



Toxic Effects of an Anionic Detergent -Linear Sodium Alkyl Benzene Sulfonate on Oxygen Consumption and Gill Structure of *Anabas Testudineus*

Jilna Alex N¹, Afifa Fathima M. A.², Sona Simon E³

¹Associate Professor, Department of Zoology, Little Flower College (Autonomous) Guruvayur, India.

^{2,3}UG student - Department of Zoology, Little Flower College (Autonomous) Guruvayur, India.

Email ID: jilna@littleflowercollege.edu.in¹, afifa10fathima05@gmail.com², sonaseleenasimon@gmail.com³

Abstract

Detergent toxicity studies are crucial for environmental risk assessment and hazard classification. Linear alkyl benzene sulfonate is a widely used anionic surfactant in domestic cleaning products such as laundry detergent and dish liquid. It is also widely used in textile and personal care industries. Its presence alters the quality of water primarily by reducing dissolved oxygen and adversely affects the existence of aquatic organisms. The present study analysed the effect of various concentrations of linear alkyl benzene sulfonate on oxygen consumption of *Anabas testudineus* and further changes induced on gill surface as a consequence to exposure. Continuous exposure to sub-lethal concentration of alkyl benzene sulfonate caused alterations in behavior responses of the fish and reduced the amount of dissolved oxygen in water. It also decreased the rate of oxygen consumption of the fish and caused damages to the gill surface. By conducting toxicological studies on detergents, researchers can provide critical insights into the potential risks and impacts of these widely used chemicals, ultimately informing strategies for safer use, disposal, and regulation.

Keyword: Linear alkyl benzene sulfonate, *Anabas testudineus*, Dissolved oxygen, Gill surface

1. Introduction

Over the past few decades detergents pose serious ecological risk and raise the mortality rate of aquatic plants and animals. Fish are the primary victims of detergent contamination. Detergent toxicity in aquatic organisms reduces the success rate of reproduction, congenital anomalies, histopathological damages to liver cells, gills, intestinal walls, and mucosa, changes in hematological parameters leading to increased mortality. Anionic surfactants change the surface tension of water and reduce the oxygen holding capacity of water. All fish need oxygen to survive, and low oxygen content in the water causes physical harm to the respiratory surfaces. When Dissolved oxygen levels drop some vulnerable species may become weaker, disappear, or move away. As a result, it is regarded as a crucial indicator that shows the animal's physiological and metabolic changes. Anionic detergents decrease oxygen levels in water by forming a film on the water surface and inhibit the absorption of oxygen from the atmosphere, essentially blocking the natural process of oxygen transfer from air to water; this disruption in gas

exchange can significantly impact aquatic life due to reduced dissolved oxygen levels. Due to their sensitivity to different xenobiotics, fishes are considered important bioindicator species and play key roles in analysing the water quality parameters.

1.1. Behavioral Responses

Behavioural responses may provide more sensitive early warnings than standard test methods. Over the last few decades concern has been growing about the deleterious effects of aquatic pollution on fresh water ecosystems. Recent developments in ecotoxicology have been shown that observations of behaviour, which represents a crucial step between biochemical and ecological responses to environmental contamination, can constitute a sensitive approach in sub lethal toxicity monitoring. [1]

1.2. Physiological Changes

All the activities of an organism are directly dependent on its physiological status. Measurements of respiration often can tell us how a fish is responding to environmental conditions and what its physiological state may be. Respirometry gives quantitative measures of how rapidly energy and



oxygen are used by an organism. Respiratory data are important in the construction of bioenergetic models that can be used to calculate capacities for growth and reproduction. It also gives indications of altered environmental conditions and describes much about a fish's recent and current activity, acclimation, and stress. [2]

2. Method

The present study was conducted to understand the effect of various concentrations of linear sodium alkyl benzene sulfonate on behavioral responses, oxygen consumption and gill structure of *Anabas testudineus*. For the present study two sublethal concentrations 2g/13Litre and 1g/13Litre of tap water (ie, 0.154 and 0.0761g/L respectively or 154.1759ppm and 79.0906ppm respectively) were selected. [1g/L = 1001.142303 part/million (ppm)]. Fish samples for the study (*Anabas testudineus*) were collected from local ponds of Guruvayur municipality and the weight of the fish were recorded ($9\pm 2g$). *Anabas* was selected for this study due to its sturdy nature with which it can withstand the stress much better than other fishes. [3] [11-15]

2.1. Measurement of Fish Activity

2.1.1. Frequency of Water Gulping

All the activities of an organism are directly dependent on its physiological status. In the present study the frequency of water intake was determined by counting the mouth movements of the fish. The fish was placed in a glass tumbler of 1000ml for 10minutes. Then the frequency of mouth opening was counted for further 1 minute. Data was collected from both experimental and control group. [4]

2.1.2. Opercular Movement

Movement of gills in fishes can be considered as a measure of respiratory activity. Fish inhabiting in pure water will get plenty of oxygen and hence will be less stressed. Here the movement of gills will be relaxed. But as the water become hypoxic the fish will become stressed and show signs of asphyxiation. A major indication of asphyxiation in fish is the random movements of the gills. In the present study the difference in movement of gills was analysed by observing the fish treated with detergent added hypoxic water and control fish. Six fish were tested in each water sample for 10 minutes. Each fish was

individually introduced in to the experimental jar. After ten minutes of acclimation data were recorded for 10 minutes. [5]

2.2. Measurement of Rate of O₂ Consumption by Fish

The ability of the fish to utilize dissolved oxygen was measured by introducing both the test fish and control fish to a respirometer containing fresh water. The data were taken separately. The metabolic rate in fish is usually estimated by oxygen consumption measurements. The apparatus used was a modification of Fry's respirometer (Kutty et al., 1971). The construction of the apparatus has been done following the principles described in the literature. Data were expressed as individual oxygen consumption (mg O₂/h/g). The iodometric or Winkler method of measuring O₂ concentrations (mg/O₂/L) water is highly accurate when fresh reagents are available to fix and titrate water samples.

2.3. Gill structure and Morphometry

Prior to structural analysis of gills, fish were killed by severing the spinal cord behind the head. Gills were collected from the second gill arch. Tissues for light microscopy were fixed in Bouin's fluid for 24 h, transferred to 70 % alcohol. Later width of the filaments was taken by Micrometry. [6]

3. Results and Discussion

3.1. Results

Variations in the water gulping tendency were observed between control fish and the treated fish. The frequency of water gulping through mouth was high in control fish around 70 times per minute. The rate was low in experiment group II (1g/13L) and further reduced in experimental group I (2g/13L). No consistent decrease in frequency was observed as the days of experiment (7, 14 and 21) progressed. Differing from the water gulping through mouth, frequency of opercular movement of experiment groups of fish was found to be increasing. In the control group frequency of opercular movement was similar to the water gulping frequency through mouth. Increased rate of opercular movement was observed in both experiment group. However, a consistent increase in the rate of opercular movement was not observed as the days of treatment progressed. The rate of oxygen consumption by the fish in the

experimental group was observed as low compared to the control group. In the control group a consistent rate of oxygen consumption was observed throughout the experimental period. No marked change in oxygen consumption was observed as the days of experiment advanced. In experiment 1 (2g/13L), rate of oxygen consumption decreased remarkably compared with the control group. Further a regular decrease was also observed as the days of treatment advanced. Such a drastic change was not observed in experiment II (1g/13L) when compared to the control. However there was a remarkable change in oxygen consumption as the days of treatment advanced. Changes in the structure of gill filaments were very significant in both the treatment groups. Gills showed severe spongiosis and necrosis, and there was much lamellar fusion. Spongiosis and necrosis were more widespread and severe in gills of fish exposed to 2g/13L. Related lesions were also more severe. These included cell membrane vesiculation, exfoliation and hydropic degeneration

of lamellar and interlamellar epithelium, and delamination of lamellar epithelium. Cells beneath sloughed epithelium were characterized by their reduced number of microridges. Reduced width of gill filaments evident from the measurements taken by micrometry [Control fish: Average width 452μ, Experiment 1 (2g/13L): Average width 292.6 μ, Experiment II (1g/13L): Average width 399 μ] [7]

3.2. Discussion

In aquaculture, dissolved oxygen (DO) is the most important chemical parameter in determining water quality. Low DO levels are more responsible for fish mortality than other variables including salinity, alkalinity, and Table 2 shows Frequency of Movement – 1, Table 3 shows Frequency of Movement – 2, Table 4 shows Frequency of Movement – 3 [8]

4. Measurement of Fish Activity

4.1. Measurement of Rate of O₂ Consumption by Fish

Table 1 Control and Experimental Water

a) Control water (mean value of 6 fish)

Days of analysis (n=6)	Initial amount of oxygen		Final amount of oxygen		Amount of oxygen consumed by the fish during experimental period (mg)	Rate of oxygen consumption mg/h/g (wet weight)
	in the sample mg/L	in the experimental volume mg/ml	in the sample mg/L	in the experimental volume mg/ml		
7th	9.9	69.3	1.7	11.9	57.4	12.34
14th	7.4	51.8	1.5	10.5	41.3	9.60
21st	8.4	58.8	1.5	10.5	48.3	11.23

b) Experimental water I (2g/13L) (mean value of 6 fish)

Days of analysis (n=6)	Initial amount of oxygen		Final amount of oxygen		Amount of oxygen consumed by the fish during experimental period (mg)	Rate of oxygen consumption mg/h/g (wet weight)
	in the sample mg/L	in the experimental volume mg/ml	in the sample mg/L	in the experimental volume mg/ml		
7th	7.7	53.9	5.8	40.6	13.3	2.86
14th	8.2	57.4	6.2	43.4	14	3.25
21st	8.4	58.8	7.1	49.7	9.1	1.78

c) Experimental water II (1g/13L) (mean value of 6 fish)

Days of analysis (n=6)	Initial amount of oxygen		Final amount of oxygen		Amount of oxygen consumed by the fish during experimental period (mg)	Rate of oxygen consumption mg/h/g (wet weight)
	in the sample mg/L	in the experimental volume mg/ml	in the sample mg/L	in the experimental volume mg/ml		
7th	8.5	59.5	4.2	29.4	30.1	6.47
14th	8.2	57.4	4.8	33.6	23.8	5.53
21st	8.5	59.5	5.4	37.8	21.7	4.25

4.2. Frequency of Water Through Mouth

Table 2 Frequency of Movement - 1

7th Day of treatment	Frequency of movement (in 1 minute)		
	Control	Exp I (2g/13L)	Exp II (1g/13L)
1	74	61	63
2	76	58	65
3	74	58	66
4	71	55	67
5	69	50	70
6	72	56	61
Mean	72.66±2.5	56.33±3.72	65.33±3.14

4.3. Frequency of Opercular Movement

Table 5 Frequency of Opercular Movement

7th Day of treatment	Frequency of movement (in 1 minute)		
	Control	Exp I (2g/13L)	Exp II (1g/13L)
1	75	60	84
2	73	80	78
3	62	67	74
4	72	82	75
5	65	78	80
6	70	79	70
Mean	69.5±5.01	74.33±8.78	76.83±4.92

Table 3 Frequency of Movement - 2

14th Day of treatment	Frequency of movement (in 1 minute)		
	Control	Exp I (2g/13L)	Exp II (1g/13L)
1	71	57	69
2	69	57	61
3	72	59	62
4	69	54	60
5	70	60	62
6	71	65	60
Mean	70.33±1.21	58.67±3.72	62.33±3.39

Table 6 Frequency of Opercular Movement - 1

21st Day of treatment	Frequency of movement (in 1 minute)		
	Control	Exp I (2g/13L)	Exp II (1g/13L)
1	66	62	68
2	71	80	68
3	72	77	80
4	70	82	77
5	75	77	65
6	68	76	70
Mean	70.33±3.14	75.67±7.06	71.33±5.85

Table 4 Frequency of Movement - 3

21st Day of treatment	Frequency of movement (in 1 minute)		
	Control	Exp I (2g/13L)	Exp II (1g/13L)
1	68	56	62
2	70	50	58
3	70	50	60
4	75	54	59
5	70	61	55
6	71	55	57
Mean	70.67±2.34	54.33±4.13	58.5±2.43

Table 7 Frequency of Opercular Movement - 2

21st Day of treatment	Frequency of movement (in 1 minute)		
	Control	Exp I (2g/13L)	Exp II (1g/13L)
1	66	62	68
2	71	80	68
3	72	77	80
4	70	82	77
5	75	77	65
6	68	76	70
Mean	70.33±3.14	75.67±7.06	71.33±5.85

4.4. Gill Structure and Morphometry

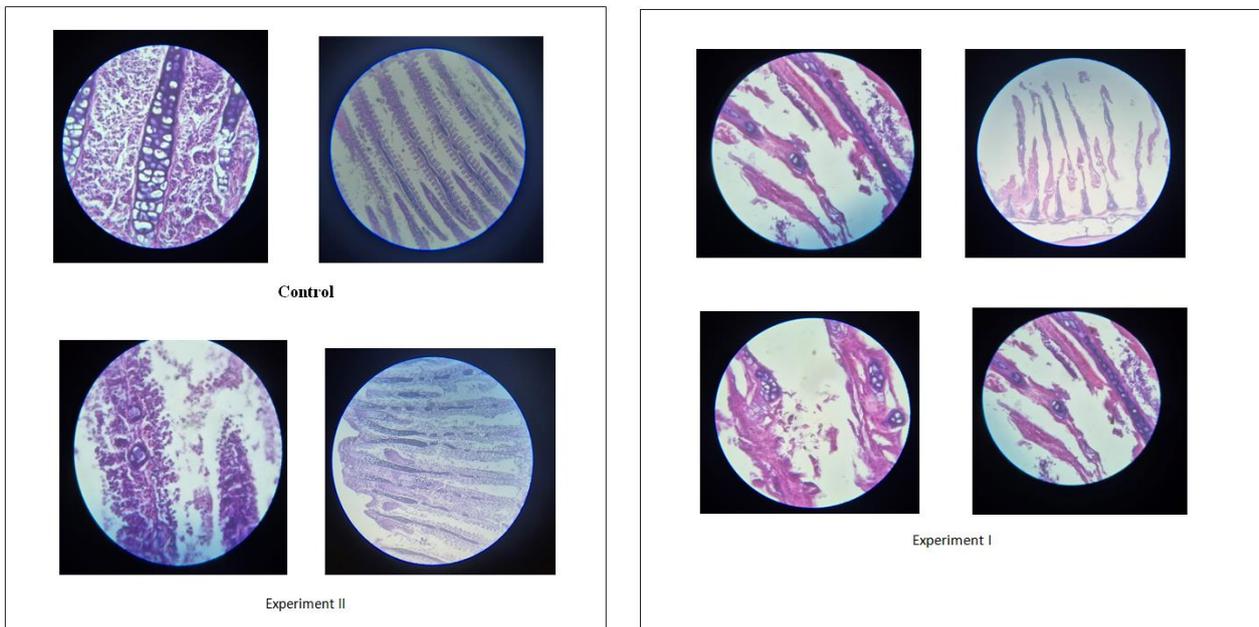


Figure 1 Experiments Vision

temperature. Dissolved oxygen is usually measured in milligrams per liter (mg/L) or as a percentage (%) of oxygen saturation (Matsumoto, 2006). In fish, the gills absorb dissolved oxygen from the water as it passes over them. Anthropogenic activities, in particular, can have a significant impact on the processes involved in this vital oxygen transport. Man-made synthetic chemicals in detergents are thought to have certain constituents that may disrupt the endocrine physiology of different organisms. Heavy metals can find their way into aquatic habitats through industrial discharges, mining activities, and atmospheric deposition (Adnan et al., 2022, Khoshnood et al., 2012). These pollutants are persistent and can accumulate in aquatic organisms, posing significant impacts to both aquatic ecosystems and human health (Al-Rudainy et al., 2014, Al-Rudainy and Al-Samawi, 2017, Badawi and Badawi and Magdy, 2023, Mustafa, 2020). The present study reveals that the general behavioral responses of fish reduced with respect to the dissolved oxygen content in the water. The decrease in water gulping through mouth and increased gill movement indicates respiratory distress. In hypoxic

water, fish close and open their mouth and gill covers at a faster rate than normal. For example, three-spine sticklebacks *Gasterosteus aculeatus* move their gill covers at a rate of 165 openings per minute when the water has only half of its normal oxygen content. This is a substantial increase over the normal rate of 95 openings per minute (Dembele K, 2005). After encountering an area of low oxygen content, fish often start to move rapidly and erratically. They probably do this to escape the danger zone. Table 5 shows Frequency of Movement – 1, Table 6 shows Frequency of Movement – 2, Table 7 shows Frequency of Movement – 3, Figure 1 shows Experiments Vision. However, if hypoxic conditions persist, most fish greatly reduce their general activity. Three-spine sticklebacks in natural ponds become rather sluggish during hypoxia, even failing to show normal flight responses when there is a risk of predation (Sen, 2007). Guppies in the laboratory usually swim less, eat less, and court less when oxygen levels are lower than normal (Rahimi, 2020). However, in all the experiments, the fishes appeared inactive later and rested at the bottom of the experimental chamber. Behavioral abnormalities in



various fish species on exposure to pollutants have been reported by several researchers. Simon (2017) reported that fish fed pollutant contaminated phytoplankton and zooplankton comes up with damaged peripheral organs and reduction in swimming activities. Rejeki, (2008) observed frequent surfacing with irregular opercular movement and loss of equilibrium in *Tilapia mossambicus* when exposed to different concentrations of cadmium. These behavioural features provide useful measures of sub lethal toxicity because they represent the integrated results of any biochemical and physiological processes (Simon, 2017). Alkyl benzene sulphonate is a widely used component of synthetic detergents. When reached in aquatic ecosystem, it penetrates the cell membrane and cause non-specific polar narcosis (Schwuger and Bartnik, 1980). The present study shows that the presence of surfactants severely damaged the gill tissues, resulting in noticeable behavioural changes. Further it changed the alkalinity of water facilitating growth of micro organisms and reduced the dissolved oxygen content of water. The normal process of oxygen moving from the atmosphere into the water can be disrupted by alkyl benzene sulphonate molecules. By forming a barrier on the water's surface, surfactants can prevent oxygen from the air from diffusing into the water. Histological examination showed that the lamellae of the fish were severely damaged. When the fish were kept in hypoxic water, a large reduction in this cell mass occurred, making the lamellae protrude and increasing the respiratory surface area. As the number of days of exposure increased severe necrosis occurred and width of lamellae decreased. Further, when ingested, its metabolism and biotransformation occurs via monooxygenases in liver, leading to Reactive Oxygen Species, ROS, production and consequently express oxidative stress by disturbing cellular antioxidant enzymatic equilibrium (Sunil, 2017). This further brings about histopathological impairments in exposed fish. [9]

Conclusion

The extensive usage of detergents and their possible effects on the environment and human health make toxicological research on these compounds extremely

pertinent. Wastewater can carry detergents into streams, impacting aquatic life and ecosystems. The food chain may be impacted by detergents' toxicity, bioaccumulation, and biomagnification in aquatic creatures. Studies in these areas provide vital insights into the possible hazards and effects of the commonly used chemicals and ultimately help to shape policies for safer usage, disposal, and regulation. [10]

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