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The effect of textile dyeing effluents on the total free sugar levels of the Haemolymph of the different larvae of *Bradinopyga geminata*

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Abstract
The rapid industrialization of India is vital for the progress of the Indian economy. At the same time it is pathetic to see that the environmental deterioration is also equally on the rise along with the speed of industrialization due to different pollutions. Among the various industrial effluents, the textile dyeing effluent (TDE) poses a significant threat to the aquatic environment, by altering the pH, BOD, COD, TDS, heavy metals, organic and inorganic to an alarming level which affects the normal life of aquatic organisms. The present study analyses the impact of six significant stations where the major confluents of TDE take place in the area of Pallipalayam, Namakkal District, on the total sugar level in the haemolymph of the different larval stages of dragonfly *Bradinopyga geminata.* The results indicate that the highest and lowest reduction of total estimated free sugars were evident at ST5 and ST2 respectively.

Keywords: Textile dyeing effluent, *Bradinopyga geminata,* haemolymph and total free sugars

1. Introduction
The textile dyeing industries may produce gorgeous fabrics by its vibrant colours to influence the soul. But, the resulted effluents are the devils that devour the spirit of the environment. Indian textile industry is one of the largest contributors to the Indian economy. The textile industrial sector of India is one of the largest among the world industries in terms of providing employment opportunities and production of textile materials [1]. The textile manufacturing and processing industries are the second largest industrial body in India, where 35 millions of people both skilled and unskilled get direct employment as a result of vast advancement in the textile technology.

In India, 81% of total textile dyeing industries are present in Tamil Nadu, Gujarat, Punjab and Maharashtra [2, 3]. There are more than 2000 textile units are effectively functioning in Tamil Nadu. Among them, there are 694 units in Erode District and 270 units in Namakkal District are functioning [4, 5].

The major share of the TDE arises, from the certain stages like desizing, scouring, bleaching, mercerizing, dyeing, printing etc. The TDE consists of a vast range of chemical salts, dye stuffs, chemicals, unused or escaped dye stuffs, pigments, resins, gums, starch etc. In addition to that, many toxic chemical compounds, formaldehyde based dye fixing agents, chlorinated stain removers, hydro carbon based softeners, non bio-degradable dyeing chemicals may also present in a typical TDE [6]. The TDE have heavy metals like, copper, arsenic, lead, cadmium, mercury, nickel, cobalt, manganese, zinc, lead, chromium and aluminum [7, 8].

When the TDE mixes with the aquatic ecosystems, they alter the physico-chemical properties of water. The water becomes, turbid with flocculation owing to the presence of the dissolved solids of the TDE. There is a wide pH fluctuation takes place from time to time depending upon the quality of the TDE that mingles with the water body. There is a high salinity, undesirable colouration, increased BOD and COD are common in the TDE [9, 10].

Studies revealed that the most of the TDE challenge the sustainability of the aquatic ecosystem [8]. The waste effluent produces hostile effects on the inhabiting organisms of water bodies. The growing need to satisfy the demand for the textile materials for ever expanding population leads to the establishment of more textile dyeing and processing units. The toxic nature of the dye and indiscriminate discharge of TDE, pollute the aquatic environment and its organisms are of great environmental concern.

The composition of all the above components of the TDE makes it toxic to the aquatic organisms.

TDE interferes with the biological systems in a number of ways, and resulting in series of physiological, biochemical, genotoxic problems and mortality in the aquatic organisms [11]. The synergic effect of TDE could be biomagnified by the aquatic organisms and it may be transferred to even human being through the food chain and resulting in serious health disorders like hypertension, sporadic fever, renal damage, muscular cramps and even cancer [12].

There were several works conducted in different organisms, such as fishes to assess the impact of the textile dyeing effluents. But the macro invertebrates are the less studied organisms, for the evaluation of the TDE. The *B. geminata* is a common dragonfly species found all over India [13]. It is one of the best indicator species. The presence of dragonflies indicates the good quality of the freshwater [14]. Hence, the present work is attempted to analyze the alterations in the total sugar levels in effluent treated larvae of *B. geminata.*

2. Materials and Methods

2.1. Collection of textile dyeing effluents

The major TDE confluences were identified as Aavarankadu (ST1), Pallipalayam (ST2), Aavathipalayam (ST3), Komarapalayam (ST4), Vediyarasanpalayam (ST5) and Kaliyanur (ST6) of Namakkal District, Tamil Nadu, India. The TDE samples were collected in clean and sterile plastic containers and brought to the laboratory for further investigation.

2.2. Collection and identification of different larval stages of *B. geminata*

The different larval stages such as antepenultimate, penultimate and final instars of the dragonfly *B. geminata* were collected from their natural breeding sites such as irrigation tanks from Thiruchengode, Namakkal District, Tamil Nadu, India, using wide rectangular shaped and fine meshed hand nets and brought to the laboratory using a suitable sterile polypropylene container for laboratory acclimatization. The bottom of the container was laid with a thin layer of sterilized fine gravels for a substratum. In the laboratory, they were kept in large cement tanks and separated by fine wire mesh to avoid cannibalism. The dragonfly larvae were fed with mosquito larvae *ad libitum.* The collected different larval stages of *B. geminata* were identified [15] and confirmed by Zoological Survey of India, Western Ghats, Kozhikode, Kerala, India.

2.3. Experimental design

Six individuals of selected healthy larvae from each instar such as antepenultimate, penultimate and final instar of *B. geminata* were introduced into the each different concentrations (0.4, 0.8, 1.2, 1.6, 2.0 and 2.4%) of the TDE of various stations (ST1 to ST6) in suitable petri dishes. Appropriate control (0%) was also maintained. The larvae were fed with mosquito larvae *ad libitum* from 7.30am to 8.30am regularly throughout the experimental period. The unfed mosquito larvae were discarded. After the 15 days of exposure the haemolymph were collected for estimation of total free sugars.

2.4. Estimation of total free sugars

The total free sugar content in haemolymph was determined by adopting the method of
Roe [16]. To 0.05ml of each homogenate, 10ml of 80% ethanol was added and centrifuged (3000 rpm) for 5 minutes. Each supernatant was made up to 5ml by the addition of distilled water. To 1ml of each supernatant, 10ml of Anthrone reagent was added, heated in a water bath for 15 minutes and cooled in a dark place for 30 minutes at room temperature. The optical density (OD) values were read at 620nm. The amount of total free sugars was calculated using the given formula,

$$= \frac{\text{OD of the sample}}{\text{OD of the standard}} \text{ x concentration of standard x } 10$$

where, 10 refers to dilution factor.

2.5. Statistical analyses

The data obtained from the present investigations were tabulated, statistically analyzed. Two-way analyses of variance (ANOVA) were calculated using SPSS (version No.10) to test the level of significance of difference between various effluents of different stations.

3. Results and Discussion

The total free sugar contents in the haemolymph of the control antepenultimate, penultimate and final larval instar were estimated as 86.29±6.21mg/100g, 115.65±8.82mg/100g and 137.52±10.56 mg/100g respectively (Table 1, 2 and 3). In all the stations, the level of total free sugars decreased with the increment in the concentrations of the TDE. The highest reduced level of total free sugars were noticed at 2.4% concentration of ST5 and the recorded values found in antepenultimate, penultimate and final instars were 54.09±6.12mg/100g, 71.40±6.85mg/100g and 94.65±8.77mg/100g respectively (Table 1, 2 and 3). At the same time the same concentration of ST2 was proved to be least effective in reduction of total free sugars and the estimated values were 61.22±5.20mg/100g, 76.11±7.15mg/100g and 102.75±9.45mg/100g in antepenultimate, penultimate and final instars larvae respectively (Table 1, 2 and 3). The values are significant at 5% (P<0.05) levels.

Table 1: Total free sugars (mg/100ml) in the haemolymph of control and effluent treated antepenultimate larvae (15 days of exposure) of *B. geminata* (each value is the mean ±SD of 5 observations)

S. No.	STATIONS	Different concentrations of textile effluent							P value (<0.05)
		Control (0%)	0.4%	0.8%	1.2%	1.6%	2.0%	2.4%	
1	ST 1	86.29±6.21	84.36±5.65 (-2.23)	78.86±4.65 (-9.18)	73.91±5.33 (-14.34)	68.05±5.12 (-21.14)	66.32±5.25 (-23.14)	56.42±6.14 (-34.61)	0.000728*
2	ST 2	86.29±6.21	80.36±4.25 (-6.87)	77.62±5.24 (-10.05)	70.39±4.20 (-18.43)	65.94±4.85 (-23.58)	63.32±4.62 (-26.62)	61.22±5.20 (-29.05)	
3	ST 3	86.29±6.21	83.68±5.20 (-3.02)	79.48±5.12 (-7.89)	74.95±4.40 (-13.14)	69.29±4.75 (-19.70)	66.48±4.75 (-22.96)	59.48±5.78 (-31.07)	
4	ST 4	86.29±6.21	85.94±4.23	80.24±5.17	76.02±4.25	73.94±4.62	69.29±4.12	60.84±4.12	

			(-1.41)	(-7.01)	(-11.90)	(-14.31)	(-19.70)	(-29.49)	
5	ST 5	86.29±6.21	83.84±5.12 (-2.83)	79.68±5.02 (-7.67)	72.58±5.20 (-15.89)	69.05±5.21 (-19.98)	65.18±6.21 (-24.46)	54.09±6.12 (-37.31)	
6	ST 6	86.29±6.21	81.64±5.02 (-5.39)	77.48±5.22 (-10.21)	73.65±5.01 (-14.65)	68.01±5.33 (-21.18)	65.00±5.28 (-24.67)	55.94±5.42 (-35.17)	

Two-way ANOVA of total free sugar in the haemolymph of antepenultimate larvae
* Significant ($P< 0.05$)
NS - Non-significant

Table 2: Total free sugars (mg/100ml) in the haemolymph of control and effluent treated penultimate larvae (15 days of exposure) of *B. geminata* (each value is the mean ±SD of 5 observations)

S. No.	STATIONS	Different concentrations of textile effluent							P value (<0.05)
		Control (0%)	0.4%	0.8%	1.2%	1.6%	2.0%	2.4%	
1	ST 1	115.65±8.82	112.68±7.78 (-2.57)	96.34±7.25 (-16.70)	84.02±8.02 (-27.35)	81.35±7.25 (-29.66)	78.19±6.98 (-32.39)	73.05±6.15 (-36.83)	0.108788*
2	ST 2	115.65±8.82	109.36±7.20 (-5.46)	99.05±7.21 (-14.35)	87.65±8.12 (-24.21)	82.36±7.78 (-28.79)	78.49±6.12 (-32.13)	76.11±7.15 (-35.05)	
3	ST 3	115.65±8.82	110.48±7.25 (-4.47)	94.32±7.02 (-18.44)	86.91±7.65 (-24.85)	81.69±7.12 (-29.36)	77.18±7.12 (-33.26)	74.69±7.11 (-35.42)	
4	ST 4	115.65±8.82	106.95±6.98 (-7.52)	95.48±7.62 (-17.44)	81.34±7.15 (-29.70)	78.45±7.18 (-32.19)	76.64±7.15 (-33.73)	75.42±8.02 (-34.79)	
5	ST 5	115.65±8.82	102.48±6.54 (-11.39)	94.35±7.12 (-18.42)	88.47±7.46 (-23.50)	82.48±7.16 (-28.68)	76.84±7.15 (-33.56)	71.40±6.85 (-38.26)	
6	ST 6	115.65±8.82	109.61±7.71 (-5.22)	98.38±6.18 (-14.93)	87.25±7.25 (-24.56)	83.61±7.02 (-27.70)	79.78±8.12 (-31.01)	72.08±7.45 (-37.67)	

Two-way ANOVA of total free sugars in the haemolymph of penultimate larvae
* Significant ($P< 0.05$)
NS - Non-significant

Table 3: Total free sugars (mg/100ml) in the haemolymph of control and effluent treated final instar larvae (15 days of exposure) of *B.geminata* (each value is the mean ±SD of 5 observations)

S. No.	STATIONS	Different concentrations of textile effluent							P value (<0.05)
		Control (0%)	0.4%	0.8%	1.2%	1.6%	2.0%	2.4%	
1	ST 1	137.52±10.56	133.35±9.66 (-3.03)	128.64±9.25 (-6.46)	121.28±8.12 (-11.81)	118.46±9.12 (-13.86)	106.34±8.12 (-22.67)	98.69±3.68 (-28.24)	0.710648^{NS}
2	ST 2	137.52±10.56	135.64±8.78 (-1.37)	127.66±9.55 (-7.17)	120.69±9.12 (-12.24)	113.52±10.20 (-17.45)	103.85±9.14 (-24.48)	102.75±9.45 (-25.28)	
3	ST 3	137.52±10.56	132.95±9.12 (-3.23)	125.98±8.45 (-8.39)	122.05±9.14 (-11.25)	117.64±9.48 (-14.46)	107.69±9.41 (-21.69)	97.42±8.75 (-29.16)	
4	ST 4	137.52±10.56	135.52±10.11 (-1.45)	129.57±9.12 (-5.78)	124.98±8.75 (-9.12)	115.48±9.75 (-16.03)	107.12±8.65 (-22.11)	99.65±9.12 (-27.54)	
5	ST 5	137.52±10.56	133.08±10.14 (-3.23)	128.68±9.10 (-6.43)	123.84±8.86 (-2.68)	118.49±9.68 (-13.84)	105.48±8.87 (-23.30)	94.65±8.77 (-31.39)	
6	ST 6	137.52±10.56	134.68±10.24 (-2.07)	128.63±9.14 (-6.49)	120.51±8.96 (-12.37)	113.84±10.23 (-17.22)	108.29±8.80 (-21.25)	96.84±7.86 (-29.58)	

Two-way ANOVA of total free sugars in the haemolymph of final instar larvae
* Significant ($P< 0.05$)
NS - Non-significant

The total free sugars of the haemolymph were also found to be in reduced in all the larval forms of the experimental larvae under the effluent pressure of each station. This can be due to the impact of the heavy metals found in the textile dyeing effluents. Similar significant reduction of glycogen was observed in the tissues of *Lymantria dispar* larvae under the exposure of the heavy metals zinc and cadmium. This might be due to the interference of the heavy metals with the pathways of glycolysis and Kreb's cycle [17]. Another investigation also supported the present findings that the reduction of sugar levels in the haemolymph of insects resulted due to the distorted glycogen metabolism [7]. From the above investigations, it can be ascertained that the glycogen metabolism might be affected severely under the pollution stress of textile effluents and the free sugars found in the haemolymph might be utilized to manage the energy requirement which resulted in the decline of the total sugar level in the haemolymph [18]. Researchers also came across the fact that the influence of cadmium which is a common pollutant found in the dyes, also play a significant role in the sugar reduction of the test organisms as they cause hindrance in the pathway of the glycolytic enzymes [19].

Many investigators claimed that the industrial effluents are the rich source of heavy metal residues, which interfere with the biochemical cycle of the living organisms and distort their normal life activities. Our findings also lay in the same line of the findings of other workers, which clearly indicates that heavy metals are the most significant factors in the reduction of free sugar levels in the haemolymph of insects.

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