniform increase throughout the experimental period and in pesticides-treated animals also they showed an increase in oxygen consumption.[28]



(Average value of oxygen consumption in all dosed groups and in control in a specific time period) during different time intervals (dosed and controlled).

Fig 3. Average Consumption of oxygen (mg/ml/gm body weight) by fishes

It was noted that in the initial period of exposure to normal water the consumption of oxygen by the dosed fish was very high when compared to control, whereas in later it was somewhat equal to control (Fig .3). The observed increase in oxygen consumption by the whole animal may be due to respiratory distress as a consequence of the impairment of oxidative metabolism.[29-32] The rate of oxygen consumption increased in the lower concentration of pesticide.[33] 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.

5. CONCLUSION

The aim of the present study was to throw more light on the effect of sub-lethal chlorine toxicity on respiratory stress of *Oreochromisniloticus*. Chlorine and compounds that release active chlorine into water are used as disinfectants in both public health and veterinary medicine. In all dosage experiments as the concentration increased the rate of gill movement decreased. Consumption of oxygen increased with increase in dosage and decreased with increased period of exposure. This study can be used as baseline to assess the respiratory stress due to sublethal concentration of Chlorine on *Oreochromis niloticus* and this can be used to initiate further research in this aspect.

6. AUTHORS CONTRIBUTION STATEMENT

Dr. A. U. Arun designed the topic and experiment, research students Smt. Asna Salam and research scholar Smt. Shalu Soman carried out the laboratory work. These two research students drafted the paper and Dr. A. U. Arun corrected it after incorporating and using different statistical tools.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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