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Assessment of biochemical effects of acute exposure of Basic blue 3 dye in fresh water bivalve *Lamellidens marginalis*

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Abstract: **In present investigation, the acute toxicity of Basic blue 3 dye for fresh water bivalve *Lamellidens marginalis* (7-8 cm) for 96 hours. The static bioassay was conducted at 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 ppm on fresh water bivalve *Lamellidens marginalis.* The mortality rate was recorded and the LC_0 and LC_{50} values for 96 hours were found at 40 ppm and 70 ppm respectively. The physico chemical parameters of test water like temperature, pH, total hardness, Dissolved O_2, free CO_2, inorganic phosphate and nitrate was measured. By examining the biochemical profile against control in different tissues like gill, mantle, hepatopancrease and gonad, the effects of LC_0 and LC_{50} concentration were evaluated. There was highly significant depletion ($p < 0.001$) in glycogen content at both the concentration (LC_0 and LC_{50}) in all tissues. Maximum protein content depletion ($p < 0.001$) was in Gill at LC_0 concentration compared to other tissues. While at LC_{50} concentration, decrease in protein content was significant in hepatopancrease, gill, mantle and gonad. The gill and hepatopancrease showed maximum decrease ($p < 0.001$) in lipid content at both the concentration (LC_0 and LC_{50}).**

Keywords: **Basic blue 3 dye, Biochemical content, *Lamellidens marginalis*, LC_0, LC_{50}.**

1. INTRODUCTION

The dyes are used to modify the colour characteristic of different substrates, such as fabric, paper, leather or other material [2]. Natural dyes were completely replaced by synthetic dyes in the beginning of twentieth century.

Today, almost all dyes which are commercially available are synthetic substances, with exception of some inorganic pigments. Every year hundreds of new coloured compounds are released in aquatic system. During the textile process, inefficiency in the coloring generates large amount of dye residues, which are directly released into the water bodies. The residues of dyes that passed by treatment in the industries may be directly discharged in water bodies, causing severe contamination of water bodies [3, 4] . In dye removing process, the use of a single process may not be completely decolourise the waste water or degrade the dye molecule [5] . Therefore several methods are used in the treatment of decolourisation. Some physicochemical methods such as filtration, coagulation, flocculation and precipitation are used to decolorize. From these methods, some are quite effective but quite expensive [6, 7] . The high concentration of textile dyes in water bodies stop the reoxygenation in water and ceases the sun light penetration in water [8] .

Due to release of dye effluents, the physico chemical parameters of that water system will be changed. The altered parameter will affect the aquatic biota of that system. The basic dyes are easily soluble in water produces colouring cationic compounds in solution and constituted by compounds azo, anthraquinone, methane, oxazine, acridine and quinoline with application in modified acrylic, nylon, polyesters and papers and some of them having biological activity are used in medicine as antiseptics [9] . Like other basic dyes the basic blue 3 dye is readily soluble in water gives dark blue coloration. The basic blue 3 dye is used for cotton, wool and nitriles, stick and acrylic blended fabric graft copolymerization dyeing, also can be used for direct printing acrylic carpet. The toxicity of this dye was reported by occupational safety and health administration USA in 2012. While acute toxicity of this dye was reported in fat head minnows[10].

Many species of molluscs are the key species to determine the impact of pollutant on marine, fresh water and terrestrial ecosystems which will affect the mollusc population, results in negative impact on entire ecosystem [11]. In present investigation the acute toxicity of this dye was determined for *Lamellidens marginalis* with respect to their biochemical alterations after acute exposure.

2. MATERIAL AND METHODS

The fresh water bivalve *Lamellidens marginalis*, of about 7 to 8 cm in size, were collected from Rajaram tank, Kolhapur (16˚40'43.4"N 74˚15'53.0"E). They were brought to the laboratory and cleaned to remove fouling algal biomass, mud and acclimatized in glass aquaria containing dechlorinated tap water for 7 days. The physico chemical parameters of this water was measured (Table No. 1) by standard methods APHA [12].

Test Media:

Basic blue 3 dye was used for preparation of stock solution. The stock solution was prepared by dissolving 10 mg dye in 10 ml distilled water. The stock solution was diluted up to desired concentration.

Exposure System:

The primary tests were carried out to determine the LC_0 and LC_{50} values of the bivalves to basic blue 3 for 96 hours by static bioassay method. For determining these values 10 bivalves were kept in each container containing 10 liter tap water at different concentrations of basic blue 3 from 40 ppm to 85 ppm. After every 24 hours, water was changed from containers by maintaining it's concentration. The control group was run simultaneously. The percent mortality was recorded with concentrations after each change. The LC_0 was observed at 40 ppm while the LC_{50} mortality was observed at 70 ppm.

Biochemical Estimations:

After 96 hour of exposure period the animals from three groups i.e control, LC_0 and LC_{50}, were sacrificed the tissues like gill, mantle, hepatopancrease and gonad were taken out for biochemical stimations. The glycogen estimation was done by using anthron reagent [13] . The total protein content was done by follin phenol reagent [14]. The lipid content was estimated by vanillin reagent [15].

Statistical analysis:

The data obtained from rate of mortality was statistically analyzed by using probit analysis. The data on biochemical profile was analyzed for statistical significance between the control and experimental bivalves by Student't' test. Significant difference was established at 0.05, 0.01 and 0.001 level.

3. RESULTS

The physicochemical parameters of test water were measured by standard method by APHA [16] . The physicochemical parameters of test water were shown in Table **1**. All physicochemical parameters were relatively remain constant throughout the experiment.

Table No. 1: Physico chemical properties of experimental water

Sr No.	Parameters	Mean values ± SD
1	pH	7.77 ± 0.057
2	Temperature (^{0}C)	29.4 ± 1.14
3	DO (mg/l)	3.79 ± 0.208
4	Free CO_2 (mg/l)	23.23 ± 1.12
5	Hardness (mg/l)	184. 67 ± 0.578
6	Phosphate (mg/l)	4.38 ± 0.125
7	Nitrate (mg/l)	15.17 ± 0.76

Ten individuals of *Lamellidens marginalis* were tested for Basic blue 3 dye concentration for 96 hours with the above physico chemical parameters. The bivalves were more susceptible with increased concentration of dye. The percentage mortality was increased with increase in concentration of basic blue 3 dye and exposure period. In (Table 2**),** the graph represents the relation between Probit (Y) and Log of concentration (X). The observed LC_0 and LC_{50} values

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were 40 ppm and 70 ppm respectively. According to probit analysis by Finney[17], The median lethal concentration (LC_{50}) of basic blue 3 dye for *Lamellidens marginalis* at 96 hours of exposure was calculated. The calculated LC_{50} value was 71 ppm and regression line equation was Y = 12.27x - 17.69. The results of this study were useful for determination of LC_{50} value for basic blue dyes for bivalves which will be helpful as early indicator in dye toxicity to fresh water bivalves.

Table No.2: LC_{50} value for Lamellidens marginalis exposed to different concentrations of Basic Blue 3 dye for 96 hours

Sr. no	Concentration of Basic blue 3 (mg/l)	Log of concentration	No. of bivalves exposed	No. of bivalves died at 96 hours	Probit kill	Percent kill (%)
1	40	1.6020	10	00	0.00	00
2	45	1.6532	10	01	3.72	10
3	50	1.6989	10	01	3.72	10
4	55	1.7404	10	02	4.15	20
5	60	1.7781	10	03	4.48	30
6	65	1.8129	10	04	4.74	40
7	70	1.8451	10	05	5.00	50
8	75	1.8751	10	05	5.00	50
9	80	1.9030	10	06	5.25	60
10	85	1.9294	10	06	5.25	60

Biochemical studies:

Carbohydrates are the important energy source which was decreased in stress condition. Under exposure of basic blue 3 dye, there was significant depletion at $p < 0.001$ in glycogen content in gill, hepatopancrease, gonad and mantle of for 96 hours at both the concentrations (LC_0 and LC_{50})Table 3 . At LC_0 concentration in hepatopancrease there was maximum percentage decrease (-9.31 %) as compared to control. Gill showed highly significant depletion at LC_{50} concentration (-20 %) as compared to control and compared to other tissues. While gonad showed minimum percentage depletion (-7.57 %) at LC_0 concentration and mantle showed minimum depletion (-11.63%) at LC_{50} concentrations against the control. The protein content was affected by many factors, which may be increase or decrease response to stress. After exposure of basic blue 3, there was significant depletion observed in all tissues at both the concentrations, LC_0 (40 ppm) and LC_{50} (70 ppm). The results obtained are presented in Table 4. At LC_0 concentration, there was significant depletion in protein content was observed in gill (- 17.91%) against the control (at $p < 0.001$). While gonad and mantle also showed significant depletion at LC_0 concentration compared to control (-8.18 %), (-12.2%) respectively (at $p < 0.01$). At LC_{50} concentration, there was highly significant depletion observed in all tissues. In hepatopancrease, the maximum decrease in protein content (-26.46%) was maximum as compared to other tissues.

Table No. 3: Glycogen content in fresh water bivalve *Lamellidens marginalis* exposed to LC_0 and LC_{50} concentrations of Basic blue 3 Dye (values are in mg / 100 mg wet tissue)

Tissues	Control	LC_0 (40 ppm)	%change over control	LC_{50} (70 ppm)	%change over control
Gonad	3.837±0.016	3.5417±0.040***	-7.57	3.341±0.070***	-12.79
Hepatopancrease	4.271±0.022	3.895± 0.0507***	-9.31	3.535±0.0835***	-17.21
Gill	2.425±0.022	2.228± 0.0202***	-8.45	1.948±0.0125***	-20
Mantle	2.751±0.032	2.52 ± 0.015***	-8.36	2.43±0.03122***	-11.63

Table No. 4: Protein content in fresh water bivalve *Lamellidens marginalis* exposed to LC0 and LC50 concentrations of Basic blue 3 (values are in mg / 100 mg wet tissues

Tissues	Control	LC_0 (40 ppm)	%change over control	LC_{50}(70 ppm)	%change over control
Gonad	6.916±0.1191	6.352±0.158**	-8.18	5.542±0.117***	-19.89
Hepatopancrease	4.784±0.078	4.1687±0.039***	-13.04	3.518±0.083***	-26.46
Gill	3.35±0.091	2.75±0.030***	-17.91	2.565±0.079***	-23.58
Mantle	3.857±0.1432	3.38±0.026**	-12.2	2.956±0.105***	-23.37

Lipid is an important fuel in aquatic organisms. During stress it is metabolized to meet the energy needs. After acute exposure to basic blue 3, the lipid content showed depletion in all tissues(Table 5). The maximum depletion in lipid content was observed in gill at both the concentration LC_0 (-23.36%) and LC_{50} (-38.85%) compared to control. While in

gonad, the minimum depletion in lipid content was observed at both the concentrations, LC_0 (-4.36%) and LC_{50} (-14.38%) compared to other tissues. At both, the LC_0 (-15.22%) and LC_{50} (-23.85%) concentrations the hepatopancrease showed significant depletion.

Table No. 5: Lipid content in fresh water bivalve *Lamellidens marginalis* exposed to LC_0 and LC_{50} concentrations of Basic blue 3 dye (values are in mg / 100 mg wet tissue)

Tissues	Control	LC_0 (40 ppm)	%change over control	LC_{50} (70 ppm)	%change over control
Gonad	5.96±0.022	5.70±0.09*	-4.36	5.03±0.127**	-14.38
Hepatopancrease	5.39±0.028	4.629±0.124***	-15.22	4.15±0.095***	-23.85
Gill	3.72±0.023	2.823±0.239***	-23.36	2.26±0.059***	-38.85
Mantle	4.95±0.007	4.56±0.079**	-7.87	4.03±0.127***	-18.58

4. DISCUSSION

As dyes are being used in number of products, the excessive use can make over lives miserable at very low concentration, while some are resistance to light, heat, chemicals hence are difficult to biodegrade in the environment[18]. The direct release of dyes in aquatic system will alter the physicochemical parameters which directly or indirectly affect the biota of that system. Several workers have been determined the LC_{50} values for many commercial dyes at different time intervals by using static bioassay test for various fishes[19.] While LD_{50} values of different synthetic dyes for mice, rabbit, different human cell lines were also determined[20, 21]. The toxicity of dyes was affected by the physicochemical parameters like temperature, pH, hardness, dissolved O_2 and CO_2 of that aquatic system[22, 23, 24]. The toxicity of basic dyes was reported by some workers on fishes. Little[25] first reported the LC_{50} values for 46 commercial dyes on Fathead minnows. Among these dyes the basic, acidic and disperse dyes having harmful effects. From the basic dyes, the toxicity of basic green 4 (malachite green) were reported on some fishes like Silver barb, Snake head fish, Channel catfish, Rainbow trout, Carp, Atlantic salmon, Nile Tilapia etc [26] while Kirandeep Kaur (2016)[27] observed the detrimental effects in gills of *Labeo rohita* exposed to basic violet 1. The contents of basic blue 3 dye are Sodium Dithionite and 3-diethylaminophenol. Sodium Dithionite acts as strong reducing agent which is used in dye in and in bleaching the paper pulp. When it comes in contact with moisture it get oxidised into hydrogen sulfite, hydrogen sulphate and sulphites. The sulphites reduce the Thiamine content in food. The second content of this dye is 3-diethylaminophenol formed by amines and phenols. From these, phenols showed injurious effects in Tilapia[28]. The presence of phenols in water alters the pH which may changes the other physiological parameters [29] while the toxicity of phenols were influenced by change in temperature in Rain bow trout [30] . Due to presence of these compounds the basic blue 3 dye may showed mortality in bivalves. This dye is easily soluble in water. So that content from these may be readily absorbed by the aquatic animals during respiration or may be in filter feeding. After observing the decrease in biochemical content, this dye can alter the biochemical reactions.

Glycogen is the best suitable storage product in invertebrates as well as in vertebrates. Carbohydrates are the primary and immediate source of energy. In stress conditions, to meet energy demands the reserve level get depleted. This demand was caused by active compound induced hypoxia. The glycogen content in hepatopancrease was significantly depleted during acute hypoxia and physiological disturbances in bivalves[31]. The muscular exersion in gills showed decrease in glycogen content[32.] The glycogen depletion was observed in all tissues, which clearly indicates that excessive utilization of glycogen to cope up basic blue 3 dye induced toxicity and stress condition (Figure 2).

Proteins are the important constituent of animal tissue, which regulates cell metabolism. In bivalves, it is the most fundamental and abundant biochemical constituent and the estimations of protein is considered to be important[33]. From the results of total protein contents in all tissues, digestive gland was the most affected organ followed by gill, mantle and gonad (Figure 3). The higher depletion of protein in digestive gland might be due to high metabolic potency of the gland compared to other tissues 34. The digestive gland is the site of action of pollutants in the body of bivalves to degrade or to detoxify the toxicants, hence the largest demand of energy for metabolic processes resulting into increasing utilization of protein. After acute exposure the decrease in amount of protein in all tissues indicates that the dye contents inhibit the synthesis of protein, results in increase in number of free amino acids in the cell to cope up with the high energy demands under toxic stress[35, 36.] Svobodova (1997)[37] have observed the increase in hypoxia and impairs protein synthesis in certain fishes after therapeutic bath to basic green 4 dye. After acute exposure to basic green 4 dye, there was depletion in serum protein level in *Heteropneustis fossilis* [38.]

Like carbohydrates and proteins, lipids are also important constituent of the animal body. Decrease in lipid content of all tissues of bivalve clearly indicates that there was basic blue 3 induced toxicity stresses (Figure 4) . Gill showed maximum depletion in lipid content compared to other tissues. Decrease in total lipid content in animal tissue after exposure to various pollutants were reported by several investigators[39 40]. The lamellibranch mollusc exposed to different pollutants showed decrease in level of lipid content[41 42]. Changes in lipid content of all targeted organs was because of utilization of lipid component at cellular level by increase in lipolysis process or may be due to mitochondrial structural damage impairs the function of citric acid and fatty acid mechanism43 44 . During the present work it was observed that the dye basic blue 3 have induced toxicity in fresh water bivalve leads to alteration in their biochemical content.

5. CONCLUSION

In this investigation, the biochemical parameters were evaluated as indices of basic blue 3 dye induced stress and lethal concentration of this dye for fresh water bivalve was determined. Our findings suggest that the fresh water bivalves are more susceptible to this dye, which may affect the bivalve productivity. Release of this dye in aquatic system may causes harmful effects to aquatic organisms and it will create health problems in human being also.

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