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Effect of Soil Contamination by Some Azo Dyes on the Seed Germination and Plant Growth of Bengal Gram (*Cicer arietinum*)

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Abstract **Original Research Article**

Azo dyes and pigments are widely used for dyeing and printing in textile industries owing to their good fixative properties and cost affectivity. Azo dyes may enter the soil through the discharge of untreated effluents of dye and textile industries. Soil, contaminated/polluted by the azo dyes, is known to be phytotoxic. Presently, we have studied the effect of soil contamination by some azo dyes at different concentrations, on the seed germination and plant growth of Bengal gram (*Cicer arietinum*). Four azo dyes viz., 1-(2'-carboxyphenylazo)-2-naphthol, 5-(2'-carboxyphenylazo)-8-hydroxyquinoline, 4-(2'-carboxyphenylazo) salicylate and 4-(2'-carboxyphenylazo) resorcinol were synthesized and characterized. Calculated quantities of the above dyes were separately mixed with the known weight of soil in separate earthen pots, so as to obtain a dye concentration (w/w) of 200, 400, 600, 800 and 1000 ppm in the soil. Thus, a total of twenty experimental sets (dye-contaminated soil) and one control set (pure soil) were set up. The dyes were thoroughly mixed with the soil so as to obtain a uniform distribution of the dye in the soil. Ten seeds of Bengal gram (*Cicer arietinum*) were sown in each of the experimental pots as well as in the control pot. Proper sunlight and moisture were provided to the pots. Seed germination and plant growth were observed regularly. The morphogenic parameters of the plants such as, root length shoot length and number of leaves was found out at the end of 25 days. Mean value of each of the parameters for each of the set were determined. Percentage inhibition of the parameters, as compared to the control, were also determined. Results revealed a mild to severe phytotoxicity of the azo dye-contamination of soil on the plants of Bengal gram (*Cicer arietinum*). Chemical nature of the azo dye also seemed to rule the level of toxicity. Percentage germination ranged from 40 to 80% in the experimental sets. The plants in the experimental sets had generally a poor/stunted growth. Percentage inhibition of root length, shoot length and number of leaves per plant in the experimental sets, compared to the control set, were quite high, ranging from 11.49 to 54.02% for root length, 6.54 to 29.61% for shoot length and 2.00 to 13.04% for the number of leaves. In general, all the four dyes exhibited a uniform and comparable toxicity. However, 4-(2'-carboxyphenylazo) resorcinol was found to be slightly more toxic. The mechanism behind phytotoxicity of the dyes might be either through complexation/chelation of some nutrient metal ion and resultant inhibition/depletion of its uptake by the plants from soil; or it might be through the extraction of some vital metal ion from the root by the dye, thus depleting the nutrition to the plant. Phytotoxicity of the azo dyes might also have been due to their (dye's) effect on soil microbiological processes and/or plant's physiological processes such as the synthesis of chlorophyll and other organic nutrients.

Keywords: Soil pollution, Phytotoxicity, Azo dye phytotoxicity, Azo dyes, Bengal gram, *Cicer arietinum***.**

INTRODUCTION

Azo dyes are the most versatile class of dyes. About 50% of the synthetic dyes and pigments produced annually belong to the class of azo dyes. They are mainly used because of their stability to light, washing and resistance to microbial attack. They are also cost effective. However, owing to their high resistance to degradability, they often enter the environment through the effluents of dye and textile industries [1,2]. It is estimated that about 10% of the dye in the dying process remain unattached to the fiber and this finds its way to the environment [3-5]. The azo dyes released to the environment contaminate the aquatic bodies and soils in the vicinity of dye and textile industries. Dye factory effluents alter the colour and quality of the water bodies and are hazardous to the aquatic organisms [6-9]. Azo dyes have been reported to be severely toxic to the plant and animal kingdom. They have been found to be cytotoxic, mutagenic as well as carcinogenic [10,11].

Irrigation of agricultural lands with water, polluted with the industrial effluents, contaminate/pollute the soil, and affect the plant growth adversely. Azo dye-contaminated effluents have been reported to affect the seed germination and plant growth parameters of crop plants [12-15].

With the above views in mind, we have presently studied the effect of soil contamination by some azo dyes viz., 1-(2’-carboxyphenylazo)-2-naphthol (Dye-1), 5-(2’-carboxyphenylazo)-8-hydroxyquinoline (Dye-2), 4-(2’-carboxyphenylazo)salicylate (Dye-3) and 4-(2’-carboxyphenylazo)resorcinol (Dye-4) at different concentrations, on the seed germination and plant growth of Bengal gram (*Cicer arietinum*).

MATERIAL AND METHODS

Synthesis of Azo Dyes

The azo dyes, 1-(2’-carboxyphenylazo)-2-naphthol, 5-(2’-carboxyphenylazo)-8-hydroxyquinoline, 4-(2’-carboxyphenylazo)salicylate and 4-(2’-carboxyphenylazo)resorcinol were synthesized by the standard method of diazotization[16] of a known weight of of anthranilic acid (19.7 gm) dissolved in 10 ml HCl and 30 ml water at 0-5^{0}c using a solution of sodium nitrite (10.8 gm) in water. The diazotized solution was coupled with an ice cold alkaline solution of β-naphthol (20.7 gm) or 8-hydroxyquinoline (20.9 gm) or salicylic acid (19.9 gm) or resorcinol (15.8 gm), as the case may be. The separated dye was left over night, filtered, washed with distilled water and dried. They were recrystallised from acetone. The dyes were characterized by elemental analysis as well as infrared (FTIR) and ^{1}H NMR spectral studies.

Amendment/Contamination of the soil with the dyes

Soil was collected from a local agricultural field. 4 kg of soil was contaminated with calculated quantities of dyes separately, so as to achieve the desired concentration (in ppm, w/w) of the dye in soil. Contamination at five concentrations viz., 200, 400, 600, 800 and 1000 ppm were studied in separate sets.

Seeds of Bengal gram (*Cicer arietinum*) were procured from local market, and washed thoroughly with Rozar solution (fungicide, prepared by dissolving 10 mg in 200 ml distilled water) and followed by washing with distilled water. Ten seeds were sown in each of the azo dye-contaminated soil (contained in an earthen pot). Ten seeds were also sown in a set with 4 kg of pure (uncontaminated) soil, which served as the control set. Thus, a total of 21 sets, including one control set and 20 experimental sets, were set up as follows:

1. 4 Kg soil + 10 seeds (**Control Set**)
2. 4 kg soil + 200 ppm Dye-1 + 10 seeds.
3. 4 kg soil + 400 ppm Dye-1 + 10 seeds.
4. 4 kg soil + 600 ppm Dye-1 + 10 seeds.
5. 4 kg soil + 800 ppm Dye-1 + 10 seeds.
6. 4 kg soil + 1000 ppm Dye-1 + 10 seeds.
7. 4 kg soil + 200 ppm Dye-2 + 10 seeds.
8. 4 kg soil + 400 ppm Dye-2 + 10 seeds.
9. 4 kg soil + 600 ppm Dye-2 + 10 seeds.
10. 4 kg soil + 800 ppm Dye-2 + 10 seeds.
11. 4 kg soil + 1000 ppm Dye-2 + 10 seeds.
12. 4 kg soil + 200 ppm Dye-3 + 10 seeds.
13. 4 kg soil + 400 ppm Dye-3 + 10 seeds.
14. 4 kg soil + 600 ppm Dye-3 + 10 seeds.
15. 4 kg soil + 800 ppm Dye-3 + 10 seeds.
16. 4 kg soil + 1000 ppm Dye-3 + 10 seeds.
17. 4 kg soil + 200 ppm Dye-4 + 10 seeds.
18. 4 kg soil + 400 ppm Dye-4 + 10 seeds.
19. 4 kg soil + 600 ppm Dye-4 + 10 seeds.
20. 4 kg soil + 800 ppm Dye-4 + 10 seeds.
21. 4 kg soil + 1000 ppm Dye-4 + 10 seeds.

All the pots were kept in an open place where proper sunlight would be available to them during the day hours. From time to time the pots were treated/irrigated with tap water so as to have proper moisture. The germination and growth pattern of the plants were observed for 25 days. At the end, the surviving plants were dug out and the morphogenic aspects of the plants viz., root length, shoot length and number of leaves was recorded. Mean values of the parameters were calculated separately for each set. Percentage germination of seeds as well as germination cum survival of plants in the sets was also recorded. Percentage inhibition of the parameters, compared to that of control, was calculated out.

RESULTS AND DISCUSSION

All the four presently synthesized azo dyes were characterized by elemental analysis, infrared (FTIR) and ^{1}H NMR spectral studies. Satisfactory elemental analyses were found corresponding to the molecular formula of $C_{17}H_{12}N_2O_3$ for Dye-1, $C_{16}H_{11}N_3O_3$ for Dye-2, $C_{14}H_{10}N_2O_5$ for Dye-3 and $C_{13}H_{10}N_20_4$ for Dye-4.The N=N band of the azo dyes was observed at 1590-1600 cm^{-1} in the infrared spectra. The ^{U}OH of the dyes showed as weak band at rather low position at ~3400 cm^{-1}, suggesting its (OH) involvement in hydrogen-bonding [17,18]. The ^{1}H NMR spectra of the azo dyes showed the phenylic and naphthylic protons as a complex pattern of several multiplets in the region δ6 to 8.5 ppm. The carboxylic protons showed at δ7.21 to 7.90. The phenolic protons of the azo dyes were positioned at δ6.22 to 7.87 ppm. The rather down field positions of phenolic proton signals indicate their involvement in hydrogen-bonding [17,18].

Percentage germination of Bengal gram (*Cicer arietinum*) and morphogenic characteristics (root length, shoot length and number of leaves) of plants in the control set (pure soil) and in the experimental sets (dye-contaminated soil) at different concentrations are recorded in Tables-1 to 4. Percentage germination of seeds and survival of plants as well as percentage inhibition of morphogenic parameters, as a function of concentration of the dye in the soil for different dyes, are shown in Fig.-1 to Fig.-5. Phytotoxicity of a pollutant in the soil would generally be reflected in the germination of seeds and the survival of the plants, as well as, their morphogenic characteristics. A study of Tables-1 to 4 reveal that the azo dyes (in the soil) have exhibited toxicity towards the germination and plant growth of Bengal gram (*Cicer arietinum*). The exhibited toxicity, however, has shown-up to be a function of the concentration of the dye in the soil, as well as, the chemical nature of the dye.

The dyes have mostly affected the germination of seeds. At 200 ppm of the dye, the percentage germination was in the range 70 to 80%, and it gradually decreased, with increasing concentration of the dye. At 1000 ppm, the percentage germination had been 40% for Dye-1, 50% for Dye-2, 40% for Dye-3 and Dye-4. The germinated plants mostly continued to survive, however, with depressed morphogenic parameters.

Besides germination cum survival, morphogenic characteristics of plants are also the indicators of toxicity expression of soil and environment. Presently (Tables-1 to 4), the morphogenic parameters viz., root length, shoot length and number of leaves have been found to be severely affected by the increasing dye concentration in the soil. The percentage inhibition of root length ranged from 30 to 50% for Dye-1, 22 to 54% for Dye-2, 11 to 50% for Dye-3 and 22 to 45% for Dye-4. Root length is a vital parameter for plant growth. Inhibition of root length generally affects the other parameters of the plants because of a depleted uptake of nutrients from soil by the plants. Presently, we have also observed the inhibition of shoot length and number of leaves. Percentage inhibition of shoot length of the plants, compared to that of control, has shown-up to be in the range of 6 to 29% for different dyes in the concentration range of 200 to 1000 ppm. The inhibition has more or less been comparable for all the dyes. The number of leaves has also been found to be inhibited as a result of the dye toxicity. The inhibition of the number of leaves per plant ranged from 2 to 13% for different dyes, in the range of 200 to 1000 ppm concentration. Besides inhibition in the number, the leaves were also found to be quite unhealthy as compared to that of control plants.

So far as chemical nature of the dye is concerned, the Dye-4 (containing resorcinol moiety) was found to be more toxic as compared to the other three dyes (Dye-1, Dye-2 & Dye-3). Reduced morphogenic parameters and related reduced level of growth, as result of toxicity of the dye in the soil, might have been due to a depletion of nutrition to the plants.

Though it is difficult to elucidate the exact mechanism of nutritional depletion and expression of toxicity by the dyes, a role of complexation/chelation of vital/nutrient metal ions by these polydentate dyes may tentatively be speculated. The dyes are probably either depleting the nutrient metal ion uptake, by holding the later in the soil by strong complexation ; or the dye might be extracting out (by complexation) some vital metal ion from the root and thus becoming detrimental to the plant growth. The dyes might even have expressed toxicity by effecting the physiological changes in plants such as destabilization of chloroplast and decrease in chlorophyll content [1, 15]. Interaction of azo dyes with the soil microbes and consequent effect on microbial biomass and their properties might also adversely affect the nutrition and growth of plants [19-21].

Table-1: Effect of Soil Contamination by 1-(2'-carboxyphenylazo)-2-naphthol (Dye-1) on the Seed Germination and Plant Growth of Bengal Gram (*Cicer arietinum*) at 25 Days

Concentration of azo dye in soil (ppm, w/w)	Percentage germination of seeds	Percentage germination cum survival of plants	Mean root length (cm)	Percentage inhibition of root length (compared to control)	Mean shoot length (cm)	Percentage inhibition of shoot length (compared to control)	Mean number of leaves per plant	Percentage inhibition of number of leaves per plant (compared to control)
0 (Control)	100	100	8.7	--	26	--	115	--
200	80	80	6	30.03	21.7	16.54	112.7	2.00
400	70	70	5.7	34.48	21.3	18.08	105.3	8.43
600	60	60	5.0	42.53	21	19.23	104.3	9.30
800	60	60	4.7	45.98	19.7	24.23	104	9.56
1000	40	40	4.3	50.57	19	26.92	103.7	9.83

Table-2: Effect of Soil Contamination by 5-(2'-carboxyphenylazo)-8-hydroxyquinoline (Dye-2) on the Seed Germination and Plant Growth of Bengal Gram (*Cicer arietinum*) at 25 Days

Concentration	Percentage	Percentage	Mean	Percentage	Mean	Percentage	Mean	Percentage

of azo dye in soil (ppm, w/w)	germination of seeds	germination cum survival of plants	root length (cm)	inhibition of root length (compared to control)	shoot length (cm)	inhibition of shoot length (compared to control)	number of leaves per plant	inhibition of number of leaves per plant (compared to control)
0 (Control)	100	100	8.7	--	26	--	115	--
200	70	70	6.7	22.99	24.3	6.54	108.7	5.48
400	70	70	6.3	27.59	22.3	14.23	104.7	8.96
600	70	70	5	42.52	21.3	18.08	104	9.56
800	60	60	4.7	45.98	21	19.23	103.3	10.17
1000	50	50	4	54.02	19.3	25.77	103	10.43

Table-3: Effect of Soil Contamination by 4-(2'-carboxyphenylazo) salicylate (Dye-3) on the Seed Germination and Plant Growth of of Bengal Gram (*Cicer arietinum*) at 25 Days

Concentration of azo dye in soil (ppm,w/w)	Percentage germination of seeds	Percentage germination cum survival of plants	Mean root length (cm)	Percentage inhibition of root length (compared to control)	Mean shoot length (cm)	Percentage inhibition of shoot length (compared to control)	Mean number of leaves per plant	Percentage inhibition of number of leaves per plant (compared to control)
0 (Control)	100	100	8.7	--	26	--	115	--
200	80	80	7.7	11.49	23.3	10.38	112	2.61
400	50	50	6.3	27.59	21.7	16.54	111.3	3.22
600	50	50	5.3	39.08	21.3	18.08	107	6.96
800	60	60	4.7	45.98	19.7	24.23	105.3	8.43
1000	40	40	4.3	50.57	18.3	29.61	104.7	8.96

Table-4: Effect of Soil Contamination by 4-(2'-carboxyphenylazo) resorcinol (Dye-4) on the Seed Germination and Plant Growth of of Bengal Gram (*Cicer arietinum*) at 25 Days

Concentration of azo dye in soil (ppm, w/w)	Percentage germination of seeds	Percentage germination cum survival of plants	Mean root length (cm)	Percentage inhibition of root length (compared to control)	Mean shoot length (cm)	Percentage inhibition of shoot length (compared to control	Mean number of leaves per plant	Percentage inhibition of number of leaves per plant (compared to control)
0 (Control)	100	100	8.7	--	26	--	115	--
200	70	70	6.7	22.99	23.3	10.38	110.3	4.09
400	60	60	6.3	27.59	23	11.54	108	6.09
600	50	50	5.3	39.08	22.3	14.23	106	7.83
800	50	50	5.0	42.53	20.6	20.77	103	10.43
1000	40	40	4.7	45.98	18.3	29.61	100	13.04

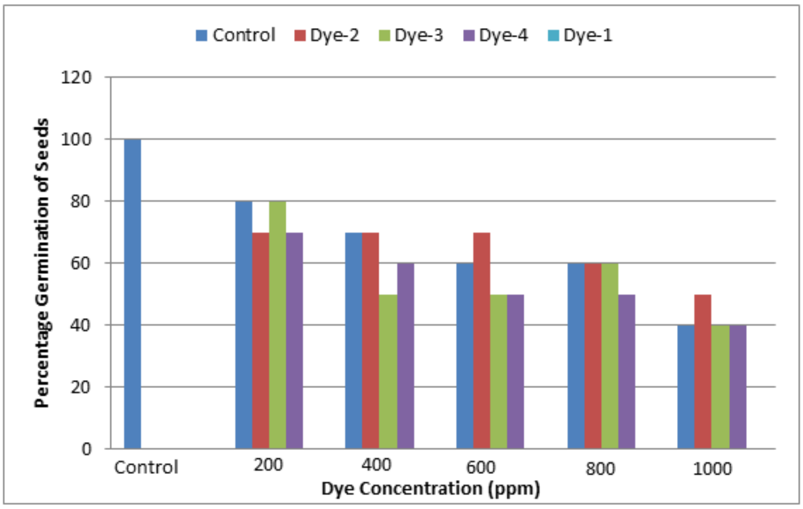

Fig-1: Percentage Germination of the Seeds of Bengal Gram (*Cicer arietinum*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes

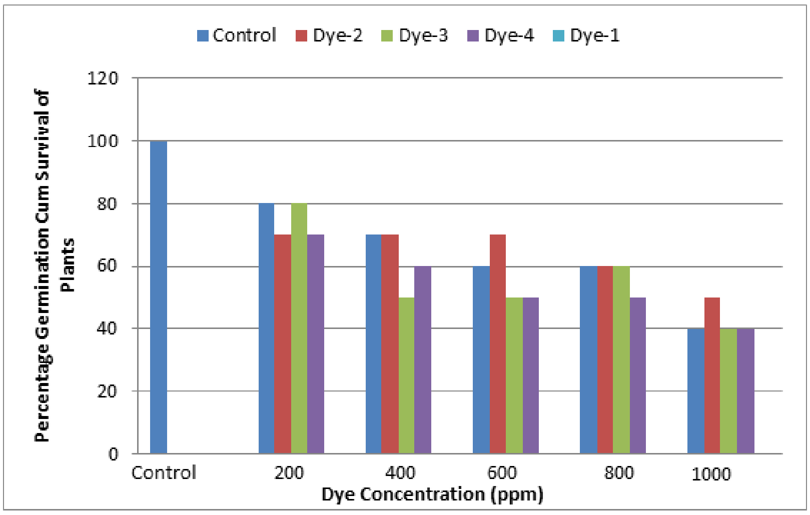

Fig-2: Percentage Germination Cum Survival of the Plants of Bengal Gram (*Cicer arietinum*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes

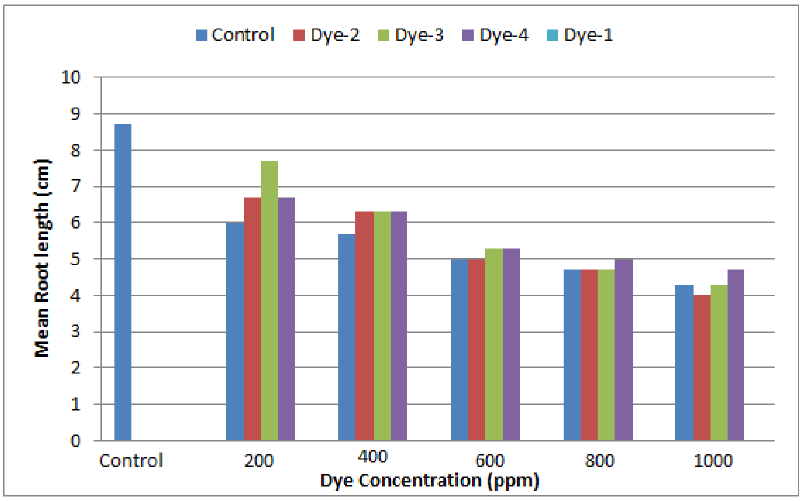

Fig-3: Mean Root Length of the Plants of Bengal Gram (*Cicer arietinum*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes

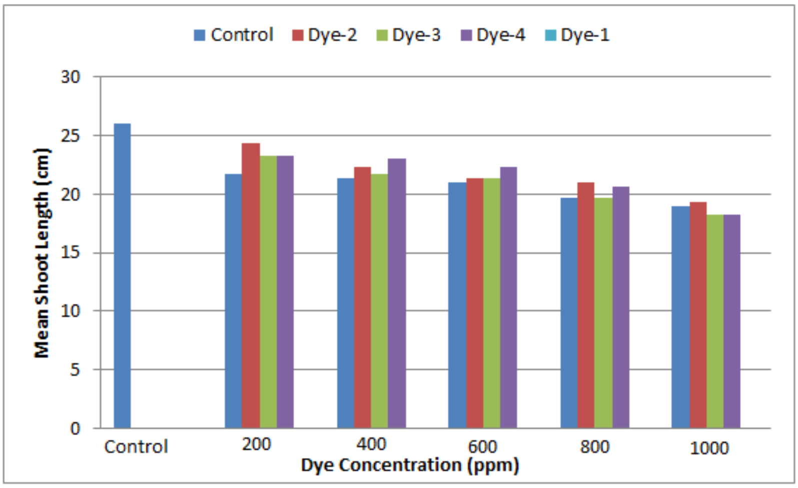

Fig-4: Mean Shoot Length of the Plants of Bengal Gram (*Cicer arietinum*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes

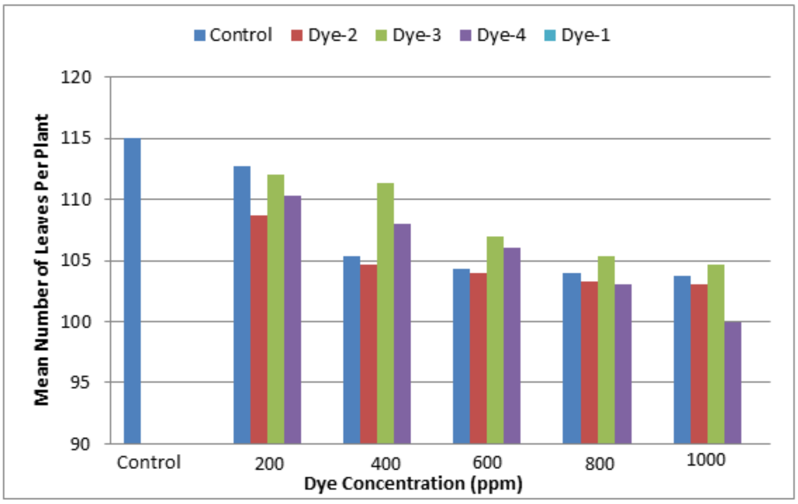

Fig-5: Mean Number of Leaves of the Plants of Bengal Gram (*Cicer arietinum*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes

CONCLUSION

Our present studies suggest that the soils contaminated by the azo dyes are detrimental to the germination and growth of the plants of Bengal gram (*Cicer arietinum*). Survival of the plants and their morphogenic parameters (root length, shoot length and number of leaves) were found to be severely affected from 600 ppm onwards of the dye concentration in the soil. The results, thus, show that pollution of the soil by the azo dyes results in severe phytotoxicity. The azo dyes generally enter the environment, particularly the soil and the aquatic bodies, through the discharge of untreated effluents from the dye and textile industries. The dyes, thus, contaminate the soil either directly or through polluted irrigation-water. The soil quality/productivity of the agricultural lands, as observed in our present study, would be affected. This, in turn, will affect the quality and quantity of crop. As such, measures should be taken to avoid a direct discharge of untreated effluents from the dye and textile industries into the environment. The related industries should, mandatorily, be made to treat the effluents properly for complete degradation of the dyes and intermediates, before discharging to the environment. The soil pollution of the agricultural lands around the dye and textile industries should be properly monitored and remedied before crop production.

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REFERENCES

1. Puvaneswari N, Muthukrishnan J and Gunasekaran P. Toxicity assessment and microbial degradation of azo dyes. Indian J Exp Biol. 2006; 44: 618-626.
2. Bruna de Campos Ventura-Camargo, Maria Aparecida Marin-Morales. Azo Dyes: Characterization and Toxicity – A Review. Textile and Light Industrial Science and Technology (TLIST). 2013; 2 (2): 85-103.
3. Hildenbrand S, Schmahl FW, Wodrarz R, Kimmel R & Dartsch P C. Azo dyes and carcinogenic aromatic amines in cell culture. Int Arch Occup Environ Hlth. 1999; 72: 52.
4. Nam S and Renganathan V. Non enzymatic reduction of azo dyes by NADH. Chemosphere. 2000; 40: 351-357.
5. Jarosz-Wilkołazka A, Kochmańska-Rdest J, Malarcžyk E, Wardas W, Leonowicz A. Fungi and their ability to decolourize azo and anthraquinonic dyes. Enzyme and Microbial Technology. 2002 Apr 16;30(4):566-72.
6. Khan I T and Jain V. Effects of textile industry waste water on growth and some biochemical parameters of *Triticum aestivum var.* Raj 3077. J Environ Poll. 1995; 2: 50.
7. Sponza DT. Toxicity studies in a chemical dye production industry in Turkey. Journal of Hazardous Materials. 2006; 138: 438-447.
8. Zhang W, Liu W, Zhao H, Zhang Y, Quan X, Jin Y. Characterisation of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish. Journal of Environmental Sciences. 2012; 24: 2019-2027.
9. Li Y, Shi JQ, Qu RJ, Feng MB, Liu F, Wang M, Wang ZY. Toxicity assessment on three direct dyes (D-BLL, D-GLN, D-3RNL) using oxidative stress bioassay and quantum parameter calculation.

Ecotoxicology and Environmental Safety. 2012; 86: 132-140.
10. Clonfero E, Vermir P, Granell N and Leis AG. La Medicina Del Lavoro. 1990; 81: 222-229.
11. Garg A, Bhat KL and Bock CW. Dyes and Pigment. 2002; 55: 35-52.
12. Sahai R Agarwal N & Khosala N. Effect of fertilizer factory effluent on seed germination, seedling growth and chlorophyll content of *Phaseolous radiates*. Trop Ecol. 1983; 20: 135.
13. Saxena R M Kewal P F, Yadav R S & Bhatnagar A K. Impact of tannery effluents on some pulse crops. Indian J Environ Hlth. 1986;28: 345.
14. Nirmalarani J & Janardhanan K. Effect of South India Viscose factory effluent on seed germination seedling growth and chloroplast pigments content in five varieties of Maize (*Zea mays* I) Madras. Agric J. 1988;75:41.
15. Ameta C, Punjabi P B, Kothari S & Sancheti A. Effect of untreared and photo catalytically treated dyeing industry effluent on growth and biochemical parameters of *Allium cepa*. Pollut Res. 2003; 22: 389.
16. Vogel A I. Text Book of Practical Organic Chemistry (4^{th} Edition, Reised) ELBS & Longman, London, P. 1978; P.716.
17. Rao T.V.R.K. and Kumud SNP. Mixed Ligand Aluminium (lll) Complexation Involving Some Chelating Organic Acids and Acidic Azo Dyes. J Indian Council of Chemists. 2002; 19: 50-55.
18. Rao TVRK and Yadav UP. Mixed Ligand Complexes of Magnesium and Calcium with Some Chelating Organic Acids as Primary Ligands and Some Polydentate Azo Dyes as Secondary Ligands. J Institution of Chemists (India). 2005; 77: 161-165.
19. Oranusi A, Nathaiel, Ogugbue JC. Toxic response of Nitrobactor to Orange II and Direct Blue 71. Journal of Applied Science and Environment Management. 2001; 6: 87-90.
20. Topac FO, Dindar E, Ucaroglu S, Baskaya HS. Effect of a sulfonated azo dye and sulfanilic acid on nitrogen transformation processes in soil. Journal of Hazardous Materials. 2009; 70: 1006-1013.
21. Imran M, Shaharoona B, Crowley DE, Khalid A, Hussain S, Arshad M. The stability of textile azo dyes in soil and their impact on microbial phospholipid fatty acid profiles. Ecotoxicology and Environmental safety. 2015; 120: 163-168.