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Effect of Soil Contamination by Some Azo Dyes on the Seed Germination and Plant Growth of Broad Bean (*Vicia faba*).

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ABSTRACT

Effect of soil contamination by some azo dyes at different concentrations, on the seed germination and plant growth of Broad bean (*Vicia faba*) has been studied. 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. Ten seeds of Broad bean (*Vicia faba*) were sown in each of the experimental pots as well as in the control pot. Seed germination and plant growth were observed regularly. The morphogenic parameters of the plants such as, root length , shoot length and number of leaves were 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 Broad bean (*Vicia faba*). Chemical nature of the azo dye also seemed to rule the level of toxicity. Many of the plants in the experimental sets died within a few days after germination. Percentage germination ranged from 50 to 100% in the experimental sets. Percentage germination cum survival of plants at 25 days ranged from 0 to 100% in the experimental sets compared to 100% in the control set. At the dye concentration of 600 ppm and above, the germination cum survival of plants was very poor, ranging from 0 to 60%. 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 7.69 to 100% for root length, 5.71 to 100% for shoot length and 7.55 to 100% for the number of leaves. Higher toxicity was shown by the azo dyes, 5-(2'-carboxyphenylazo)-8-hydroxyquinoline and 4-(2'-caboxyphenylazo)resorcinol. Phytotoxicity of the azo dyes might also have been due to their (dyes) effect on soil microbiological processes and/or plant's physiological processes such as the synthesis of chlorophyll and other organic nutrients, thus, depleting nutrition to the plant.

Keywords: Soil pollution, Phytotoxicity, Azo dyes, Broad bean, *Vicia faba.*

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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 Broad bean (*Vicia faba*).

MATERIALS 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^{o}c using a solution of 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 Broad bean (*Vicia faba*) 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:

(I) 4 Kg soil + 10 seeds (**Control Set**)
(II) 4 kg soil + 200 ppm Dye-1 + 10 seeds.
(III) 4 kg soil + 400 ppm Dye-1 + 10 seeds.
(IV) 4 kg soil + 600 ppm Dye-1 + 10 seeds.
(V) 4 kg soil + 800 ppm Dye-1 + 10 seeds.
(VI) 4 kg soil + 1000 ppm Dye-1 + 10 seeds.
(VII) 4 kg soil + 200 ppm Dye-2 + 10 seeds.
(VIII) 4 kg soil + 400 ppm Dye-2 + 10 seeds.
(IX) 4 kg soil + 600 ppm Dye-2 + 10 seeds.
(X) 4 kg soil + 800 ppm Dye-2 + 10 seeds.
(XI) 4 kg soil + 1000 ppm Dye-2 + 10 seeds.
(XII) 4 kg soil + 200 ppm Dye-3 + 10 seeds.
(XIII) 4 kg soil + 400 ppm Dye-3 + 10 seeds.

(XIV) 4 kg soil + 600 ppm Dye-3 + 10 seeds.
(XV) 4 kg soil + 800 ppm Dye-3 + 10 seeds.
(XVI) 4 kg soil + 1000 ppm Dye-3 + 10 seeds.
(XVII) 4 kg soil + 200 ppm Dye-4 + 10 seeds.
(XVIII) 4 kg soil + 400 ppm Dye-4 + 10 seeds.
(XIX) 4 kg soil + 600 ppm Dye-4 + 10 seeds.
(XX) 4 kg soil + 800 ppm Dye-4 + 10 seeds.
(XXI) 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 were 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 were also recorded. Percentage inhibition of the parameters, compared to that of control, were 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 $C_{13}H_{10}N_2O_4$ for Dye-4.The N=N band of the azo dyes was observed at 1590-1600 cm^{-1} in the infrared spectra. The νOH 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 Broad bean (*Vicia faba*) 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 Broad bean (*Vicia faba*) seeds. 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 affected the germination as well as the survival of germinated plants. At 200 ppm of the dye, the percentage germination was 100%, and it gradually decreased, with increasing concentration of the dye. At 1000 ppm, the percentage germination had been 60% for Dye-1, 80% for Dye-2, 50% for Dye-3 and 90% for Dye-4. So far as survival of the germinated plants are concerned, the toxicity was seen to be more severe. In most of the cases, at 600 ppm and above, many of the germinated plants have died within a few days. Only a few plants survived till 25 days, at these concentrations. In case of 1000 ppm concentration of Dye-1, Dye-2 and Dye-4, none of the plants survived till 25 days. In case of Dye-3 even, only one plant survived till 25 days at 1000 ppm level. The percentage germination cum survival of plants at 600 ppm and above was from 60 to 0% for Dye-1 and Dye-2, 50 to 10% for Dye-3 and 30 to 0% for Dye-4. Thus, the Dye-4 seemed to be highly toxic from the plant survival point of view. The presence of resorcinol moiety in the dye structure might have been the reason behind this.

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 in the concentration range of 200 to 800 ppm, has ranged from 15 to 35% for Dye-1, 7 to 38% for Dye-2, 7 to 46% for Dye-3 and 7 to 38% 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 5 to 50% for different dyes in the concentration range of 200 to 800 ppm. The inhibition has more or less been comparable for all the dyes. The number of leaves have also been found to be inhibited as a result of the dye toxicity. The inhibition of the number of leaves per plant ranged from 7 to 56% for different dyes, in the range of 200 to 800 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-2 (containing 8-hydroxyquinoline moiety) and Dye-4 (containing resorcinol moiety) were found to be more toxic as compared to the other two dyes (Dye-1 & 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 Broad Bean (*Vicia faba*) 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	6.5	--	7.0	--	10.6	--
200	100	90	5.5	15.38	6.0	14.29	9.2	13.21
400	100	90	5.3	18.46	5.5	21.43	9.1	14.15
600	90	60	5.o	23.08	4.6	34,29	8.7	17.92
800	70	20	4.2	35.38	4.0	42.86	6.0	43.40
1000	60	00	--	--	--	--	--	--

Table 2: Effect of Soil Contamination by 5-(2'-carboxyphenylazo)-8-hydroxyquinoline (Dye-2) on the Seed Germination and Plant Growth of Broad Bean (*Vicia faba*) 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	6.5	--	7.0	--	10.6	--
200	100	100	6.0	7.69	6.0	14.29	8.8	16.98
400	90	80	5.5	15.38	5.5	21.43	8.2	22.64
600	80	60	4.5	30.77	4.5	35.71	6.8	35.85
800	90	30	4.0	38.46	3.5	50.00	4.6	56.60
1000	80	00	--	--	--	--	--	--

Table 3: Effect of Soil Contamination by 4-(2'-carboxyphenylazo)salicylate (Dye-3) on the Seed Germination and Plant Growth of Broad Bean (*Vicia faba*) 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	6.5	--	7.0	--	10.6	--
200	90	90	6.0	7.69	6.5	7.14	8.6	18.87
400	80	70	5.0	23.08	5.5	21.43	7.8	26.42
600	70	50	4.5	30.77	4.5	35.71	7.4	30.19
800	70	40	3.5	46.15	4.0	42.86	6.8	36.85
1000	50	10	3.0	53.85	2.0	71.43	6.0	43.40

Table 4: Effect of Soil Contamination by 4-(2'-carboxyphenylazo) resorcinol (Dye-4) on the Seed Germination and Plant Growth of Broad Bean (*Vicia faba*) 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	6.5	--	7.0	--	10.6	--
200	100	100	6.0	7.69	6.6	5.71	9.8	7.55
400	70	70	5.5	15.38	6.2	11.43	9.2	13.21
600	90	30	4.5	30.77	5.7	18.57	8.2	22.64

800	80	30	4.0	38.46	5.3	24.28	6.6	37.74
1000	90	00	--	--	--	--	--	--

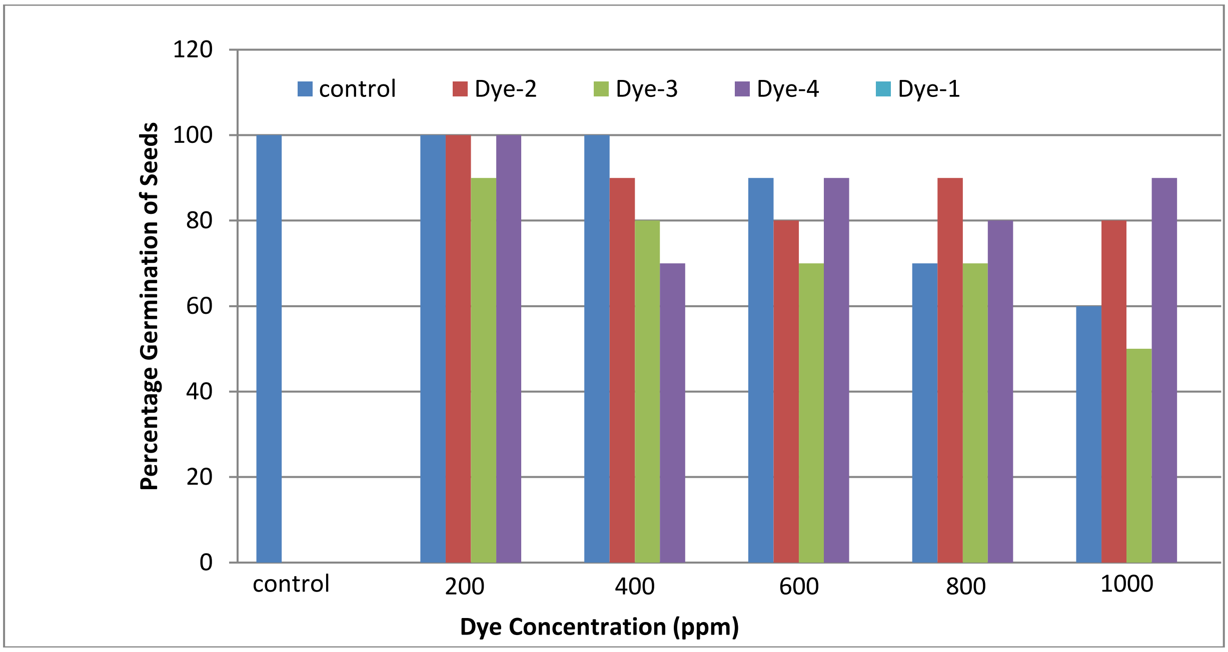

Figure 1: Percentage Germination of Seeds of Broad Bean (*Vicia faba*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes.

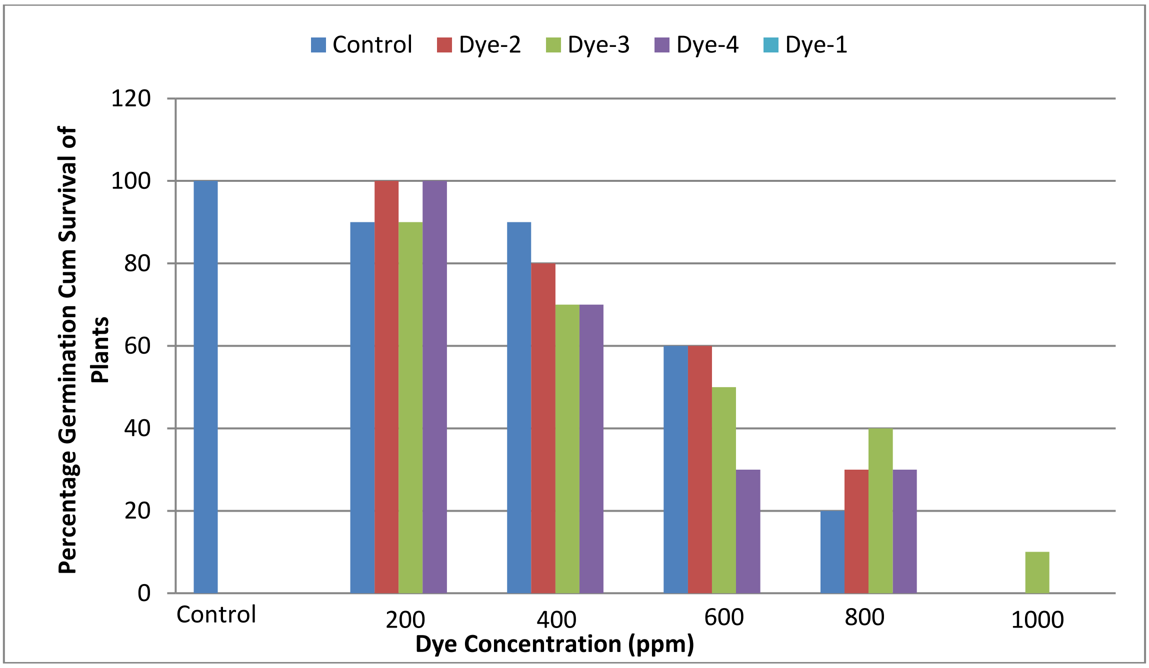

Figure 2: Percentage Germination Cum Survival of plants of Broad Bean (*Vicia faba*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes.

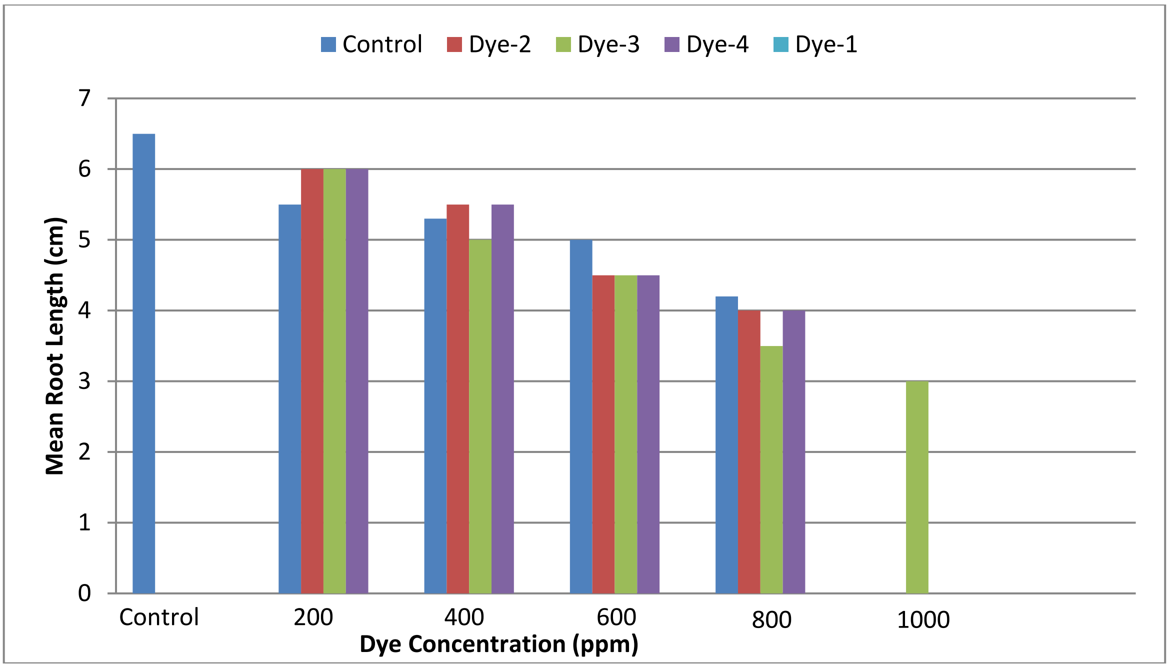

Figure 3: Mean Root Length of the plants of Broad Bean (*Vicia faba*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes.

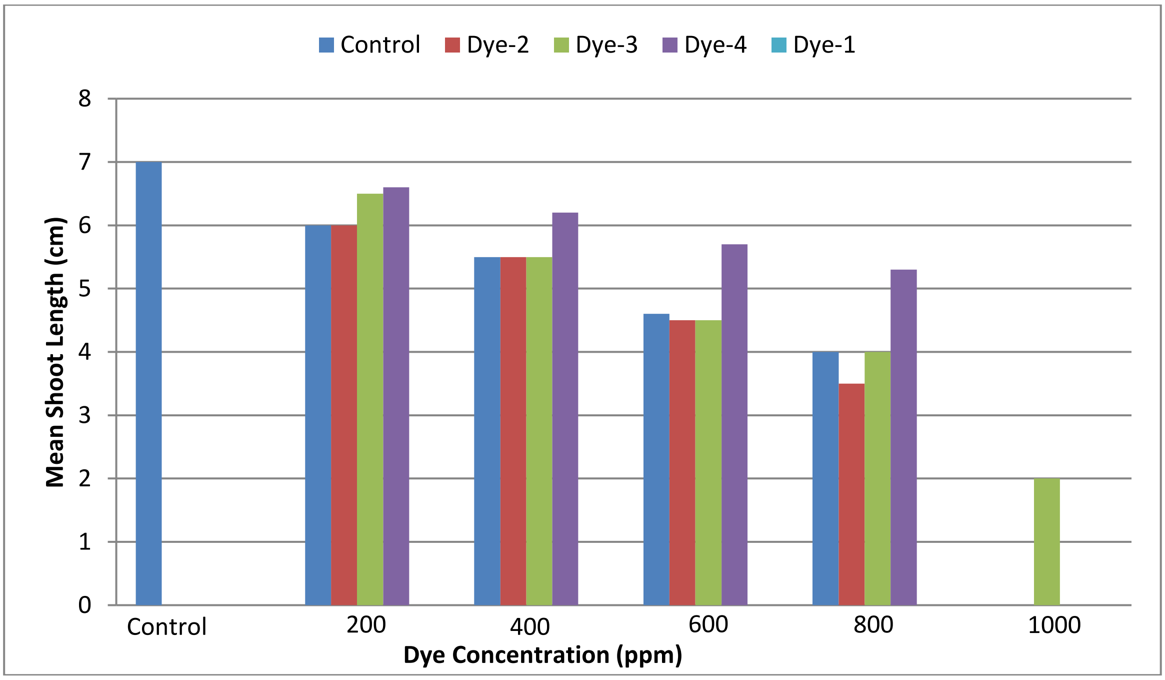

Figure 4: Mean Shoot Length of the plants of Broad Bean (*Vicia faba*)) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes.

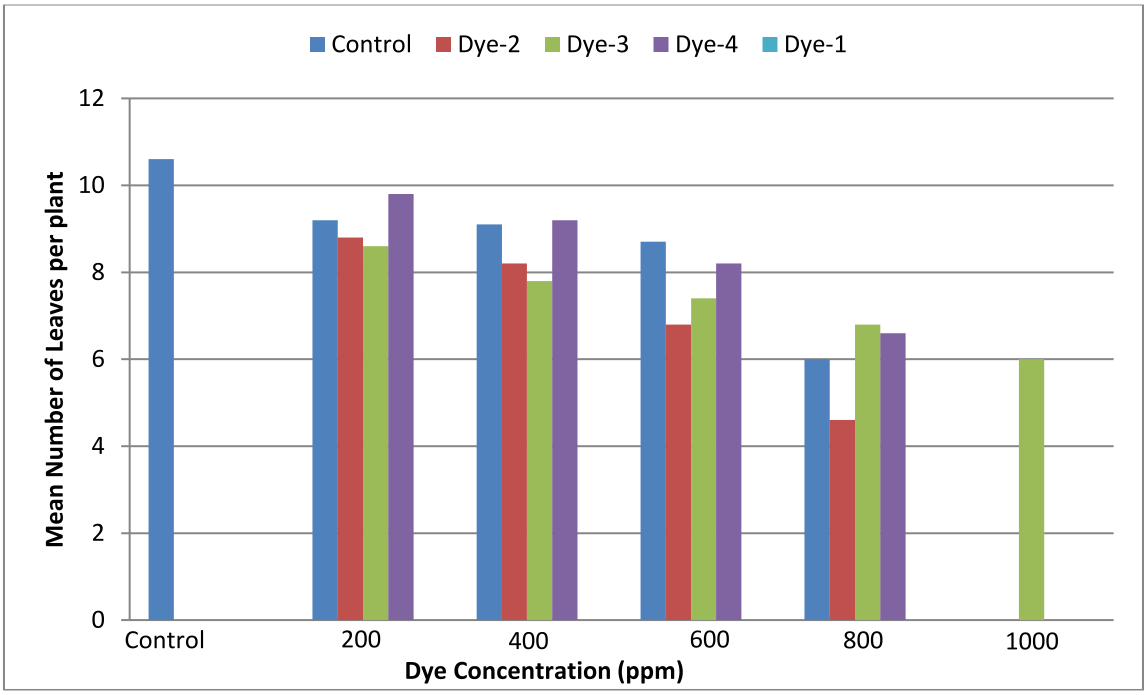

Figure 5: Mean Number of Leaves of the plants of Broad Bean (*Vicia faba*) in Azo Dye-Contaminated Soil at Different Concentrations of Dyes.

CONCLUSION

Our present studies suggest that the soils contaminated by the azo dyes are detrimental to the germination, survival and growth of the plants of Broad bean (*Vicia faba*). Survival of the plants and their morphogenic parameters (root length, shoot length and number of leaves) were found to be severely affected from 400 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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