Germination and Seedling growth of Field Pea *Pisum sativum* Malviya Matar-15(HUDP-15) and Pusa Prabhat (DDR-23) under varying level of Copper and Chromium

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Abstratct: The heavy metal gradual impact on field pea (Pisum sativum) Var. Malviya Matar-15 (HUDP-15) and Pusa Prabhat (DDR-23) was assessed at germination, seedling growth and pigments concentration after having subjected it to different concentration of $CuSO_4$ and K_2CrO_4 .The germination percentage seedling growth and pigment concentrations were affected by elevating concentration, where Pusa Prabhat (DDR-23) is more sensitive to the elevated concentration of $CuSO_4$ and K_2CrO_4 solutions concentrations then Malviya Matar-15 . But the negative stress of the non essential Cr is more vigorous than essential Cu.This justifies that the field pea variety Pusa Prabhat is less suitable for the cultivation under situation where water and soil suffer from intermittent and momentary metal pollution like Copper and Chromium. [The Journal of American Science. 2008;4(1):33-47]. (ISSN: 1545-1003).

Keywords: Heavy Metals, Seed Germination, Chemical Treatments, Field Pea.

1. Introduction:

Heavy metals are the intrinsic component of the environment with essential and non essential both types. It is the unplanned municipal waste disposal, mining and use of extensive pesticides, other agro-chemicals uses are the significant cause of elevation in environment, its persistence is the cause of most concern. Copper is essential element required in trace amount to plants. Copper concentration increasing in the environment is the cause of disposal of copper containing waste water, sludge and combustion of fossil fuel. Its source may be mining, metal production, phosphate fertilizer production. It has been also incorporating by natural agencies like wind blown dust, decaying vegetation forest fire and sea spray. A very common practice sewage sludge amendment to agricultural soil about 1:20 ratio can expose >100 ppm of Cu to the plants, Singh, R.P.et.al. (2007). Since Cu does not break down in the environment and is continually accumulated by plants and animals thus Cu can influence plant diversity depending on acidity of soil and presence of organic matter, Anonymous b (2004). Cu is essential plant nutrients in trace in excess it is causing stunted growth, cholorsis and root malformation. Cook et.al. (1997) Foy et.al. (1978), Imaculada, Y. (2005).

Chromium is a non-essential and toxic element to plants; Chromium is found in all part of the environment, including air, water and soil naturally occurring in soil, normal range of Cr is from 10 to 50 mg/ kg depending on the parental material. In ultramafic soils (serpentine), it can reach up to 125 mg/ kg,

Adriano (1986). The leather industry is the major cause for the high invasion of Cr to the biosphere, accounting for 40% of the total industrial use Barnhart (1997). In India, about 2000–32,000 tons of elemental Cr yearly escapes into the environment from tanning industries. Average 1:20 ratio of soil and sewage sludge blending can expose >20 ppm of cr to the plants Singh,R.P et.al. (2007). Since seed germination is the first physiological process affected by Cr, the capability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal Peralta et al., (2001). High levels (500ppm) of hexavalent Cr in soil reduced germination up to 48% in the bush bean Phaseolus vulgaris Parr and Taylor,(1982). Peralta et al. (2001). The maximum quantity of element contaminant was always contained in roots and a minimum in the vegetative and reproductive organs. In bean, only 0.1% of the Cr accumulated was found in the seeds as against 98% in the roots Huffman and Allaway (1973). Decrease in root growth is a well-documented effect due to heavy metals in trees and crops Breckle, (1991); Goldboldand Kettner, (1991); Tang et al., (2001) Prasad et al.(2001) reported that the order of metal toxicity to new root primordia in Salix viminalis is Cd>Cr>Pb, whereas root length was more affected by Cr than by other heavy metals studied. Adverse effects of Cr on plant height and shoot growth have been reported (Rout et al., 1997), Shankar Arun,et. al. (2005).Barcelo,et.al.(1986) studied the chlorophyll-a,chlorophyll-b,total carotenoids and trace elements of initial and first trifoliate leaves of Phoseolus vulgaris. Plants grown with or without Cr(IV) in the nutrient solutions showed negative linear relation between chlorophyll and carotinoid contents due to chromium. High correlation coefficient was also obtained between pigment contents and Iron and Zinc contents. Chromium induced inhibition of Fe and Zn transport was considered to cause chlorosis in plants. Rai et.al.(1990) studied the effect of Cr,Pb,Ni, & Ag on growth, pigments DNA,RNA,heterocysts,frequency uptake of NH_4^+ and NO_3^- nitrate reductase and glutamine synthetase activities of Nostic muscorum and revealed a direct positive correlation between molal concentration and inhibition of different processes. The inhibition of pigments was recorded in the order as Chll.<Phycocyanin<Carotinoid. No generalized trend for inhibition of macromolecules was observed. Field peas are the most consumable pulses crops, Malviya Matar-15 and Pusa Prabhat DDR-23 are the recently developed high yielding varieties. The required attribute for plants are metal uptake avoidance and accumulation without toxic symptoms development, these are the symptoms of tolerant plants, meanwhile the sensitive plant shows more stress symptoms, To determine tolerance capabilities, the plants were tested by germination test and seedling growth, chlorophyll content these are the key issues for sustenance of plants in any pre-existing conditions. The present work is to assess the effect of increasing concentration of heavy metals like Cu and Cr their possible gradual impact on physiology, biochemistry and heavy metal tolerance of the plants and also to find out the correlation between elevated concentration of heavy metals to pigments concentration and intra pigments dependencies on different concentration of heavy metals.

2. Materials and Methods:

2.1 Study area

The experiment was conducted at Deptt. of Evironmental Science, P.G.College, Ghazipur a suburban area of district head quarter, located in the eastern Gangetic plain of the Indian sub continent at $25^0 19$\`and $25^0 54$\`N latitude, $83^0 4$\`and $83^0 58$\`E longitude and 67.50 m above the sea level. The experiment was carried out between Sept.-Oct 2005. This period of the year is characterized by mean monthly maximum temperatures between 34.8 and 36.1 ^{0}C and mean monthly minimum temperatures between 16 and 23.5 ^{0}C. Maximum relative humidity varied from 95% to 100% and minimum from 69% to 71%.

2.2 Selection of Seed

Seed of Field Pea Malviya Matar-15 (HUDP-15) and Pusa Prabhat (DDR-23) was chosen for the experimental work has developed by B.H.U. Varanasi and Indian Agricultural Research Institute (IARI); New Delhi, both are resistant to PM, adoptive to the site of the study (Eastern U.P.) India. And a recently developed and exceedingly espouse high yield variety respectively.

2.3 Experimental Design and Set up

Forty seeds of uniform size for each variety were selected, the seeds were surface sterilized in 5% sodium hypochlorite solution to remove the microbial contamination then seed were thoroughly washed with di-ionized water. Water soluble salts of copper ($CuSo_4$) and Chromium were (K_2CrO_4) were taken for making the solutions by the A.R. Grade reagents first stock solution for 1000 ppm were made and desirable 25,50 and 100 ppm obtained by diluting them by distilled water. Seeds were spread on sterlised petri dishes lined with filter paper whatman No.-1 . The seed were irrigated with equal volume (20ml.) of different concentration of solutions for each treatment , the petri dishes were arranged in a completely randomized block design with three replicates and in each replicates 40 seeds .The experiment was carried out in a growth chamber at 25^0C , 12 hours dark and 12 hours light period with illumination of 2500 lux. Their germination were recorded on each day at fixed time and fixed intervals. The germination percentage was recorded in tabular form. After 15 days only 12 germinated seedlings of similar morphology and age groups of each variety were transferred in plastic trays with size 24\`\`×10\`\` size with soil and irrigated on alternate days with 250 ml of respective concentration of 25, 50 and 100 % of Cu and Cr solutions. Each treatment having three replicates and each replicates contains 12 seedlings. The seedlings were harvested after 15 days from the date of seedling transferred in Trays.

2.4 Estimation of Germination %, Root length, Shoot length and No. of lateral roots

Germination percentage was calculated by dividing the seed germinated on each day by total no. of seed taken × 100. And finally adding the total percentage. Root, and shoot length measured by normal scale.

2.5 Estimation of Chlorophyll and Carotinoids

The chlorophyll and carotinoids contents of primary leaves were estimated with 80% acetone with help of spectrophotometer (Hitachi, Ltd. Tokyo) Jayaraman,J.(1981).

Chlorophyll a = (.0127) (OD663)-(.00269) (OD 645) gm/lit.

Chlorophyll b = (.0229) (OD 645) – (.00488) (OD 638)gm/lit.

Carotenoids = (OD 490)-(.114) (OD663) – (.638) (OD 645) gm/lit.

3. Results:

Table 1 (a): Effect of Copper and Chromium on percentage germination of Malviya Matar-15. (Mean±Stand.Error)

S.No.	Treatment	Concentration	% Germination	% Reduction in germination
1.	Control	------	$100\pm.00^{a}$	------
2.	$CuSO_4$	25 50 100	88 ± 3.0^{b} 80 ± 1.1^{c} 70 ± 1.5^{d}	12 20 30
3.	K_2CrO_4	25 50 100	83 ± 1.5^{b} 75 ± 1.5^{c} 66 ± 1.8^{d}	17 25 44

Different letters in each group shows significant difference at P<0.05 levels.

Table 1 (b): Effect of Copper and Chromium on percentage germination of Pusa Prabhat (DDR-23). (Mean±Stand.Error).

S.No.	Treatment	Concentration	% Germination	% Reduction in germination
1.	Control	------	$100\pm.00^{a}$	------
2.	$CuSO_4$	25 50 100	85 ± 2.5^{b} 78 ± 2.5^{b} 67 ± 2.5^{c}	15 22 33
3.	K_2CrO_4	25 50 100	83 ± 3.6^{b} 72 ± 1.5^{c} 63 ± 3.0^{d}	17 28 37

Different letters in each group shows significant difference at P<0.05 levels.

Table-2(a): Effect of Copper and Chromium on Root length, Shoot length and Number of lateral roots of Malviya Matar-15.(Mean±Stand.Error)

S.No.	Treatment	Concentration%	Root length(Cm.)	Shoot length(Cm.)	No. of lateral roots
1.	Control	------	$6.5\pm.40^{a}$	$9.9\pm.26^{a}$	$9.2\pm.10^{a}$
2.	$CuSO_4$	25 50 100	$5.4\pm.01^{b}$ $4.6\pm.20^{bc}$ $3.2\pm.15^{d}$	$6.2\pm.20^{b}$ $5.6\pm.35^{b}$ $3.8\pm.11^{c}$	$7.3\pm.40^{b}$ $6.2\pm.15^{c}$ $4.2\pm.15^{d}$
3.	K_2CrO_4	25 50 100	$2.9\pm.45^{b}$ $2.2\pm.15^{bc}$ $1.3\pm.15^{c}$	$3.6\pm.26^{b}$ $2.9\pm.26^{bc}$ $1.6\pm.30^{c}$	$7\pm.40^{b}$ $5\pm.20^{c}$ $3.2\pm.20^{d}$

Different letters in each group shows significant difference at P<0.05 levels.

Table-2(b): Effect of Copper and Chromium on Root length, Shoot length and Number of lateral roots of Pusa Prabha(DDR-23).(Mean±Stand.Error).

S.No.	Treatment	Concentration	Root length(Cm.)	Shoot length(Cm.)	No. of lateral roots
1.	Control	------	6.8±.20[a]	9.1±005[a]	8.7±.11[a]
2.	$CuSO_4$	25 50 100	5.0±.75[b] 4.2±.15[bc] 2.9±.23[c]	6.0±.30[b] 5.2±.15[c] 3.7±.15[d]	7.0±.30[b] 5.9±.005[c] 3.9±.008[d]
3.	K_2CrO_4	25 50 100	2.5±.005[b] 1.8.±.15[c] 1.1±.1[d]	3.3±.01[b] 2.0±.25[c] 1.4±.26[c]	6.6±.20[b] 4.7±.11[c] 2.9±.26[d]

Different letters in each group shows significant difference at P<0.05 levels.

Table-3(a): Effect of Copper and Chromium on pigment content of Malviya Mater-15. (Mean±Stand.Error)

S.No.	Treatment	Concentration	Cholophyll'a'(g/lit.)	Chlorophyll 'b'(g/lit.)	Carotenoids (g/lit.)
1.	Control	------	.0042±.00002[a]	.0056±.00003[a]	.345±.00005[a]
2.	$CuSO_4$	25 50 100	.0036±.000005[a] .0029±.00002[b] .0021±.00002[c]	.0052±.000015[a] .0049±.00002[b] .0038±.000015[c]	.221±.00026[b] .168±.00015[c] .0978±.000046[d]
3.	K_2CrO_4	25 50 100	.0028±.000015[b] .0019±.000057[c] .0012±.000015[d]	.0050±.000050[a] .0037±.000020[b] .0025±.000015[c]	.208±.000025[b] .144±.00040[c] .0975±.000028[d]

Different letters in each group shows significant difference at P<0.05 levels.

Table-3(b): Effect of Copper and Chromium on pigment content of Pusa Prabhat (DDR-23). (Mean±Stand.Error).

S.No.	Treatment	Concentration	Cholophyll'a'(g/lit.)	Chlorophyll 'b'(g/lit.)	Carotenoids (g/lit.)
1.	Control	------	.0040±.000025[a]	.0054±.000016[a]	.339±.00011[a]
2.	$CuSO_4$	25 50 100	.0031±.000025[b] .0024±.000026[bc] .0019±.000015[c]	.0047±.000015[b] .0040±.00002[b] .0036±.000013[c]	.218±.00011[b] .162±.00011[c] .0973±.000017[d]
3.	K_2CrO_4	25 50 100	.0022±.000030[b] .0020±.000015[b] .0011±.0000[c]	.0050±.000026[a] .0031±.00001[b] .0022±.000015[c]	.200±.0000[c] .140±.00028[c] .0965±.00010[d]

Different letters in each group shows significant difference at P<0.05 levels.

Percentage germination of field pea (Malviya Matar-15) and Pusa Prabhat (DDR-23) has affected by the copper and chromium solutions. The rate of germination have highly retrograde in presence of higher concentration of solution than preceding lower concentration and control as mentioned in Table No. 1(a)and (b).

Root and Shoot length , No. of lateral roots and pigments of 15 days old seedlings at different concentrations of Cu and Cr are presented in Table No. 2(a),(b) and 3 (a),(b) respectively. The highest value of Root length, Shoot length and No. of lateral roots and amount of pigments reported in control condition for both species and treatments and least values were reported in case of 100 PPM concentration. There was considerable difference in root length shoot length, no. of lateral roots for both species treatments with Copper sulphate and Potassium chromate , the extent of decline was significantly greater in case of Pusa Prabhat than Malviya Matar-15. The root length was significantly influenced by varying concentration of copper sulphate and vigorously affected by potassium chromate solution. The comparative negative effect was higher in case of Pusa Prabhat than Malviya Matar-15. The two different metal treatments had a significant effect on shoot development. The shortest shoot length was observed at highest concentration 100 PPM of potassium chromate in Pussa Prabhat. However comparatively longer shoot length in case of Malviya Matar-15 reported in both chemical treatments as mentioned in Table No.-2. Negative effect of chromate solution is more on both crops then copper sulphtae solution. Percentage germination and number of lateral roots have reduced with each elevation in concentration in all the treatments. Effect of metal treatment on pigments had been significantly seen as the copper sulphate solutions decreases in the pigment formation like Chlorophyll a, Chlorophyll b and formation of carotenoids. While increase the concentration potassium chromate causes a more adverse effect on the vegetations. As lesser formation of chlorophyll a, chlorophyll b and carotinoids. ANOVA analysis indicates the $P<.05$ level of significance in all the treatment cases compare to control.

Table:4a. Co-relationship between varying level of copper concentration and pigments concentration in leaf of Malviya Matar-15.

Correlations

		CHLOROPH	CHLB	CARATINO
CHLOROPH	Pearson Correlation	1.000	.780**	.921**
	Sig. (2-tailed)	.	.003	.000
	N	12	12	12
CHLB	Pearson Correlation	.780**	1.000	.848**
	Sig. (2-tailed)	.003	.	.000
	N	12	12	12
CARATINO	Pearson Correlation	.921**	.848**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

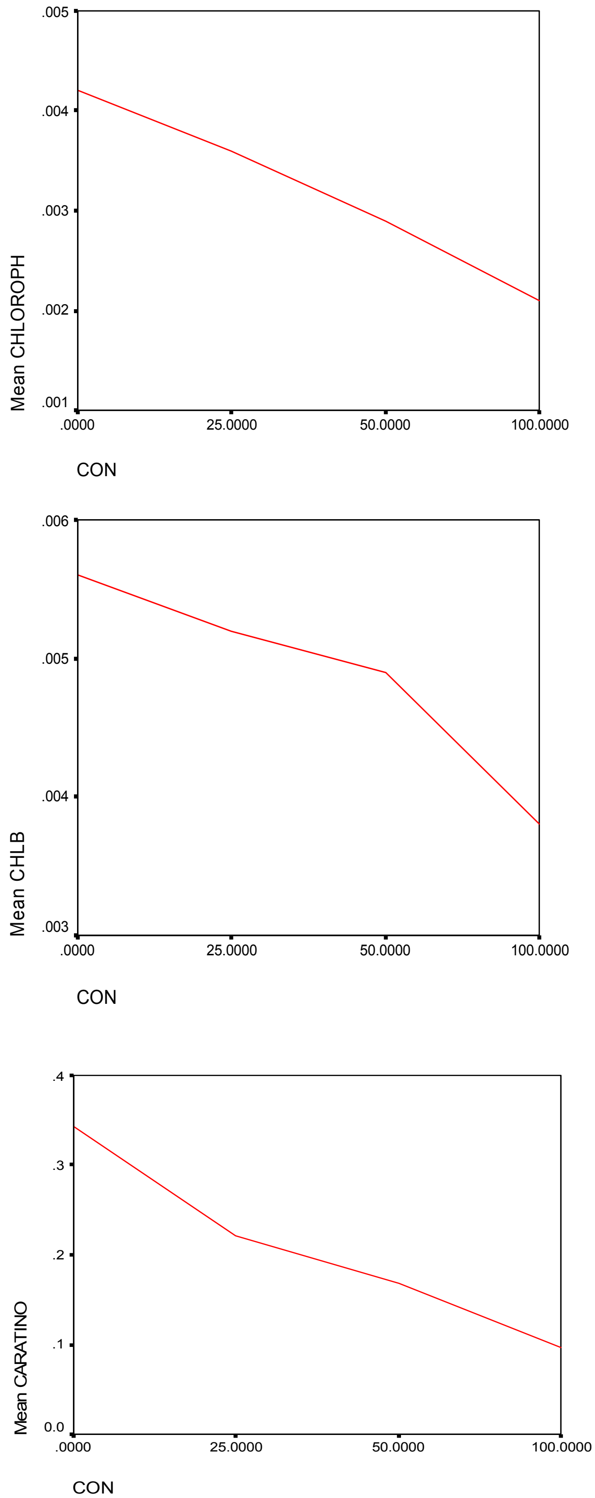
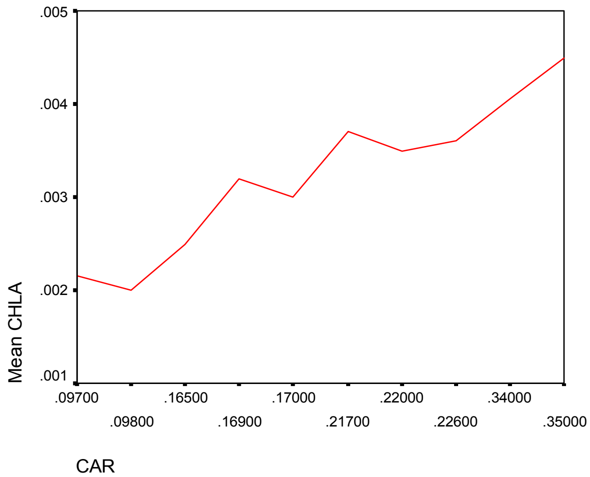
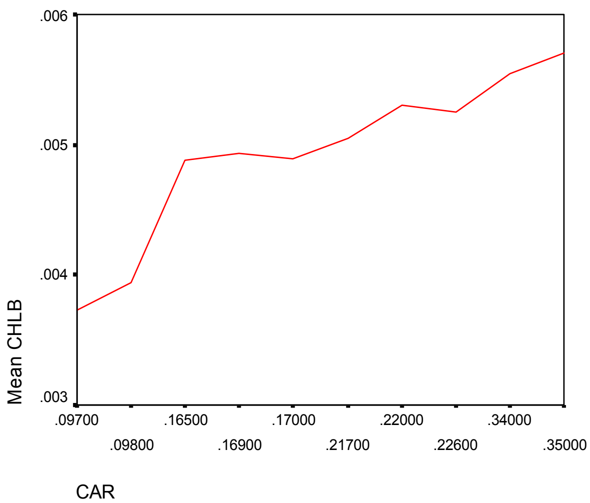


Fig.:1a. Relationship between varying level of copper concentration and pigments concentration in leaf of Malviya Matar-15.





.Fig.:1b. Rerelationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Malviya Matar-15.

Table: 4b.Co-relationship between varying level of copper concentration and pigmentsconcentration in leaf of Pusa Prabhat.

Correlations

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.913**	.918**
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
CHLB	Pearson Correlation	.913**	1.000	.929**
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
CAR	Pearson Correlation	.918**	.929**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

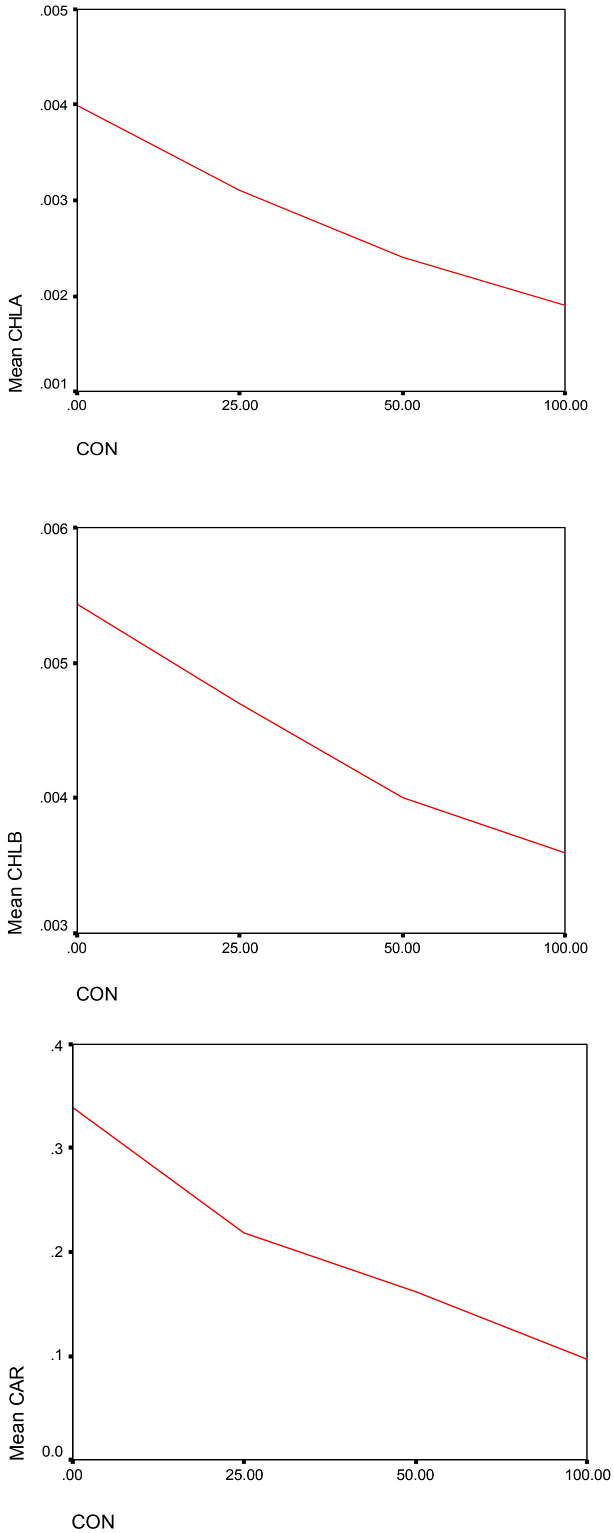
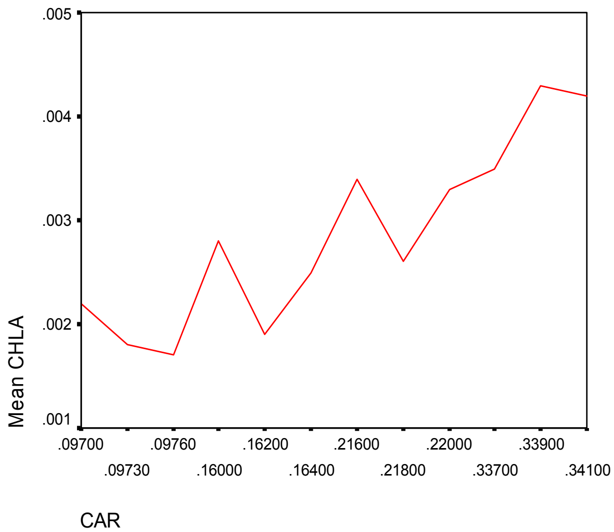


Fig.:2a. Relationship between varying level of copper concentration and pigments concentration in leaf of Pusa Prabhat.



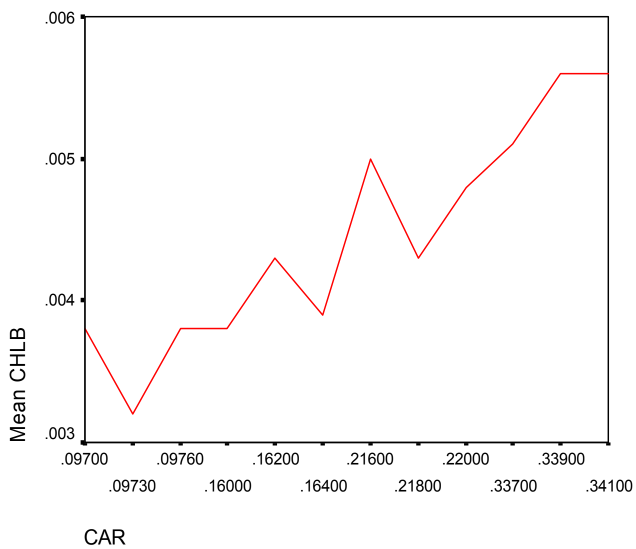


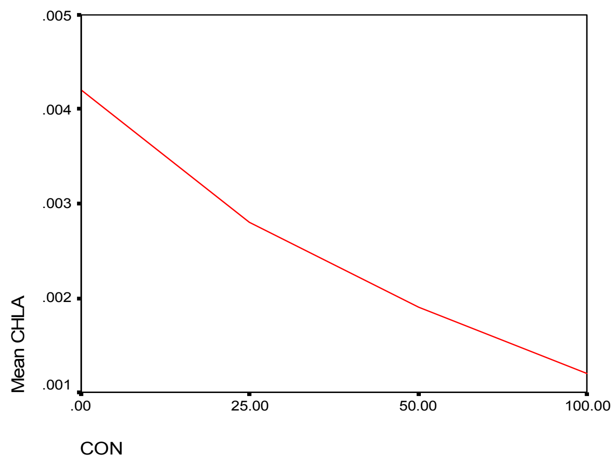
Fig.:2b. Rerelationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Pusa Prabhat.

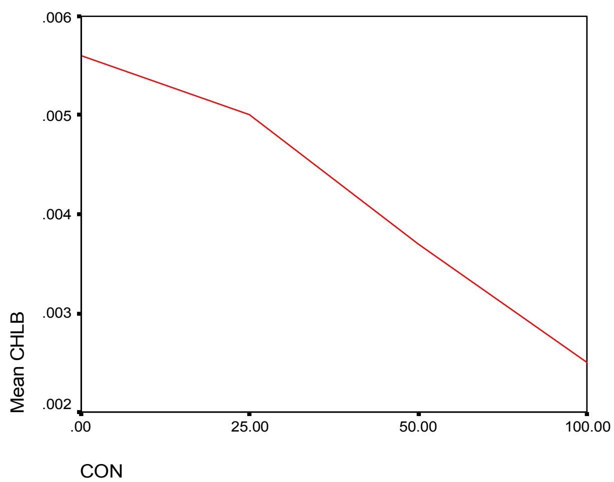
Table:4c. Co-relationship between varying level of Chromium concentration and pigments concentration in leaf of Malviya Matar-15.

Correlations

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.875**	.977**
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
CHLB	Pearson Correlation	.875**	1.000	.889**
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
CAR	Pearson Correlation	.977**	.889**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).





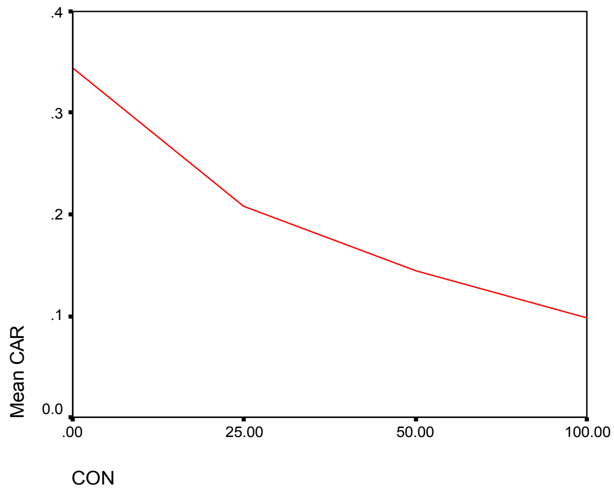


Fig.:3a. Relationship between varying level of Chromium concentration and pigments concentration in leaf of Malviya Matar-15.

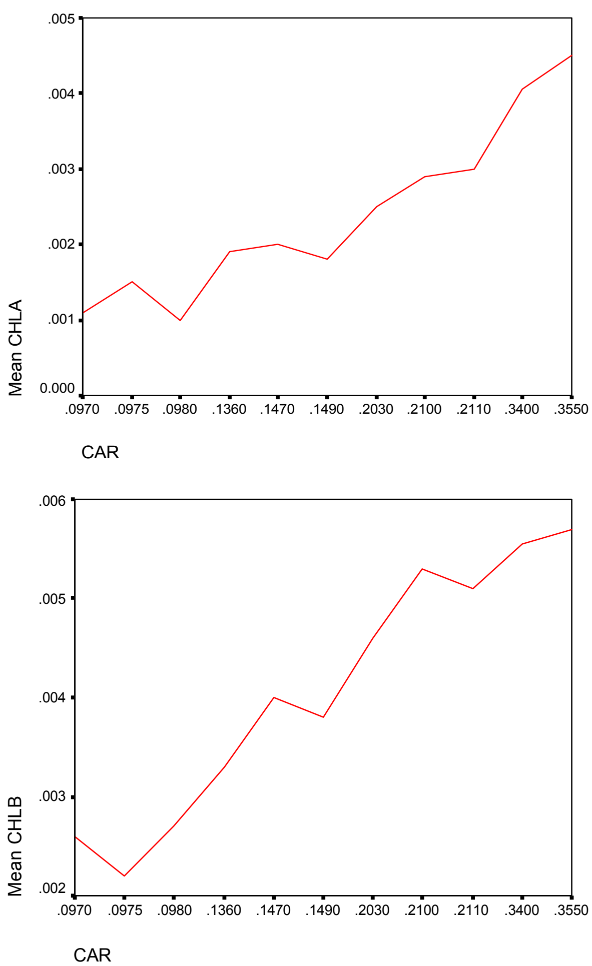


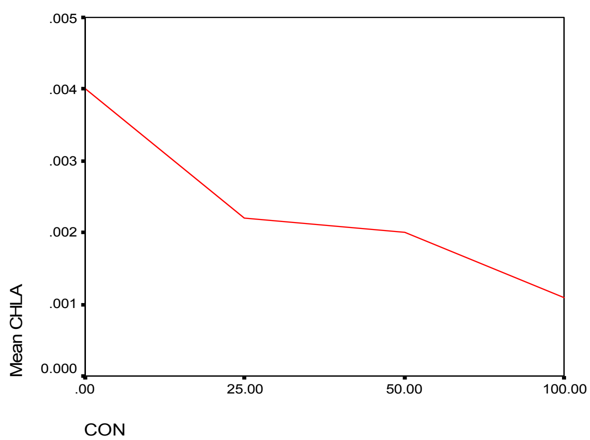
Fig.:3b. Rerelationship between varying level of carotinoids concentration with chlorophyll ‘a’ and ‘b’ concentration in leaf of Malviya Matar-15.

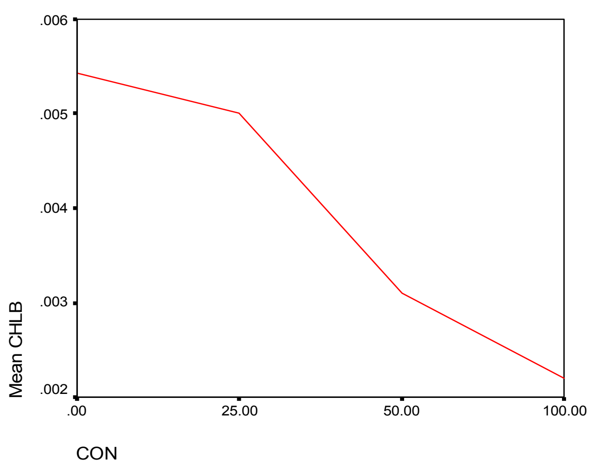
Table:4d. Co-relationship between varying level of Chromium concentration and pigments concentration in leaf of Pusa Prabhat.

Correlations

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.787**	.948**
	Sig. (2-tailed)	.	.002	.000
	N	12	12	12
CHLB	Pearson Correlation	.787**	1.000	.882**
	Sig. (2-tailed)	.002	.	.000
	N	12	12	12
CAR	Pearson Correlation	.948**	.882**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).





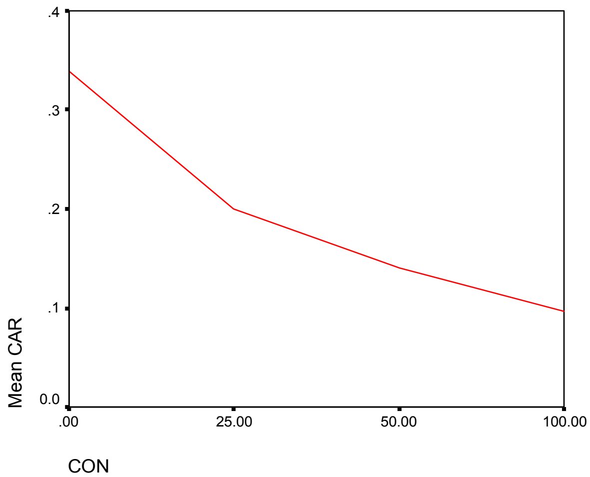
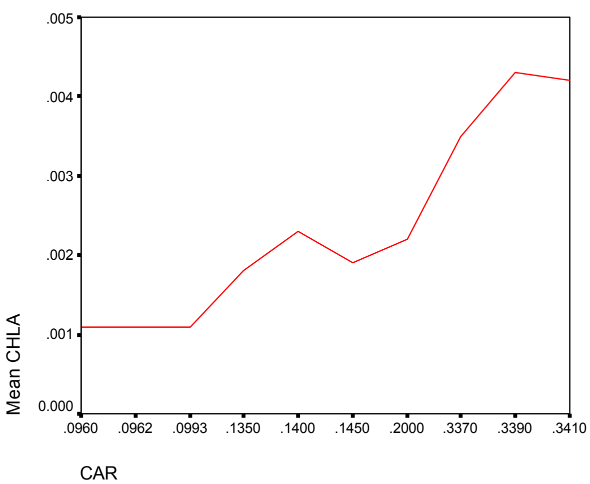


Fig.:4a. Relationship between varying level of Chromium concentration and pigments concentration in leaf of Pusa Prabhat.



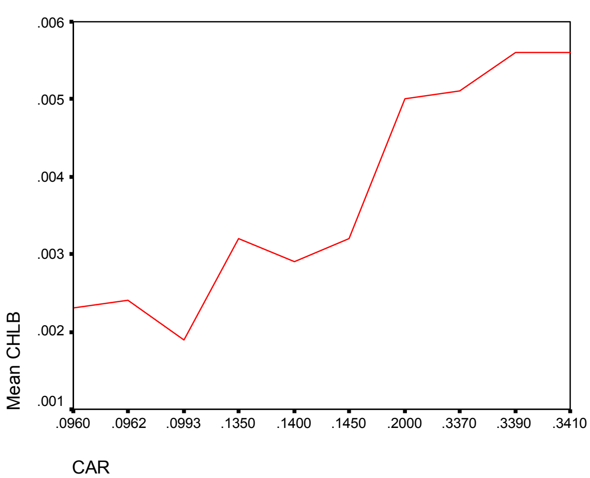


Fig.:4b. Relationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Pusa Prabhat.

Correlation studies shows that the increase in concentration of solution is inversely related to the concentration of the all pigments while the correlations between carotinoids and chlorophyll 'a' and 'b' is positive as increase in concentration of chlorophyll increase in concentration of carotinoids.

4. Discussion and Conclusions:

The present result showed that the germination of both varieties was significantly influenced by both chemical treatments. The results clearly indicates that increasing concentration of these two metals retrogrades the germination with its percentage increase. The result was consistent with Rout et. al. (2000). Jain et. al.(2000) Peralta (2001). The reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and on subsequent transport of sugars to the embryo axes Zeid (2001).Heavy metal causes a significant adverse effect on seedling growth and pigment formation. The study showed reduction in root length, shoot length, no. of lateral roots and pigment formation. The effect of negative stress is max.in case of Cr then Cu. This could be due to the inhibition of the root cell division/root elongation or to extension of the cell cycle in roots Barcelo et. al. (1986).Result similar to Cu toxicity on root growth has been reported by Sheldon,A. and Neal W. Menzies (2004). Shoot growth reduction through the toxicity of Cr has been reported by Rout et. al. (1997). Barton (2000). The reduction in shoot growth is due to stunted root growth results lesser nutrients and water supplied to aerial parts, where Cr and Cu causes direct impact on the cellular metabolism causes shortning of shoot height. Hanus and Tomas(1993).At elevated level of Cu treatment of seedlings the Chl a, Chl b and carotinoids content decreases supported by Miller (1938), Patsikka (2002). Where author suggested that reduced chlorophyll contents observed in the plants grown in presence of very high copper content due to iron deficiency. Meanwhile the elevated level of chromium shows decreased level of Chl a,Chl b and carotinoids as supported by Ganesh Shankar,K. et. al. (2006).Where author have suggested that morphological germination study parameters were decreased with respect to increase of chromium concentrations. In concluding remark we can say that both metal treatments are harmful for the plant germination and seedling growth behaviors at elevated concentration level from its optimum. Where Cr is comparatively more toxic than Cu. The Pusa Prabhat is more sensitive to the metal pollution like Cr and Cu pollution than Malviya Matar-15.

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