The Physico-Chemical Characteristics of Different Textile Dyeing Effluents and Their Influence on the Total Protein Levels of Dragonfly Larvae *BradinopygaGeminata*

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ABSTRACT

The textile dyeing units of Pallipalayam area of Namakkal District play a vital role in polluting the river Cauvery directly and spoiling the underground water levels indirectly by letting off their dyeing effluents. This leads to serious environmental hazards of aquatic organisms. In the present study, the textile dyeing effluents of six major textile dyeing units (ST1, ST2, ST3, ST4, ST5 and ST6) were selected, analysed the physico-chemical parameters and their impact on the total muscle protein levels of the dragonfly larvae *Bradinopygageminata*. From the results it was observed that the textile dyeing effluent of six stations contain high level of several organic and inorganic pollutants, which include toxic metals and chemicals. The levels of proteins were observed in decreasing trend in all the concentrations of effluent in different larval stages. It was concluded thatthe improper/without treatment of effluent when let out would results in adverse effects on aquatic and terrestrial ecosystems.

Key words: Textile dyeing effluent, *Bradinopygageminata*, muscle protein.

I. INTRODUCTION

Pollution in the river Cauvery is a big and perennial problem. Pallipalayam is a small town of Namakkal district which is situated at the banks of river Cauvery, contributes a lot in polluting the river by discharging the textile dyeing effluents of numerous textile dyeing units that are functioning in and around the town. The effluents are almost untreated and finally merge with the river water along with the sewage drains. For the past two decades the revolution in textile industry induced the proliferation of countless textile processing units in this area and polluted the river by their effluents beyond control.

The textile dyeing effluents carry dyes particularly harmful synthetic dyes such as direct dyes, mordant dyes, vat dyes, sulfur dyes, insoluble azo dyes and fibre-reactive dyes are the widely used common dyes to dye cellulosic fibres for dyeing and colouring process (Herman and Fletcher, 1966) and also used a number of chemicals are in the textile processing units, like sulphur, naphthol, nitrates, acetic acid, soaps, enzymes, chromium compounds and heavy metals like copper, arsenic, lead, cadmium, mercury, nickel, cobalt and certain auxiliary chemicals. Synthetic dyes and other chemicals are complex substances most of them produce an adverse effects on all forms of living/non-living things.The chemicals are discharged as waste water and contribute to the effluents strong colour, high temperature, turbidity, varying pH, high COD and BOD levels, increased turbidity, high level of suspended solids and total dissolved solids and the toxic nature of the effluents proves fatal to the aquatic organisms directly or indirectly (Elango, 2017).The textile dyeing effluents induce many diseases in human beings and animals. Reactive dyes cause allergic dermatoses, respiratory diseases, mutagenicity (Rannung*et al.,* 1992), genotoxicity (Wollin and Gorlitz, 2004), embryo teratogenic effects (Birhanli*et al.,* 2005), etc.The detergents, starches and other chemicals generate toxic metabolites which are poisonous to fish and other aquatic animals (Sun and Hu, 2014).

Aquatic insects like dragonfly larvae are considered as an excellent ecological indicator in monitoring water quality worldwide, as it is susceptible to anthropogenic changes of aquatic systems. The study of the dragonfly larvae can reveal the physico-chemical changes in aquatic ecosystems more quickly than studying any other animal or plant.

The impacts of the pollutants are best reflected in insect physiology and biochemistry, and can be successfully used bio-indicators to monitor the environmental pollutions by metal contaminants and other pollutants(Davis *et al.,* 2001; Saunders *et al.,* 2002; Chen *et al.*, 2005; Lee *et al.,* 2006).The extended larval duration of the larvae and comparatively larger body size make it easy to study the chronic effects of the effluent. Hence, the larvae of *Bradinopygageminata* are used in the present investigation to study the impact of effluents of textile dyeing industries.

II. MATERIALS AND METHODS

Collection and characterization of textile dyeing effluent

The textile dyeing effluents were collected from six stations namely, Aavarankadu (ST1), Pallipalayam (ST2), Aavathipalayam (ST3), Komarapalayam (ST4), Vediyarasanpalayam (ST5) and Kaliyanur (ST6) of Namakkal District, Tamil Nadu, India. The collected effluent samples were subjected to analyses of physico-chemical parameters such as colour, odour, pH, EC, BOD, COD, TDS, TSS, total nitrogen, total phosphorous, total potassium, chloride, sodium, sulphate, calcium, magnesium, cadmium, lead, copper, zinc and chromium as per standard methods (APHA, 2005).

Collection and identification of larval stages of *Bradinopygageminata*

The different larval stages of the dragonfly larvae were collected from their natural breeding sites such as irrigation tanks from Thiruchengode of Namakkal District, Tamil Nadu, India, using hand nets and brought to the laboratory. The larvae of *Bradinopygageminata* have minute antennae, large compound eyes, slender abdomen with posterior tracheal gills and a prehensile labium with dark brownish green back and pale abdomen. They are hemimetabolous insects which moult averagely fifteen times before it reaches adult stage. The final instar larva is about the size of 21mm in total length. The previous larval instar to the final instar (penultimate larva) and the one with one moult younger (antepenultimate) to the penultimate, having 16mm and 12mm in total length respectively.

Determination of total proteins

The total proteins in the tissues (muscle, gut and rectal gills) of antepenultimate, penultimate and final instar larvaewere determined by following the method given by Gornall*et al.* (1949).Muscle, gut and rectal gills (100mg)were weighed and homogenized with distilled water. The haemolymph (0.05ml) was centrifuged (2500 rpm) for 20 minutes. The homogenates and supernatant were used for analyses.To 0.05ml of each tissue homogenate, 10 ml of 80% ethanol was added and centrifuged for 15 minutes at 5000 rpm. To 2ml of each supernatant, 3ml of Biuret reagent was mixed.The mixture was kept in a water bath at 37°C for 10 minutes and then cooled. The optical density (OD) of the sample was recorded at 540 nm.A protein standard curve was prepared and referred to the analysis.

III. RESULTS AND DISCUSSION

Physico-chemical characteristics of textile effluents of different stations (ST1 to ST6) are given in the Table 1. The parameters of the textile dyeing effluents show a drastic deviation from the standards prescribed by the World Health Organization (WHO) and Bureau of Indian Standards(BIS). In the present investigation, the effluent samples released from different stations displayed different colours such as grey, brown, red and blue with an offensive odour. The colour of the effluent might be due to the presence of biodegradable and nonbiodegradable high-molecular weight organic compounds and high amount of chemicals used during the processing. The textile waste water is highly coloured showing the presence of high concentrations of unused dyes.The different stations of the textile dyeing the odour may be due to the various chemical processing of textile dyeing effluents (Ravibabu*et al*., 2007).The offensive odour could be due to the presence of volatile compounds (Ogunlaja and Aemere, 2009; Arul *et al.*, 2011).From the results higher pH of all the effluents indicates that the effluents were highly alkaline due to the presence of various colouring agents (Senthilkumar*et al.,* 2016).The increased EC of all the stations were observed,mainly due to the excessive usage of sodium chloride (Venkatesh*et al.*, 2009; Sathiyaraj*et al.*, 2017).

High levels of BOD and COD were noticedin all effluent samples.The high BOD may deplete dissolved oxygen, causing death of aerobic organisms and increase anaerobic properties of water (Jody and Dons, 2003). A high level of COD implies toxic conditions and the presence of biologically resistant organic substances in textile dyeing effluents. It determines the oxygen required for the chemical oxidation of organic matter and assesses the quantity of chemically oxidizing matter in water (Vigneshpriya, 2015).The observed TDS and TSS values were higher than the level prescribed by WHO and BIS. This may be due to the presence high level of heavy metals (Yusuff and Sonebare, 2004).

Total nitrogen and total phosphorus levels were quite high in the effluent of all the stations because of the microbial action of the nitrogen fixing microflora and phosphobacteria(Moorthi and Nagarajan, 2011).Chloride andsodium were also found highin the effluent may be on account of water softening processes. The high concentration of sulfateis evident in the untreated effluentas a result of using sulfuric acid in various steps of dyeing and printing process. The high levels of calcium and magnesium were witnessed. Calcium hydroxide and magnesium salts were used to increase the alkalinity to enhance the dyeing processes which increase the hardness of the effluents and soil salinity at the end(Hussain and Hussain, 2012).The heavy metals such as cadmium, lead, copper, zinc and chromium in the effluents were estimated higher than the standard level. This may be due to the application of some dye stuffs which have metallic compounds (Correia, 1998;Vigneshpriya, 2015).

The present study also investigated all these physico-chemical alterations in the effluent that influenced the protein metabolism of antepenultimate, penultimate and final larval instar of dragonfly *B.geminata.* From the results, it is inferred that the textile dyeing effluents of the all the stations have a negative effect on the protein levels of the test animals. The level of total proteins in the muscles of the antepenultimate larvae exposed to the textile dyeing effluents decreased significantly ($p<0.05$) in the larvae of ST5 at 3.5%(Table 2) as well as the least impact was observed in the dyeing effluent of ST2. The same trend was noticed in the penultimate and final instar larvae (Table 3 and 4).

The changes in biochemical parameters such as carbohydrates, proteins and lipids are important to indicate the susceptibility of organ systems to pollutants by altering their functions. Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism(Remia, 2008). The protein metabolism of the larvae includes protein synthesis, degradation and growth. From the above observations, it is well clear that the protein metabolism is disturbed due to the pollutants present in the textile dyeing effluents. Proteins can be expected to be involved in the compensatory mechanism of stressed organisms. Even the experimental animals were fed adequately the total protein levels show a decreasing trend in the effluents of all the stations. Sponza (2006) studied the toxicity of textile dyeing effluents using several toxicity tests and found that besides the dyes themselves, ions like Cr^{6+}, Cd^{2+}, Zn^{2+}, Pb^{2+} also contribute to toxicity of textile effluents. The results of the present study showed that when the three different larvae were exposed to different concentrations of different stations of textile dyeing effluents the muscle protein contents were found to have decreased when increasing the concentrations. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress. It was reported that reduction in protein contents could be due to utilizing protein to meet the energy demand when the experimental animals are under stress (Sandhya *et al.*, 2006, Gehan, 2012).

IV. CONCLUSION

The organic pollutants and metal pollutants may interfere with the cellular mechanism of the aquatic insects and cause a hitch in protein synthetic routines. This may be resulted in decreased protein content of the *Bradinopygageminata*. Besides the protein in tissues might have underwent hydrolysis and oxidation for the need of energy, due to the suppressed protein synthesis in cells under pollutant stress as suggested by the earlier investigators.

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TABLE 1. PHYSICO-CHEMICAL PARAMETERS OF THE TEXTILE DYEING EFFLUENTS OF VARIOUS STATIONS

Parameters	Textile dyeing effluents in various stations						WHO/BIS
	ST1	ST2	ST3	ST4	ST5	ST6	
Colour	Grey	Brown	Red	Red	Blue	Blue	Colourless
Odour	Offensive	Offensive	Offensive	Offensive	Offensive	Offensive	Odourless
pH	8.34	8.78	7.95	8.81	9.28	9.36	6-9
EC (s/cm)	4.68	2.39	3.67	3.49	5.68	3.52	2-3
BOD (mg/l)	462	423	491	343	532	215	100

COD (mg/l)	2790	1281	3974	1698	3874	1221	250
TDS (mg/l)	8454	3321	3484	9985	10264	2517	1500
TSS (mg/l)	349	131	459	434	826	572	100
Total nitrogen (mg/l)	140	211	98	112	154	103	50
Total phosphorous (mg/l)	743	719	935	624	786	703	500
Cl^- (mg/l)	1105	1384	1446	2140	1489	2648	1000
Na (mg/l)	1702	1657	1812	1926	2115	1623	10
SO_4^{-2} (mg/l)	1589	734	1496	613	1638	1845	1000
Ca (mg/l)	115	127	110	124	138	102	200
Mg (mg/l)	16	15	14	18	21	17	30-100
Cd (mg/l)	3.24	2.68	3.25	2.43	3.40	2.15	1.00
Pb (mg/l)	1.30	1.78	0.42	0.30	1.00	1.00	1.00
Cu (mg/l)	3.08	2.02	3.01	4.29	4.03	3.09	3.00
Zn (mg/l)	15	34	10	47	32	18	15
Cr (mg/l)	3.80	3.31	2.93	3.87	4.03	2.34	2.00

TABLE 2. BIOCHEMICAL ESTIMATION OF TOTAL MUSCLE PROTEINS (mg/100g) IN ANTEPENULTIMATE LARVAL INSTAR OF DRAGONFLY *BRADINOPYGA GEMINATA* IN DIFFERENT CONCENTRATIONS OF TEXTILE EFFLUENTS TO 15 DAYS OF EXPOSURE

S. No.	Stations	Concentrations of the textile dyeing effluents (% is derived from the $LC_{50/96}$hr value of the respective stations)							P value (<0.05)
		Control (0%)	1.0%	1.5%	2.0%	2.5%	3.0%	3.5%	
1	ST 1	10.41±0.12	09.37±0.12	09.54±0.18	09.49±0.18	07.30±0.12	07.45±0.34	07.31±0.42	0.000001*
2	ST 2	10.41±0.12	09.68±0.05	09.70±0.46	09.64±0.32	08.94±0.18	08.60±0.48	08.21±0.21	
3	ST 3	10.41±0.12	09.55±0.41	09.37±0.39	09.18±0.14	08.24±0.36	08.18±0.91	07.45±0.32	
4	ST 4	10.41±0.12	09.67±0.37	09.10±0.28	08.24±0.51	08.13±0.15	08.20±0.78	07.32±0.15	
5	ST 5	10.41±0.12	09.24±0.48	08.27±0.13	08.02±0.23	07.54±0.14	07.13±0.18	06.65±0.62	
6	ST 6	10.41±0.12	09.19±0.24	08.92±0.90	08.40±0.35	07.65±0.84	07.21±0.33	06.97±0.13	

Two-way ANOVA of total muscle proteins in antepenultimate larval instar
* Significance (P< 0.05)
** Insignificance (P>0.05)

TABLE 3. BIOCHEMICAL ESTIMATION OF TOTALMUSCLE PROTEINS (mg/100g) IN PENULTIMATE LARVAL INSTAR OF DRAGONFLY *BRADINOPYGA GEMINATA*IN DIFFERENT CONCENTRATIONS OF TEXTILE EFFLUENTS TO 15 DAYS OF EXPOSURE

S. No.	Stations	Concentrations of the textile dyeing effluents (% is derived from the $LC_{50/96}$hr value of the respective stations)							P value (<0.05)
		Control (0%)	1.0%	1.5%	2.0%	2.5%	3.0%	3.5%	
1	ST 1	13.54±0.34	13.10±0.74	12.57±0.54	12.10±0.14	11.23±0.41	10.69±0.62	10.27±0.88	0.000391*
2	ST 2	13.54±0.34	13.04±0.82	12.88±0.62	12.61±0.22	12.34±0.13	12.13±0.55	11.84±0.32	
3	ST 3	13.54±0.34	13.38±0.13	13.34±0.17	12.78±0.15	12.08±0.92	11.17±0.18	11.02±0.64	
4	ST 4	13.54±0.34	13.24±0.05	12.80±0.25	12.47±0.56	11.44±0.71	11.18±0.29	10.22±0.81	
5	ST 5	13.54±0.34	13.42±0.42	12.49±0.65	12.02±0.54	11.35±0.85	10.30±0.76	09.28±0.68	
6	ST 6	13.54±0.34	13.04±0.21	12.48±0.31	11.89±0.32	11.35±0.46	11.08±0.51	10.19±0.58	

Two-way ANOVA of total muscle proteins in penultimate larval instar
* Significance (P< 0.05)
** Insignificance (P>0.05)

TABLE 4. BIOCHEMICAL ESTIMATION OF TOTAL MUSCLE PROTEINS (mg/100g) IN FINAL INSTAR LARVA OF DRAGONFLY *BRADINOPYGA GEMINATA* IN DIFFERENT CONCENTRATIONS OF TEXTILE EFFLUENTS TO 15 DAYS OF EXPOSURE

S. No.	Stations	Concentrations of the textile dyeing effluents (% is derived from the $LC_{50/96}$hr value of the respective stations)							P value (<0.05)
		Control (0%)	1.0%	1.5%	2.0%	2.5%	3.0%	3.5%	
1	ST 1	15.41±0.61	14.80±0.48	14.27±0.21	14.64±0.24	14.87±0.55	13.26±0.51	13.12±0.25	0.000377*
2	ST 2	15.41±0.61	14.89±0.24	14.31±0.58	13.47±0.61	14.78±0.41	14.41±0.33	13.41±0.45	
3	ST 3	15.41±0.61	14.84±0.81	14.24±0.62	13.04±0.88	13.45±0.82	13.14±0.42	12.69±0.41	
4	ST 4	15.41±0.61	14.57±0.38	12.64±0.35	13.54±0.65	13.00±0.64	13.21±0.56	12.28±0.39	
5	ST 5	15.41±0.61	14.46±0.92	13.58±0.64	12.34±0.35	11.28±0.72	10.61±0.15	10.11±0.44	
6	ST 6	15.41±0.61	15.40±0.34	15.14±0.49	14.89±0.51	14.65±0.28	13.84±0.28	10.78±0.71	

Two-way ANOVA of total muscle proteins in final larval instar
* Significance (P< 0.05)
** Insignificance (P>0.05)