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Phytotoxic effects of chromium and tannery effluent on growth and metabolism of *Phaseolus mungo* Roxb.

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Abstract: *The various dilution levels of treated tannery effluent (T.E.) (10, 25, 50 and 100 %) and Cr^{6+} (0.5, 2.0, 5.0 and 10 ppm) were used in experiment to know their effect on seed germination, seedling growth, pigments and enzymes content in Black gram (Phaseolus mungo Roxb.). Chromium is known as the main toxic component of tannery effluent so its various concentration were given to know their effects. For the recovery of plant damage, protective value of 10 and 25 ppm of zinc, potassium and iron sulphate were also given with 50 % treated tannery effluent and 10 ppm Cr^{6+} levels in separate petridishes. The different concentrations of tannery effluent and Cr^{6+} showed significant reduction in germination percentage, seedling growth (plumule and radicle length, number of lateral roots, fresh and dry weight, and moisture %) and pigments (chlorophyll, pheophytin and carotenoids) with increase in concentrations. The lower doses of tannery effluent (10%) and Cr^{6+} (0.5, 2 and 5 ppm) slightly increases the pigments concentration. The amylase activity and total sugar contents were also significantly decreased while catalase and peroxidase activity showed significant increase with rise in concentrations of treatments. The lower concentrations of tannery effluent (dilution 10 and 25%) and Cr^{6+} (0.5 and 2 ppm) showed significant increase in total protein contents while decrease at higher concentrations. The zinc, potassium and iron treatments led to recover the damage caused by chromium and tannery effluent in all parameters. In recovery treatments zinc showed highest and significant recovery in maximum parameters. Iron also showed almost similar effect to the zinc while potassium showed minimum recovery.*

Key words: *Tannery effluent, Chromium (VI), Pigments, Amylase, Catalase, Peroxidase, Sugar, Protein, Phaseolus mungo Roxb*
PDF of full length paper is available with author ***(*nathkamlesh@rediffmail.com)***

Introduction

In the environment chromium exist in several oxidation states but Cr^{6+} and Cr^{3+} are the prevalent species. Among the two species Cr^{6+} is more toxic than Cr^{3+} (Sinha *et al*., 2005). Chromium is used in metal plating industries, tanneries and oil well drilling (Abbassi *et al*., 1998). Cr^{6+} (Chromium VI) compounds are highly water soluble and toxic compared to Cr^{3+} (Chromium III) compounds. The health effects of these are very well documented (WHO, 1988).

Tannery industries are one of the major sources of Cr pollution in the environment. Effluent of tanneries industries containing Cr and other nutrients is discharged into the local water bodies. It results into the contamination of water bodies which are used for irrigation purposes. Presence of chromium beyond the tolerance limit (<2 ppm) makes water unsuitable for crop growth (Bera and Bokaria, 1999; Sahu *et al*., 2007). The high level of Cr and nutrient contents in the effluent has been reported to inhibit the seed germination and seedling growth, which might be due to the presence of excess amount of dissolved solids, chlorides, sulphides, chromium, high BOD and COD values of the effluent (Mishra and Bera, 1995).

Cr^{6+} compounds are highly soluble and easily bio-available than the sparingly soluble Cr^{3+}. Chromium greater than 2 ppm has been reported to be inhibitory for plant growth resulting in stunted growth, poor development of roots and discoloring of leaves (Pratt, 1966). Transport of chromium in the root from the soil is very slow (Skeffington *et al.,* 1976), but once it enters, it can be rapidly transported. Toxic heavy metals are mostly absorbed and get accumulated in various plant parts as free metals which may adversely affect the plant growth and metabolism (Barman and Lal, 1994) and their accumulation is biomagnified at different trophic levels through food chains (Rai *et al*., 2002; Saxena *et al.,* 2007; Perez and Sarma, 2008).

Chromium has its effect on certain enzymes such as catalase, peroxidase, and cytochrome oxidase, which have iron as one of the constituent. Agarwala *et al*. (1962) has reported stimulation of catalase activity in barley at excess supply of chromium. Marked toxicity of chromium has been reported with respect to photosynthetic pigments, nitrate reductase activity and protein content in algae (Rai *et al*., 1992) and other higher plants (Nath *et al*., 2005). The direct interaction of metal with cellular components can initiate variety of metabolic responses finally leading to a shift in the developmental process of the plant (Assche and Clijsters, 1990). Chromium toxicity produces chlorosis and necrosis in plants (Cervantes *et al*., 2001).

Present study is performed to explore the toxic effect of chromium and tannery effluent on plants metabolism and growth. Study also aims at studying remedial approach through zinc, potassium and iron sulphate.

Materials and Methods

In the petridish culture experiments different concentrations of chromium were selected on the basis of amount of chromium in

treated tannery effluent, as per previous experiments (Nath *et al*., 2005) and available literature. As 2 ppm concentration of Cr^{+6} is described as acceptable to the plant system and also standard for discharge of treated tannery effluent therefore, low (0.5 ppm), high (5 ppm) and very high (10 ppm) concentrations were selected. Treatment solutions of chromium were prepared by dissolving chromic oxide (CrO_3) in distilled water. On the other hand the different dilution levels 10, 25, 50 and 100% (10% means, 10 ml effluent and 90 ml distilled water) of treated tannery effluent were selected to see the toxic effect on black gram seedlings. For the recovery of plant damage 10 and 25 ppm of zinc sulphate ($ZnSO_4$), potassium sulphate (K_2SO_4) and iron sulphate ($FeSO_4$) were also prepared and added in 50% treated tannery effluent and 10 ppm Cr levels in separate petridishes. Finally the experiment was setup with the various treatments *i.e.* Control (distilled water), 10% T.E., 25% T.E., 50% T.E., 100% T.E., 50% T.E.+10 ppm $ZnSO_4$, 50% T.E.+25 ppm $ZnSO_4$, 50% T.E.+10 ppm K_2SO_4, 50% T.E.+25 ppm K_2SO_4, 50% T.E.+10 ppm $FeSO_4$, 50% T.E.+25 ppm $FeSO_4$, 0.5 ppm Cr, 2 ppm Cr, 5 ppm Cr, 10 ppm Cr, 10 ppm Cr+10 ppm $ZnSO_4$, 10 ppm Cr+25 ppm $ZnSO_4$, 10 ppm Cr+10 ppm K_2SO_4, 10 ppm Cr+25 ppm K_2SO_4, 10 ppm Cr+10 ppm $FeSO_4$ and 10 ppm Cr+25 ppm $FeSO_4$.

The higher concentration of recovery elements were used to produce more free ions for increase availability to seedlings roots. The chromium reduces the absorption capability of various nutrients in plants, while the amount of recovery elements already present in effluent may be presented as binding form (with other elements present in effluent) or non ionic form which is not easily available for absorption by plant roots (Pandey and Sharma, 2003). At the level of 50% tannery effluent and 10 ppm chromium the significant damage were observed so these levels are chosen for recovery purpose (Nath *et al*., 2005). The nutrient solution was supplied on every third day in petridishes. In the present study the effects of tannery effluent and chromium on the seed germination, seedling growth was analyzed separately. Because tannery effluent contains reasonably higher contents of chromium, therefore, to check the efficacy of chromium present therein, exclusive chromium treatments were also undertaken, other than tannery effluent.

Black gram (*Phaseolus mungo* Roxb. c.v. – Type 35) seeds were used for the petridish experiment. Treated tannery effluent samples were taken from common effluent treatment plant, Unnao, U.P and the effluent was analyzed for various physico-chemical properties (APHA, 2005) and Metal concentration (Piper, 1942). Seeds were surface sterilized with 0.1% $HgCl_2$ for the prevention of surface fungal/bacterial contamination (Young, 1926). Twenty seeds were sown on filter paper in each petridish and 10 ml solution was used as described above and the experiment was performed in triplicate. The fresh solutions were applied every alternate day for the prevention of contaminants and also for the maintenance of concentration. The experiment was under observation for 10 days. The fresh weight was taken with the help of digital balance and dry weight was measured by seedlings in hot air oven at 80±1°C for 24 hr. The growth parameters like germination %, plumule and radicle length, number of lateral roots, pigments (chlorophyll, pheophytin and total carotenoids), amylase (total amylase, α -amylase and β -amylase), catalase, peroxidase, total protein and total sugar contents and biomass production were observed in each sample at the end of 10th day.

The method of Arnon (1949), as amended by Lichtenthaler (1987) was used for the pigments estimation. The estimation of enzymes *viz.,* amylase, catalase and peroxidase was done by using the method of Katsuni and Frekuhara (1969), Euller and Josephson (1927) and Luck (1963) respectively. Protein and total sugar was estimated by the method of Lowry *et al.* (1951) and Dubais *et al.* (1956) respectively. The data observed in the experiment were statistically analyzed for the calculation of standard error (S.E.) and student 't' test was administered for testing the hypothesis.

Results and Discussion

Table 1 shows the physico-chemical characteristics of treated effluent analysis. After treatment the tannery effluent is turned light yellow in colour. Odor of tannery effluent was foul, pH ranged between 7.58-7.89. Dissolve oxygen (DO) was found nil. The EC, BOD, COD, total hardness, total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) were found higher than permissible limits. The chromium, zinc, potassium and iron ranged between 15.48-25.10 ppm, 1.07-2.34 ppm, 22.6-31.2 ppm and 18.58-28.10 ppm respectively.

The results obtained in black gram (*Phaseolus mungo* Roxb.) petridish culture experiment are shown in Tables 2a to 4b. Table 2a clearly shows the effect of tannery effluent and its combinatorial effect with different concentrations of zinc, potassium and iron sulphate. The germination % was found to be increased only in 10% tannery effluent over control while all other treatments showed inhibition of germination. In all recovery treatments germination % increased except 25 ppm zinc sulphate in combination of 50% tannery effluent if compared with 50% tannery effluent

Table - 1: Analysis of treated tannery effluent (T.E.)

Parameters	Concentration
Colour	Light yellow
Odour	Foul
E C	14.98-17.57 dSm^{-1}
pH	7.58-7.89
Dissolved oxygen (DO)	Nil
Biological oxygen demand (BOD)	150-200 ppm
Chemical oxygen demand (COD)	250-300 ppm
Total hardness	1.8-2.0 g l^{-1}
Total solids (TS)	17.86-20.43 g l^{-1}
Total dissolved solids (TSS)	16.50-19.66 g l^{-1}
Total suspended solids (TSS)	1.0-1.1 g l^{-1}
Chromium (Cr)	15.48-25.10 ppm
Zinc (Zn)	1.07-2.34 ppm
Potassium (K)	22.6-31.2 ppm
Iron (Fe)	18.58-28.10 ppm

Table - 2(a): Effect of tannery effluent (T.E.) and its combination with Zn, K and Fe on germination percentage and seedling growth (10th day) in black gram

Treatments	Germination (%)	Plumule length (cm)	Radicle length (cm)	No. of lateral roots	Fresh weight (g)	Dry weight (g)	Moisture (%)
Control	85.00 ± 2.88	14.43 ± 0.80	5.25 ± 0.37	4.00 ± 0.28	0.707 ± 0.043	0.030 ± 0.000	95.72 ± 0.26
10% T.E.	91.66 ± 1.66	13.73 ± 1.12	3.64 ± 0.23*	3.25 ± 0.14	0.660 ± 0.034	0.040 ± 0.000	93.90 ± 0.32*
25% T.E.	81.66 ± 4.41	9.51 ± 1.02*	2.72 ± 0.29*	2.00 ± 0.28*	0.490 ± 0.005*	0.030 ± 0.005*	93.90 ± 1.10
50% T.E.	75.00 ± 2.88	5.41 ± 0.65*	2.13 ± 0.21*	1.75 ± 0.43*	0.287 ± 0.026*	0.040 ± 0.000*	85.80 ± 1.32*
100% T.E.	28.33 ± 7.26*	2.41 ± 0.46*	1.35 ± 0.26*	1.00 ± 0.57*	0.167 ± 0.017*	0.030 ± 0.005	80.89 ± 5.36*
50% T.E.+10 ppm $ZnSO_4$	80.00 ± 2.88	6.56 ± 1.10*	2.95 ± 0.20*	2.66 ± 0.33*	0.340 ± 0.023*	0.040 ± 0.000*	88.12 ± 0.81*
50% T.E.+25 ppm $ZnSO_4$	63.33 ± 4.41*	7.27 ± 0.43*	3.13 ± 0.82*	2.83 ± 0.44	0.360 ± 0.005*	0.036 ± 0.003	89.83 ± 0.80*
50% T.E.+10 ppm K_2SO_4	75.00 ± 5.77	6.68 ± 1.38*	2.24 ± 0.13*	3.16 ± 0.16	0.300 ± 0.005*	0.020 ± 0.000*	93.32 ± 0.13*
50% T.E.+25 ppm K_2SO_4	76.66 ± 4.41	7.26 ± 0.89	2.60 ± 0.15*	3.16 ± 0.44	0.317 ± 0.008*	0.030 ± 0.005	90.61 ± 1.57*
50% T.E.+10 ppm $FeSO_4$	80.00 ± 2.88	5.84 ± 0.80*	2.52 ± 0.20*	3.00 ± 0.28	0.327 ± 0.003*	0.033 ± 0.006	89.83 ± 0.95*
50% T.E.+ 25 ppm $FeSO_4$	75.00 ± 2.88	6.68 ± 0.65*	2.30 ± 0.14*	3.50 ± 0.28	0.437 ± 0.014*	0.020 ± 0.000*	95.40 ± 0.15

Values are mean of three replicates ± SE, * statistically significant at p<0.05 level, T.E. - Tannery effluent

Table - 2(b): Effect of chromium (Cr) and its combination with Zn, K and Fe on germination percentage and seedling growth (10th day) in black gram

Treatments	Germination (%)	Plumule length (cm)	Radicle length (cm)	No. of lateral roots	Fresh weight (g)	Dry weight (g)	Moisture (%)
Control	85.00 ± 2.88	14.33 ± 0.80	5.25 ± 0.37	4.00 ± 0.28	0.707 ± 0.043	0.030 ± 0.000	95.72 ± 0.26
0.5 ppm Cr	90.00 ± 2.88	13.10 ± 0.15	3.00 ± 0.17*	4.50 ± 0.57	0.660 ± 0.017	0.050 ± 0.000*	92.41 ± 0.19*
2 ppm Cr	76.66 ± 3.33	12.26 ± 0.17*	2.69 ± 0.03*	4.00 ± 0.00	0.500 ± 0.040*	0.030 ± 0.005	94.11 ± 0.68
5 ppm Cr	76.66 ± 1.66	10.24 ± 1.42*	1.95 ± 0.09*	3.50 ± 0.28	0.380 ± 0.017*	0.020 ± 0.000*	94.71 ± 0.24*
10 ppm Cr	68.33 ± 4.41*	7.66 ± 0.62*	1.50 ± 0.05*	3.50 ± 0.28	0.350 ± 0.028*	0.020 ± 0.000*	94.20 ± 0.48
10 ppm Cr+10 ppm $ZnSO_4$	75.00 ± 2.88	7.32 ± 0.38*	2.09 ± 0.01*	3.50 ± 0.28	0.400 ± 0.028*	0.050 ± 0.005*	87.15 ± 2.38*
10 ppm Cr+25 ppm $ZnSO_4$	78.33 ± 8.81	8.82 ± 0.98*	2.36 ± 0.15*	5.82 ± 0.47*	0.430 ± 0.028*	0.030 ± 0.005	93.14 ± 0.88*
10 ppm Cr+10 ppm K_2SO_4	68.33 ± 4.41*	7.21 ± 0.35*	2.03 ± 0.02*	3.50 ± 0.47	0.287 ± 0.020*	0.020 ± 0.000*	92.95 ± 0.51*
10 ppm Cr+25 ppm K_2SO_4	71.66 ± 6.00	9.90 ± 0.28*	2.69 ± 0.11*	4.66 ± 0.28	0.410 ± 0.046*	0.030 ± 0.005	92.82 ± 0.61*
10 ppm Cr+10 ppm $FeSO_4$	68.66 ± 1.66*	9.12 ± 0.29*	1.88 ± 0.21*	3.55 ± 0.66	0.330 ± 0.011*	0.030 ± 0.005	91.01 ± 1.43*
10 ppm Cr+25 ppm $FeSO_4$	71.66 ± 6.09	9.49 ± 0.48*	1.89 ± 0.01*	4.16 ± 1.16	0.370 ± 0.005*	0.020 ± 0.000*	94.59 ± 0.08*

Values are mean of three replicates ± SE, * statistically significant at p<0.05 level

Table - 3(a): Effect of tannery effluent (T.E.) and its combination with Zn, K and Fe on chlorophyll (total, a and b) a/b ratio, pheophytin (total, a and b) and total carotenoid in black gram

Treatments	Chlorophyll (mg g^{-1} fresh wt. of tissue)			a/b ratio	Pheophytin (mg g^{-1} fresh wt. of tissue)			Total carotenoid (mg g^{-1} fresh) wt.of tissue)
	Total	'a'	'b'		Total	'a'	'b'	
Control	1.623 ± 0.034	1.019 ± 0.060	0.586 ± 0.015	1.74	2.334 ± 0.086	1.674 ± 0.109	0.660 ± 0.025	0.682 ± 0.018
10% T.E.	1.774 ± 0.080	1.167 ± 0.099	0.515 ± 0.016*	2.27	2.580 ± 0.117	1.997 ± 0.166	0.583 ± 0.007*	0.722 ± 0.035
25% T.E.	1.512 ± 0.069	0.956 ± 0.069	0.503 ± 0.013*	1.90	2.166 ± 0.130	1.586 ± 0.118	0.579 ± 0.011*	0.623 ± 0.056
50% T.E.	1.241 ± 0.036*	0.793 ± 0.027*	0.409 ± 0.018*	1.94	1.774 ± 0.074*	1.281 ± 0.051*	0.493 ± 0.023*	0.542 ± 0.008*
100% T.E.	0.475 ± 0.010*	0.253 ± 0.006*	0.192 ± 0.001*	1.32	0.624 ± 0.013*	0.407 ± 0.006*	0.218 ± 0.007*	0.195 ± 0.025*
50% T.E.+10 ppm $ZnSO_4$	1.916 ± 0.266	1.287 ± 0.210	0.619 ± 0.069*	2.08	2.917 ± 0.435	2.281 ± 0.431	0.637 ± 0.004	0.637 ± 0.036
50% T.E.+25 ppm $ZnSO_4$	1.477 ± 0.046	0.918 ± 0.028	0.497 ± 0.062*	1.85	2.117 ± 0.074	1.515 ± 0.051	0.601 ± 0.022	0.622 ± 0.020
50% T.E.+10 ppm K_2SO_4	1.316 ± 0.192	0.825 ± 0.121	0.463 ± 0.056	1.74	1.254 ± 0.126*	0.883 ± 0.087*	0.371 ± 0.038*	0.535 ± 0.057*
50% T.E.+25 ppm K_2SO_4	1.738 ± 0.025	1.093 ± 0.028	0.632 ± 0.007	1.73	2.514 ± 0.051	1.800 ± 0.033*	0.714 ± 0.017	0.708 ± 0.063
50% T.E.+10 ppm $FeSO_4$	1.301 ± 0.332	0.838 ± 0.226	0.440 ± 0.090	1.90	1.651 ± 0.174*	1.176 ± 0.129*	0.475 ± 0.045*	0.446 ± 0.024*
50% T.E.+25 ppm $FeSO_4$	1.585 ± 0.384	0.905 ± 0.178	0.550 ± 0.118	1.65	1.818 ± 0.311	1.266 ± 0.204	0.553 ± 0.107	0.576 ± 0.082

Values are mean of three replicates ± SE, * statistically significant at $p<0.05$ level, TE = Tannery effluent

Table - 3(b): Effect of chromium (Cr) and its combination with Zn, K and Fe on chlorophyll (total, a and b) a/b ratio, pheophytin (total, a and b) and total carotenoid in black gram

Treatments	Chlorophyll (mg g^{-1} fresh wt. of tissue)			a/b ratio	Pheophytin (mg g^{-1} fresh wt. of tissue)			Total carotenoid (mg g^{-1} fresh wt. of tissue)
	Total	'a'	'b'		Total	'a'	'b'	
Control	1.623 ± 0.034	1.019 ± 0.060	0.586 ± 0.015	1.74	2.334 ± 0.086	1.674 ± 0.109	0.660 ± 0.025	0.682 ± 0.018
0.5 ppm Cr	1.670 ± 0.026	1.062 ± 0.027	0.588 ± 0.010	1.90	2.420 ± 0.066	1.782 ± 0.078	0.639 ± 0.011	0.729 ± 0.005
2 ppm Cr	2.117 ± 0.015*	1.445 ± 0.005*	0.649 ± 0.001	2.22	3.094 ± 0.100*	2.584 ± 0.030*	0.511 ± 0.102	0.883 ± 0.033*
5 ppm Cr	2.090 ± 0.080*	1.213 ± 0.040*	0.553 ± 0.036*	1.78	3.919 ± 0.257*	3.279 ± 0.246*	0.541 ± 0.010	1.078 ± 0.092*
10 ppm Cr	1.754 ± 0.114*	1.125 ± 0.090*	0.548 ± 0.023	1.95	2.238 ± 0.164	1.625 ± 0.135	0.513 ± 0.028	0.776 ± 0.030*
10 ppm Cr+10 ppm $ZnSO_4$	1.854 ± 0.101	1.196 ± 0.058	0.561 ± 0.026	2.13	2.666 ± 0.121	2.007 ± 0.107	0.659 ± 0.014	0.779 ± 0.028*
10 ppm Cr+25 ppm $ZnSO_4$	1.855 ± 0.087	1.189 ± 0.072	0.632 ± 0.019	1.88	2.695 ± 0.145	2.007 ± 0.138	0.688 ± 0.007	0.766 ± 0.041*
10 ppm Cr+10 ppm K_2SO_4	1.754 ± 0.193	1.128 ± 0.113	0.592 ± 0.079	1.91	2.029 ± 0.026*	1.484 ± 0.029	0.545 ± 0.023*	0.647 ± 0.003
10 ppm Cr+25 ppm K_2SO_4	1.978 ± 0.001*	1.303 ± 0.023*	0.682 ± 0.019*	1.91	2.944 ± 0.026*	2.221 ± 0.049*	0.723 ± 0.023	0.807 ± 0.015
10 ppm Cr+10 ppm $FeSO_4$	2.516 ± 0.366	1.694 ± 0.317	0.870 ± 0.112	1.95	2.930 ± 0.370*	2.156 ± 0.328	0.774 ± 0.051	0.651 ± 0.025*
10 ppm Cr+25 ppm $FeSO_4$	1.865 ± 0.047*	1.189 ± 0.020	0.622 ± 0.006*	1.91	3.048 ± 0.238*	2.317 ± 0.247*	0.731 ± 0.058	0.759 ± 0.008*

Values are mean of three replicates ± SE, * statistically significant at $p<0.05$ level

Table - 4(a): Effect of tannery effluent (T.E.) and its combination with Zn, K and Fe on amylase (total, α and β), catalase and peroxidase activity, total protein and total sugar content in black gram

Treatments	Amylase (starch hydrolyzed mg g^{-1} fresh wt. of tissue)			Catalase (ml H_2O_2 hydrolysed g^{-1} fresh wt. of tissue)	Peroxidase (Δ O.D. g^{-1} fresh wt. of tissue)	Total protein (µg g^{-1} fresh wt. of tissue)	Total sugar (µg g^{-1} fresh wt. of tissue)
	Total	α	β				
Control	12.46 ± 1.00	8.06 ± 0.63	4.40 ± 0.40	120.00 ± 11.54	24.64 ± 1.66	87.93 ± 5.02	4.75 ± 0.28
10% T.E.	11.66 ± 0.33	6.83 ± 0.44	4.83 ± 0.16	153.33 ± 6.66*	41.38 ± 3.36*	133.48 ± 11.23*	4.13 ± 0.07
25% T.E.	8.46 ± 0.29*	6.33 ± 0.33	2.13 ± 0.13*	195.00 ± 8.66*	47.26 ± 4.76*	127.77 ± 8.43*	3.53 ± 0.12*
50% T.E.	6.30 ± 0.36*	4.03 ± 0.26*	2.26 ± 0.14*	240.00 ± 11.54*	32.56 ± 4.52	99.41 ± 8.37	2.57 ± 0.04*
100% T.E.	3.40 ± 0.30*	2.26 ± 0.37*	1.13 ± 0.13*	163.33 ± 8.81	11.90 ± 1.18*	51.48 ± 9.60*	1.92 ± 0.04*
50% T.E.+10 ppm $ZnSO_4$	10.00 ± 0.46	7.06 ± 0.58	2.93 ± 0.58	220.00 ± 11.54	57.88 ± 8.66*	92.68 ± 0.45	4.37 ± 0.30
50% T.E.+25 ppm $ZnSO_4$	9.50 ± 0.76	7.00 ± 0.86	2.49 ± 0.60*	170.00 ± 5.77	20.70 ± 3.73	106.54 ± 1.60*	5.07 ± 0.10
50% T.E.+10 ppm K_2SO_4	11.30 ± 0.75	8.83 ± 0.44	2.46 ± 0.32*	130.00 ± 5.77	57.60 ± 8.33*	72.48 ± 6.17	2.63 ± 0.07*
50% T.E.+25 ppm K_2SO_4	14.10 ± 0.63	10.10 ± 0.35*	4.00 ± 0.23	160.00 ± 11.54	19.60 ± 2.19	84.36 ± 0.68	4.20 ± 0.40
50% T.E.+10 ppm $FeSO_4$	9.18 ± 1.13	5.13 ± 0.44*	4.05 ± 0.68	230.00 ± 17.32*	23.33 ± 2.37	99.02 ± 7.77	3.27 ± 0.13*
50% T.E.+25 ppm $FeSO_4$	11.82 ± 1.05	7.43 ± 2.96	4.38 ± 0.87	210.00 ± 5.77*	17.16 ± 0.48*	104.96 ± 7.09	3.60 ± 0.34*

Values are mean of three replicates ± SE, * statistically significant at $p<0.05$ level, T.E. - Tannery effluent

Table - 4(b): Effect of chromium (Cr) and its combination with Zn, K and Fe on amylase (total, α and β), catalase and peroxidase activity, total protein and total sugar content in black gram

Treatments	Amylase (starch hydrolyzed mg g^{-1} fresh wt. of tissue)			Catalase (ml H_2O_2 hydrolysed g^{-1} fresh wt. of tissue)	Peroxidase (Δ O.D. g^{-1} fresh wt. of tissue)	Total protein (µg g^{-1} fresh wt. of tissue)	Total sugar (µg g^{-1} fresh wt. of tissue)
	Total	α	β				
Control	12.46 ± 1.00	8.06 ± 0.63	4.40 ± 0.40	120.00 ± 9.54	24.64 ± 1.66	87.93 ± 5.02	4.75 ± 0.28
0.5 ppm Cr	11.60 ± 0.60	7.50 ± 0.86	4.10 ± 1.05	100.00 ± 5.77	48.34 ± 3.77*	92.68 ± 2.74	5.05 ± 0.17
2 ppm Cr	10.03 ± 0.26*	4.86 ± 0.41*	5.16 ± 0.16	155.00 ± 8.66*	49.76 ± 3.74*	111.30 ± 5.71*	4.49 ± 0.34
5 ppm Cr	8.00 ± 0.49*	4.00 ± 0.20*	4.00 ± 0.33	195.00 ± 2.88*	32.74 ± 2.25*	90.70 ± 4.34	3.16 ± 0.09*
10 ppm Cr	4.85 ± 0.02*	2.90 ± 0.20*	1.95 ± 0.23*	232.00 ± 4.61*	25.20 ± 3.94	59.41 ± 2.28*	2.52 ± 0.14*
10 ppm Cr+10 ppm $ZnSO_4$	8.46 ± 1.24	7.00 ± 0.57	1.46 ± 0.66*	203.33 ± 8.81*	43.82 ± 2.18*	88.32 ± 3.43	3.01 ± 0.20*
10 ppm Cr+25 ppm $ZnSO_4$	11.03 ± 1.13	8.33 ± 0.31	2.70 ± 0.92	240.00 ± 9.54*	44.38 ± 1.85*	92.68 ± 2.74	4.32 ± 0.47
10 ppm Cr+10 ppm K_2SO_4	8.16 ± 0.44*	4.23 ± 0.14*	3.93 ± 0.34	210.00 ± 5.77*	39.82 ± 4.49*	110.90 ± 4.57*	4.38 ± 0.58
10 ppm Cr+25 ppm K_2SO_4	13.63 ± 0.74	9.40 ± 0.87	4.23 ± 0.18	243.33 ± 8.81*	17.82 ± 0.19*	139.02 ± 15.77*	5.08 ± 0.28
10 ppm Cr+10 ppm $FeSO_4$	10.23 ± 0.66	8.00 ± 0.23	2.23 ± 0.61*	230.33 ± 6.66*	35.96 ± 7.15*	174.27 ± 11.43*	3.65 ± 0.28*
10 ppm Cr+25 ppm $FeSO_4$	14.16 ± 0.32	10.33 ± 0.33*	3.83 ± 0.61	236.00 ± 5.77*	33.50 ± 5.39	181.40 ± 5.94*	4.80 ± 0.11

Values are mean of three replicates ± SE, * statistically significant at $p<0.05$ level

alone. The plumule length, radicle length and Number of lateral roots were significantly decreased with increase in concentration of effluent. In recovery treatments they all increased if compared to the 50% tannery effluent. The fresh weight and moisture content significantly decreased with increase in tannery effluent concentration. They both increased in recovery treatments in comparison to 50% tannery effluent.

Table 2b shows the effect of different concentrations of chromium on seed germination and seedling growth. In control, seed germination was 85% where as it increased to 90% in 0.5 ppm chromium and decreases from 2 ppm of chromium. In recovery treatments the germination % was increased except 10 ppm potassium and iron sulphate which is equal to 10 ppm chromium. Plumule length and radical length were found maximum in control seedlings whereas it significantly decreased as the chromium concentration increased to 10 ppm. The Number of lateral roots increased with 0.5 ppm chromium over control and equal to control in 2 ppm chromium treatment and found decreased in 5 ppm and 10 ppm chromium treatment over control. In recovery treatments Number of lateral roots increased at 25 ppm zinc, potassium and iron sulphate if compared with 10 ppm chromium. Fresh weight decreased significantly as the chromium concentration increased. The dry weights also decreased with increasing concentration of chromium except that the dry weight significantly increased in 0.5 ppm chromium. In recovery treatments fresh and dry weight increased if compared with 10 ppm chromium alone except fresh weight in 10 ppm potassium with combination of 10 ppm chromium. Moisture % was highest in control *i.e.* 95.72%. In all recovery treatments moisture % were decreased if compared with 10 ppm chromium alone but 25 ppm iron sulphate with combination showed increase in moisture %.

In petridish experiments the germination % was inhibited in tannery effluent and also in chromium treatments with the increase in their concentrations. The poor germination was observed due to lower intake of water and poor amylase activity. Seed germination can be defined as the resumption of growth of the embryo of the matured seed; it depends on the same environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present either inside the seed or in its vicinity. In tannery effluent the dissolved oxygen was found nil due to higher biological oxygen demand (BOD) and chemical oxygen demand (COD). Due to the richness of organic and inorganic elements in effluent, the rate of water intake inside the cell is reduced (Taiz and Zeiger, 2002). Barcelo *et al.* (1986) had observed decrease in water potential with little effect on relative water content (RWC) in leaves of broad bean plants subjected to chromium toxicity and attributed it to possible changes in cell wall elasticity.

The seedling growth and biomass were found to be decreased with increase in concentration of tannery effluent and chromium. The high level of nutrients and heavy metals in the effluent has been reported to inhibit the seed germination and seedling growth (Mishra and Bera, 1995). Treated tannery effluent was found to be toxic because shoot and root length was affected and abnormal structure was observed in the root system devoid of proper root development (Garg and Chandra, 1990; Pandit and Prasanna, 1999). Chromium exposure also resulted into complete loss of growth in lateral roots while lesser concentration started damaging root cap, stomata and cotyledonary hair seemed to be collapsed and plasma membrane appeared to be detached from the cell wall under cytological studies (Mariappan *et al*., 2001). The effect of hexavalent chromium in improper functioning of plasma membrane leading to plasmolysis, has been shown by Bassi *et al.* (1990), which also support the poor moisture content inside the cell at both seedling as well as mature plant stage. Previously in 1971 Turner and Rush had suggested that the excess chromium affects uptake of several nutrients, although the internal mechanism was not explored. All nutritive elements are actively involved in various metabolic pathways, so ultimately growth and yield are reduced.

Table 3a shows pigment analysis after treatment of various concentrations of tannery effluent. The pigments concentration were significantly decreased at higher concentration of tannery effluent while lower level (10% tannery effluent) increases these pigment concentration except chlorophyll 'b' and pheophytin 'b'. In case of recovery treatments total chlorophyll, chlorophyll 'a' and chlorophyll 'b' were increased if compared to 50% alone tannery effluent. Pheophytin (Total, a and b) and carotenoid were increase in all recovery treatments except 10 ppm potassium sulphate and iron sulphate in combination with 50% tannery effluent were decreased when compared with 50% tannery effluent.

Table 3b represents the pigment analysis of black gram seedlings after treatment with various concentrations of chromium. Chlorophyll (Total, 'a' and 'b') contents significantly increased up to 2 ppm of chromium and then gradually decreased up to 10 ppm chromium. All recovery treatments shows increase in chlorophyll (Total, 'a' and 'b') contents when compared with 10 ppm chromium alone. Pheophytin (Total and 'a') and carotenoids increased up to 5 ppm chromium and rapidly decreased in 10 ppm chromium. But pheophytin 'b' decreased with increase in chromium concentration. In recovery treatments all pheophytin contents were found higher if compared with 10 ppm chromium alone except 10 ppm potassium sulphate for (Total and 'a') pheophytin contents with combination of 10 ppm chromium. But in case of carotenoid contents it was increase in 25 ppm potassium sulphate if compared with its10 ppm concentration.

The decrease in dry weight may also be attributed to the lowered photosynthesis and chlorophyll 'a', and change in ultra structure of chloroplast due to excess of chromium (Vazquez *et al*., 1987). Generally the ratio of chlorophyll 'a' and chlorophyll 'b' was found to be enhanced with the increased doses of these heavy metals (Corradi, 1993). The chlorophyll 'b' is identical to chlorophyll. 'a' and the conversion of chlorophyll 'a' and 'b' has been shown to involve oxidation of methyl to aldehyde group (Ito *et al*., 1996). This oxidation is proposed to be carried out enzymatically by iron

enzyme chlorophyll 'a' oxygenase (Tanaka *et al*., 1998). The decrease in chlorophyll contents is due to chromium, competes for iron at functional site which might be interfering with the functional metal (Mg^{2+}) in the prophyrin ring (Mengel and Kirkby, 2001).

The effects of tannery effluent on biochemical parameter of black gram are shown in Table 4a. The amylase (total, α and β) contents significantly reduced as the concentration of tannery effluent increased except β amylase which increased in 10% tannery effluent if compared with control. Amylase activity (total, β and α) found increased when compared with 50% tannery effluent. In 10 ppm, amylase activity (total, α and β) were found higher than 25 ppm zinc sulphate combinatorial treatment. Catalase and peroxidase increased as the tannery effluent concentrations was increased. Catalase activity found decreased in all recovery treatments if compared with 50% tannery effluent. Peroxidase activity was also decreased in all recovery treatments except 10 ppm of zinc and potassium sulphate when used in combination with 50% tannery effluent when compared with 50% tannery effluent. Total protein contents significantly increased at 10% tannery effluent while its started decrease from 25% tannery effluent Total sugar content significantly decreased as the tannery effluent were increased. Both protein and sugar were increased in recovery treatments if compared with 50% tannery effluent alone.

Table 4b shows the effect of chromium on various biochemical parameter of black gram seedling. Amylase activity (total, β and α) significantly decreased at higher concentration of chromium. In recovery amylase activity was higher when 10 ppm chromium used with 25 ppm of zinc, potassium and iron sulphate when compared with its 10 ppm chromium alone treatment. Catalase decreased in 0.5 ppm chromium and significantly increased from 2 ppm chromium but peroxidase activity significantly increased in 0.5 ppm and 2 ppm chromium and then it was decreased. Catalase and peroxidase were found higher in recovery treatments if compared with 10 ppm chromium except in some recovery treatments. Total proteins were increased up to 5 ppm chromium and beyond it they decreased gradually. Total sugars were increased up to 0.5 ppm chromium and then decreased gradually with increased chromium. Total protein and sugars were increased in recovery treatments if compared with 10 ppm chromium alone.

The amylase activity was found to be significantly inhibited in higher doses of the treatments due to lower intake of water in seeds. The poor germination rate and seedling growth in treatments seem due to the poor break down of starch by amylase activity (Thevenot *et al*.,1992). Amylase acts as hydrolyzing enzymes, synthesizes its maximum during germination stage under the influence of signals transduced by gibberellins. Sugar accumulation may be due to the metal-induced alteration of the carbohydrate metabolism. An alternative explanation would be that metal reduces vein loading thus inhibiting photo assimilate export with a resultant carbohydrate accumulation. Reduced sugar synthesis in plants by chromium might be due to lower synthesis or diversion of metabolite to other synthesis processes. Antioxidant systems enzyme's, are activated and produced against heavy metal stress, damage of plasma membrane and generation of ROS, H_2O_2. Several cellular functions have also been described for plant catalase. It neutralizes H_2O_2 which is produced during photorespiration and acyl CoA oxidation of fatty acids (Yamaguchi and Aso, 1977). According to Panda and Patra (2000) chromium ions increased the catalase activity in younger leaves while the activity decreased in older ones. The increase in catalase activity might be due to the increase in toxic effect of H_2O_2 and ROS produced as a result of membrane damage at higher level of heavy metals. In chromium and tannery effluent treated plants the activity of another H_2O_2 scavenging enzyme *i.e.* peroxidase increased pronouncedly as has been observed earlier in several species (Tewari *et al*., 2002), which might be attributed to rapid diffusivity of H_2O_2 produced in the cytosol or might be due to accumulation of high phenols and low protein formation in such conditions. Catalase and peroxidase are the enzymes of oxido-reductase group and usually their level goes up during any type of stress that may be even heavy metal stress while extreme higher levels of heavy metal severly inhibit their activity.

In the recovery treatments the toxic effects of tannery effluent/ chromium were over come using zinc, potassium and iron. In case of potassium, 2 to 5% of dry weight of the vegetative parts is required for normal growth while zinc and iron is required at the rate of 15 - 20 mg kg^{-1} and 100 mg kg^{-1} dry weight respectively (Epstein, 1965). The application of zinc in combination with tannery effluent/chromium seems to reduce the toxicity of chromium leading to the increased growth. Many enzymes require zinc ions for their activity, and zinc may also be required for chlorophyll biosynthesis in plants, while zinc deficiency is associated with an important carbohydrate metabolism and protein synthesis (Taiz and Zeiger, 2002). Potassium used in the recovery experiment plays an important role in regulation in osmotic potential in plant cells (Bassi *et al.,* 1990). Potassium also activates many enzymes in respiration and photosynthesis. In protein synthesis K^+ is probably involved in several steps of the translation processes, including the binding of tRNA to ribosomes (Evans and Wildes, 1971). The high potassium concentrations in the sieve tubes are probably related to the mechanism of phloem loading of sucrose. Adverse effect of chromium was found to be nullified by the supply of suitable amount of iron and zinc in moong, gram and pea plants possibly due to importance of these two essential nutrients in growth and metabolism of plants (Vazquez *et al*., 1987). Iron has an also important role as a component of enzymes involved in the transfer of electron redox reaction), such as cytochromes. In this role, it is reversibly oxidized from Fe^{2+} to Fe^{3+} during electron transfer. The most well known heme proteins are the cytochromes, which contain a heme iron porphyrin complexes as a prosthetic group. Cytochromes are constituents of the redox system in chloroplasts and mitochondria and, in the form of cytochrome oxidase, participate in the terminal step of the respiratory chain. Other heme enzymes are catalase and peroxidase. Under condition of iron deficiency, the activity of both types of enzyme declines.

As per experimental observation 10% dilution levels of tannery effluent may be used for irrigation purpose without any

significant loss to the crop at proper intervals. In our experiments it has been clearly shown that lower levels of tannery effluent and chromium can be used for irrigation safely in combination with zinc, potassium and iron sulphate which also constitutes the part of essential element for plant growth and yield.

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