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Toxicity Based Evaluation of Textile Waste Water Treatment Efficiency with Algae on the Growth and Enzyme Activity of the Seedlings of *Helianthus annuus* L.

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Abstract: Two different forms of textile effluent samples before recycling and after recycling with mixed native algal inoculum was used for the study. Effect of the effluent samples on sunflower seedlings has been investigated. Raw textile effluent at different levels affected the seedling growth and recorded poor vigour index. The treated textile effluent recorded higher germination per cent and vigour index at different concentration. The presence of the enzymes peroxidase and catalase in the young seedlings grown with untreated effluent indicated the stress imparted on the seedlings due to the pollutants.

Key words: Textile effluent · Algae · Recycling · Effect on crop · Vigour index · Enzyme activity

INTRODUCTION

Environment pollution is the most formidable dangers that confronts mankind today. The large volume of industrial wastes and exhausts generated in the process of urbanization and industrialization are discharged into atmosphere or natural water bodies or in soil and does exert a degradative effect on these life support systems harming the progress of community [1]. The Indian textile industry is the second largest in the world employing about 20 million people, second only to china. A major factor in textile industry is the obsolete technology which permits recycling and reuse of process water in an economic manner. The chemical processes that exist are not economical for treating large volume of water bodies. In such scenario, the microbial biomass has become a pet in terms of ecological and economic cultivation. The algal system, more particularly the cyanobacteria are useful in treating the wastes [2]. Algae are ideally suited to perform reclamation by virtue of their flexibility to adapt to varied environments and their simple nutritional requirement. In the present work, experiments were done to study the physico-chemical characters of the textile effluent before and after treatment with native mixed algal inoculum and its effect on vigour index and enzyme activity of sunflower seedlings as seeds and seedlings are very good indicators of environmental stress particularly the pollutants.

MATERIALS AND METHODS

Isolation and Identification of Algae from Effluent Sample: The raw effluent sample from M/s. K.G. Denim Textile and Dyeing Industry Ltd., located at Jadayampalayam, Coimbatore district was collected and blue green algae and green algae were isolated. Representative effluent sample was serially diluted to 10^{-3} and plated in N-free BG-11 medium for isolation of cyanobacteria and Benecks' medium [3] for isolation of green alga. The plates were incubated under white fluorescence light (3000 lux) at a temperature of 23° C for 21 days. Well developed colonies were observed under microscope and purified for further studies. The algal isolates were identified morphologically based on the cyanobacterial taxonomy hand book [4] and the green algae with the book 'Algae' [5]. The isolated cyanobacterial cultures were identified to molecular level by PCR technique by comparing the DNA molecular markers generated for the cyanobacterial isolates with two STRR primers (CRA 22 CCGCAGCCCAA, CRA23 GCGATCCCA) with those obtained for standard cyanobacterial cultures, obtained from National Centre for Conservation and Utilization of Blue Green Algae (NCCUBGA), Indian Agricultural Research Institute, New Delhi.

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Bioremediation of Textile Effluent with the Algal Isolates: The per cent de-colorization and reduction of various pollutants of the raw and diluted textile effluent was studied with the following treatments.

T_1	Raw effluent	+	*Anabaena* TE1
T_2	Raw effluent	+	*Nostoc* TE1
T_3	Raw effluent	+	*Chlorella* TE1
T_4	Raw effluent	+	*Anabaena* TE1 +*Nostoc* TE1
T_5	Raw effluent	+	*Anabaena* –TE1 + *Chlorella* –TE1
T_6	Raw effluent	+	*Nostoc* –TE1 + *Chlorella* –TE1
T_7	Raw effluent	+	Mixed inoculum
T_8	Diluted effluent (1:3)	+	*Anabaena* –TE1
T_9	Diluted effluent (1:3)	+	*Nostoc* TE1
T_{10}	Diluted effluent (1:3)	+	*Chlorella* –TE1
T_{11}	Diluted effluent (1:3)	+	*Anabaena* TE1 +*Nostoc* TE1
T_{12}	Diluted effluent (1:3)	+	*Anabaena* TE1 + *Chlorella* TE1
T_{13}	Diluted effluent (1:3)	+	*Nostoc* –TE1 + *Chlorella* –TE1
T_{14}	Diluted effluent (1:3)	+	Mixed inoculum
T_{15}	Raw effluent alone		
T_{16}	Diluted effluent alone (1:3)		

The experiment was carried out for 21 days in 250 ml conical flasks containing the effluent treatments as mentioned. Thirty days old homogenized cultures at 5 per cent level was inoculated and incubated at 23°C in a light intensity of 3000 lux under 16:8 h light: dark photoperiod. Mixed inoculum consisted of *Anabaena*-TE1, *Nostoc*-TE1 and *Chlorella*-TE1. Textile effluent samples were collected at weekly intervals and decolourisation was determined as suggested by Yatome *et al*., [6].

Decolourisation activity was calculated using the formula

$$D = \frac{100 \times (A_{ini} - A_{fin})}{A_{ini}}$$

where,

D - Decolourisation (in per cent)
A_{ini} - Initial absorbance at 660 nm
A_{fin} - Final absorbance at 660 nm

The biomass production at the end of the experimental period was determined and expressed as g fresh weight per litre of the sample.

At the end of the experiment, performance of mixed cultures in raw effluent was found to be on par with the performance in treated effluent and hence raw effluent with mixed inoculum was selected for further study (Table 1)

Table 1: Per cent decolourisation of textile dye by the algal isolates under *in vitro* condition

	Days after incubation				
Treatments	7	14	21	28	Mean
T_1	60.21	65.16	71.14	71.10	66.88
T_2	62.10	67.30	73.20	73.24	68.96
T_3	20.00	22.41	25.10	25.30	23.20
T_4	69.10	72.24	75.30	75.21	72.96
T_5	61.30	65.50	71.70	71.80	67.58
T_6	63.00	68.10	73.90	73.90	69.73
T_7	69.20	75.20	77.12	77.20	74.68
T_8	75.12	80.21	82.30	82.40	80.00
T_9	74.00	79.22	83.00	83.10	79.83
T_{10}	24.00	30.14	33.60	33.63	30.34
T_{11}	77.14	82.10	89.00	89.10	85.34
T_{12}	75.40	80.40	83.60	84.14	81.09
T_{13}	75.00	79.30	83.20	85.32	81.21
T_{14}	78.12	83.20	90.00	90.00	85.35
T_{15}	0.00	0.00	0.00	0.00	0.00
T_{16}	0.00	0.00	0.00	0.00	0.00
		Sed	CD (0.05%)		
D (Incubation time)		0.04	0.08		
T (Treatments)		0.07	0.14		
D x T		0.16	0.32		

Evaluation of Textile Waste Water Treatment Efficiency by Algae Through Bio-assay: A bioassay was carried out to evaluate the performance of algal systems for treatment of textile effluent. The effect of treated and untreated textile effluent on the seed germination and vigour index was studied with the following treatments in sunflower (CO4).

T_1 Raw effluent 25%
T_2 Raw effluent 50%
T_3 Raw effluent 75%
T_4 Raw effluent 100%
T_5 Treated effluent 25%
T_6 Treated effluent 50%
T_7 Treated effluent 75%
T_8 Treated effluent 100%
T_9 Nutrient solution

Germination Test [7]: The germination test was conducted for eleven days by roll towel method using 16x 50 seeds counted at random. The temperature of the germination room was maintained at 25±2°C with RH of 90±3 per cent and the normal seedlings were counted on 11^{th} day after sowing of sunflower seeds. The average of the replicates was expressed as the germination percentage.

Root Length: The root length was measured from collar region to the tip of primary root of seedlings and the mean was expressed in cm.

Shoot Length: The shoot length was measured from collar region to the tip of the plumular bud of the seedlings and the mean was expressed in cm.

Vigour Index: The vigour index values were computed by adopting the procedure of Abdul-Baki and Anderson [8] and expressed in whole numbers.

Vigour Index = Germination per cent x
(root length + shoot length)

Effect of Different Treatments on Enzyme Production by Assay Plants: Plant samples were collected on 15th day of germination for peroxidase and catalase assay.

The protein extract was prepared by homogenizing 1g of plant sample in 1ml of 0.1 m sodium phosphate buffer (pH 7.0) and centrifuged at 16,000 g for 20 min at 4°C. The method developed by Lowry *et al*.,(9) was followed for the estimation of the extracted protein. Sample (50ug protein) was loaded into 8 per cent polyacrylamide gel for the analysis of peroxidase and catalase.

Peroxidase: After electrophoresis, peroxidase isoforms were visualized by soaking the gels in 0.05 per cent benzidine in dark for 30 min (Sigma, USA) [7] in acetate buffer (20mM, pH 4.2).

Catalase: For assessing the activity of catalase, the gel was incubated with 0.01 per cent hydrogen peroxide for 20 minutes and the gel was immersed in the staining solution (potassium ferricyanide-0.5 g, ferric chloride – 0.5 g. distilled water 100 ml).

RESULTS AND DISCUSSION

The algal isolates were identified and designated as *Anabaena*-TE1, *Nostoc*-TE1, *Fischerella* –TE1 and *Chlorella* –TE1 (Plate 1). Among these isolates *Fischerella* –TE1 showed poor growth rate in the effluent and was excluded from the study. The physico-chemical nature of the raw effluent and the effluent treated with mixed inoculum are presented in Table 2. A 56.18 per cent reduction in BOD was observed compared to control wherein the initial BOD was 890 mg L^{-1} and reduced to 390 mg L^{-1}. Also due to the accumulation of salts by the algal isolates reduction in pH was found.

Table 2: Characteristics of raw effluent (T_1) and effluent treated with mixed algal isolates (T_2) after 21 days of incubation.

S.No	Characters	T_1	T_2
1	Total suspended solids (mg L^{-1})	5100.00	4620.00 (9.41)
2	Total dissolved solids	6245.00	2900.00 (53.56)
3	pH	9.32	7.21 (22.64)
4	EC (dSm^{-1})	10.32	4.10 (60.19)
5	BOD (mg L^{-1})	890.00	390.00 (56.18)
6	COD (mg L^{-1})	2100.00	1010.00 (51.90)
7	Organic carbon (%)	5.26	3.11 (41.21)
8	Total nitrogen (mg L^{-1})	431.00	303.20 (29.65)
9	Total phosphorus (mg L^{-1})	12.10	3.60 (70.25)
10	Potassium (mg L^{-1})	14.00	8.60 (38.57)
11	Sodium (mg L^{-1})	890.30	590.00 (33.71)
12	Calcium (mg L^{-1})	620.40	311.00 (49.83)
13	Magnesium (mg L^{-1})	180.30	109.00 (39.44)
14	Chloride (mg L^{-1})	810.10	340.00 (58.02)
15	Sulphate (mg L^{-1})	741.60	403.00 (45.61)
16	Carbonate (mg L^{-1})	12.00	9.10 (24.17)
17	Bicarbonate (mg L^{-1})	3.81	3.52 (7.61)
18	Copper (mg L^{-1})	1.90	0.11 (94.20)
19	Chromium (mg L^{-1})	1.00	0.08 (92.00)
20	Lead (mg L^{-1})	2.00	0.15 (92.50)

(Figures in parenthesis indicate percent reduction over control)

Table 3: Effect of different rates of untreated and treated textile effluent on the vigour index of sunflower (Var CO4)

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Vigour index
T_1 Raw effluent 25 %	70	8.10	5.30	938
T_2 Raw effluent 50 %	60	6.40	4.50	654
T_3 Raw effluent 75%	40	5.00	4.75	390
T_4 Raw effluent 100%	30	3.00	4.00	210
T_5 Treated effluent 25%	90	15.30	9.00	2187
T_6 Treated effluent 50%	90	16.00	9.20	2268
T_7 Treated effluent 75%	100	16.70	9.40	2610
T_8 Treated effluent 100%	100	17.00	9.90	2690
T_9 Nutrient solution	100	16.50	9.60	2610
SEd	0.90	9.15	0.13	71.90
CD (0.05%)	1.89	0.32	0.28	151.08

The heavy metal removal was highly appreciable compared to other pollutants. With the mixed inoculum the chromium content was reduced from 1.00 mg L^{-1} to 0.08 mg L^{-1} which is 92 % less that of the control. The lead content showed a reduction from 2.00 mg L^{-1} to 0.15 mg L^{-1} which accounted for a 92.5 per cent decline over control. The ability of algae to bio concentrate trace metals from aqueous environment is well documented and algae have been proved to be an inexpensive process for the reclamation of waste waster [10]. Mierle and Stokes [11] suggested that metal uptake by algae proceeds by a two step process: a rapid initial reaction with the surface of the cell and a slower metal accumulation which is believed to be due to intracellular transport.

Smith [12] observed that intracellular metal binding in *Nostoc muscorum* is closely related to be presence of polyphosphate granules within the cells. The production of exopolysaccharides also contribute significantly towards metal binding [13].

The performance of waste water treatment techniques are generally evaluated through bioassays. Comparative toxicological profile for sunflower at different dilutions with the raw and treated effluent revealed significant difference in germination per cent and vigour index between the treatments (Table 3). The treated effluent at all levels showed higher germination of 90-100 percent. Highest vigour index of 2690 was recorded with 100 per cent treated effluent. A vigour index of 2610 was recorded with 75 per cent treated effluent and the vigour index was on par with seedling growth and recorded the least germination percentage (30) and vigour index (210). With different dilutions of raw effluent, the treatment with 25 per cent raw effluent recorded the highest germination per cent of 70 and a vigour index of 938 (plate1). Rajula and Padmadevi [14] recorded higher vigour index in sunflower with automotive industry effluent amended with blue-green algae. Similarly the biometric as well as biochemical attributes of Soybean (Glycine max) showed a decrease with increased concentration of the textile effluent [15] and Umamaheswari *et al*. reported similar results in blackgram (*Vigna mungo*) with textile mill effluent.

Peroxidase and catalase activity is widely used as indicator in plant shoot tissues that are under stress conditions [17]. The peroxidase and catalase enzyme activities in the plant samples showed marked difference between treated and unteated effluent. Regarding the enzyme peroxidase, two isoforms were expressed in seedlings grown with 50 per cent, 75 per cent and 100 per cent raw effluent and one in 25 per cent raw effluent grown sample. No peroxidase activity was expressed in treated effluent grown plant samples at various levels and those grown in nutrient solution (plate2).

The catalase activity was also noticed in samples treated with raw effluent and variations in intensity of expression with different dilution rates was noticed. The seedling samples with 75 and 100 per cent raw effluent showed higher production of catalase compared with 25 and 50 per cent treated samples (Plate 3). The enzyme assay realized with untreated and treated effluent obviously showed the toxicity of untreated effluent to sunflower. With increased concentrations of untreated textile effluent increased expression of catalase and peroxidase was seen. The parameters studied showed better results when the seedlings were grown in effluent amended with algae. Since the textile effluent samples amended with mixed algal inoculum showed better growth, vigour index and enzyme activity, algae can be used as a cheaper means for the treatment of waste water.

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REFERENCES

1. Gupta, R. and K.G. Mukerji, 2002. Role of microbes in remediation of industrial effluents. In Microorganisms in Bioremediation, Eds.. D.K. Markandey and N. Rajvaidya Markandey, Capital Publishing Company, pp: 43.
2. Stanier, R.Y., R. Kunisawa, M. Mandal and G. Cohen-Bazire, 1971. Purification and properties of unicellular blue-green algae. Bact. Rev., 25: 171-305.
3. Smith, 1983. The role of trace metals in regulating algal growth with emphasis on Fe, Mn and Cu. In: The physiology ecology of Algae Ed. I. Morris. University of California Press, Berkeley, pp: 285.
4. Anand, N., 1989. Hand Book of Blue green algae, Bishen Singh Mahendra Pal Singh, Dehradun, pp: 78.
5. Graham, L.E. and L.W. Wilcox, 2000. Algae. Prentice Hall, New Jersey, pp: 397.
6. Vijayakumari, B., 2003. Impact of textile dyeing effluent on growth of soyabean (*Glycine max*). J. Ecotoxicol. Environ. Monit., 13(1): 59-64.
7. ISTA, 1999. International rules for seed testing. Seed Sci. Tech. Supplement Rules, 27: 25-30.
8. Abdul Baki, A.A. and P.D. Anderson, 1973. Vigour determination in soybean seed by multiple criteria. Crop Sci., 13: 630-633.
9. Levitt, K., 1972. Responses of Plants to Environmental Stress, Academic Press, New York, pp: 697.
10. Dor, I. and B. Svi, 1980. Effect of heterotrophic bacteria on green algae growing in waste water. In Algal Biomass EdS., Sheief, G. and C.J. Soeder. Elsevier /North Holland Biomedical Press, pp: 421-429.
11. Lowry, O.H., N.J. Rosebrought, A.I. Larr and R.J. Randall, 1951. Protein measurement with folin - phenol reagent. J. Biol. Chem., 193: 265-275.

12. Mierle, G.M. and P.M. Stokes, 1976. Heavy metal tolerance and metal accumulation by plaktonic algae. In Trace substances in Environmental health, Ed. D.D Hemphill, Columbia University, Missouri, pp: 113.
13. Geesey, G.G. and L. Jang, 1989. Interactions between metal ions and capsular polymers. In Metal Ions and Bacteria Eds. T.J. Bereridge and R.J. Doyle, John Wiley and Sons, New York, pp: 223-247.
14. Subramanian, G. and S. Shanmugasundaram, 1986. Sewage utilization and waste recycling by cyanobacteria. Indian J. Environ. Hlth., 28: 250-253.
15. Uma Maheswari, S., V. Balamurugan and G.S. Vijayalakshmi, 2003. Impact of textile mill effluent on germination and seedling growth of *Vigna mungo*. Jr. Ecotoxicol. Environ. Monit., 13(1): 47-51.
16. Rajula, R.G. and S.N. Padmadevi, 2000. Effect of industrial effluents without and with BGA on the growth and biochemical contents of the seedlings of *Helianthus annus* L. Asian Jr. of Microbiol. Biotech and Env. Sci., 2(3,4): 151-154.
17. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.