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Original Research Article

Effect of Heavy Metals (Ni, Cr, Cd, Pb and Zn) on Nitrogen Content, Chlorophyll, Leghaemoglobin And Seed Yield In Chickpea Plants in Aligarh City, U.P., India

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A B S T R A C T

Keywords

Chickpea, Heavy Metals, Yield

The present research had been conducted to study nitrogen content, chlorophyll, leghaemoglobin and seed yield in chickpea plants. All the plants of chickpea were grown in three pots while were as at and for each treatment there were 3 replicates were removed 60, 90 and 135 DAS after seeding of chickpea respectively. Chromium was found to be the least phytotoxic and significantly at ($P \leq 0.05$ and 0.01) among all the single metal treatments increased the percentage of nitrogen of root and shoot and chlorophyll, leghaemoglobin and seed yield at all the three concentration among the all dose. Among all the combination of metal treatments Ni+Cr+Cd+Pb+Zn was found to be the most phytotoxic and significantly ($P \leq 0.05$ and 0.01) reduced the percent of nitrogen content, chlorophyll, leghaemoglobin and seed yield in of chickpea. Plant materials were analyzed for different parameters along with the determination of heavy metal accumulation by the collected samples of *Cicer arietinum* L. The data revealed that after 60, 90 and 135 DAS maximum nitrogen content, maximum chlorophyll, leghaemoglobin and seed yield was recorded by chickpea as compare to control.

Introduction

Heavy metals are potentially toxic for plants: phyto-toxicity results in chlorosis, weak plant growth, yield depression, and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and, in leguminous plants, a reduced ability to fixate molecular nitrogen. Soil pollution with heavy metals will lead to losses in agricultural yield and hazardous health effects as they enter into the food chain (Schickler and Caspi, 1999). In heavy-metal polluted soils, plant growth can be inhibited by metal absorption. However, some plant species are able to accumulate fairly large amounts of heavy metals without showing stress, which represents a potential risk for animals and humans (Oliver, 1997). Heavy metal uptake by crops growing in contaminated soil is a potential hazard to human health because of transmission in the food chain (Fries *et al.*, 2006). There is also concern with regard to heavy metal transmission through natural ecosystems (Walker *et al.*, 2003). Heavy metals contamination attracted great interest of many scientists (Lasat, 2002) because they are one of the most important environments pollutants. In recent years, many low cost sorbets such as algae, fungi, bacteria & plants have investigated for their biosorption capacity towards heavy metals (Chehregani & Malayeri, 2007). Pulses are the most important source of nutrient of vegetarian in India as well other developing countries, wherever increasing population menace made their availability (35 gm per head per day) much lower than recommended (104 gm per head per day by WHO) World Health Organization. Moreover pulses are endowed with capacity to fix atmosphere nitrogen and improve soil fertility. Among the various crops legume for example, chickpea (*Cicer arietinum* L.) which serves as a rich source of protein, in the Indian dietary system, are popularly grown in India, and also in the region of Aligarh. Among these legumes, chickpea is the leading pulse crop occupying about 92% of the area and accounting 89% of the total production of grain legumes in semi-arid tropical countries (Ahlawal, 2002). Chickpea is also called as Bengal gram, is an important source of human food and animal feed and plays a key role in the maintenance of soil fertility in the wheat based systems of the dry rain fed areas of Indian sub-continent, West Asia and North African regions.

Materials and Methods

Biomass production and symbiotic development

All the plants of chickpea were grown in three pots while were as at and for each treatment there were 3 replicates were removed 60, 90 and 135 DAS after seeding of chickpea respectively. The plants were used for destructive plant analysis to record the extent of nodulation. The roots were carefully washed and nodules produced on the roots systems of each legume were detached, counted oven dried at 80^{0}C and weight. Plant growth, such as length of roots and shoots dry weight of roots and shoots and total dry plant biomass of all the two legumes was recorded at each sampling dates. Plant at all the uprooted sampling intervals were oven dried at 80^{0}C to measure the total plant biomass. The leghaemglobin content in fresh nodules recovered from the root systems of each legume crop raised under metal stress and metal free conditions (control) was quantified for chickpea, by the method of Sadasivam & Manickam, (1992). Fresh nodules were macerated with the help of mortar and pestle in 5 ml sodium phosphate buffer (pH 7.4) (Appendix 26) and filtered through two layers of chesse cloth. The nodule debris was discarded. The turbid reddish brown filtrate was clarified by centrifugation at 10,000rpm for 30 min. The supernatant was diluted to 10 ml with sodium phosphate buffer (pH 7.4). The extract was divided equally into two glass tubes (5ml/tube) and equal amount of alkaline pyridine reagent (Appendix 27) was added to each tube. The hexamochrome formed was read at 556 and 539nm after adding a few crystals of potassium hexacynoferrate and sodium dithionite, respectively. The leghaemoglobin formed was read at 556nm and 539nm after adding a few crystals of potassium hexacynoferrate and sodium dithionite respectively. The leghaemoglobin content was calculated using the formula-

Leghaemoglobin content (mM) = A_{556}-A_{539}×2D/23.4

Where D is the initial dilution

The remaining three pots of each treatment were maintained until harvest i.e. 135 DAS for chickpea.

Total chlorophyll and nitrogen contents

The total chlorophyll contents in fresh foliage of each experimental legume crop was quantified (chickpea) by the method of Arnon, (1949). Briefly 40 ml of 80% acetone with the help of mortar and pestle. The suspension was decanted in bunchner funnel having Whatman filter paper No. 1. The residue was ground three times with 30, 20 and 10 ml of acetone, respectively and suspension was filtered again. Contents in mortar pestle was washed with 80% acetone and filtered. The filtrate was transferred to 100 ml volumetric flask and volume was made upto 100ml. The absorbance was read at 645 and 663nm using spectronic 20D spectrophotometer. The total chlorophyll content was calculated by the formula:

Total chlorophyll= 20.2 (O.D. at 645nm) +8.02 (O.D. at 663nm) ×V/100×W

Where V= final volume of chlorophyll extract in 80% acetone and W= fresh weight of tissue extracted. The total nitrogen content of roots and shoots were measured at 60, 90 and 135 DAS (chickpea) by the micro-Kjeldahl method of Iswaran & Marwah, (1980). First, 50 ml of the sample was taken in the Kjeldahl flask, moistened with 5 ml water, containing 15 ml N/100ml H_2SO_4 and shaken thoroughly. This was followed by the addition of $NKMNO_4$ in small amount until pink colour appeared. The catalyst mixture (3g K_2SO_4, 0.3g $FeSO_4.5H_2O$ and 0.15g $CuSO_4.5H_2O$) was then added and sample was digested for 30 min. on low flame until the mixture became yellowish green.

Biomass production

All the test plants viz chickpea were harvested after 135 days of germination. Roots and shoots were dried at 80^0C for 18 h and then weighed separately. Data on nodulation were recorded 135 days after germination. The soil was gently washed from the roots and nodules were gently removed from the roots dried and weighed separately. On the basis of the current protocols on phytotoxicity tests (Purves, 1985), we evaluated that grade of growth inhibition (GGI) by the comparison of dry matter production of metal treated and control plant tissues.

GGI= {(C-T)/C} ×100

Where, C and T represent the dry weight of tissues of control (C) and treated plants (T)

Seed yield and grain protein

Chickpea was finally harvested at 135 DAS respectively, and seed yield was also measured.

Results and Discussion

Nitrogen content

The nitrogen content in root was significantly decreased in metal-treated plant of chickpea (Table 1). Percent nitrogen of root varied inversely with the amount of metals added, cadmium causing the greatest effect comparison to other single metals added. The percent nitrogen in root of chickpea was reduced at three concentrations 0.5x (6.62mg/kg soil) 1.0x (13.24mg/kg soil) and 2.0x (26.48mg/kg soil) at 60 days, (84.57, 91.80 and 94.16%), (76.22, 87.84 and 92%) at 90 days, (68.51, 81.45 and 91.09%) at 135 days respectively as compared to control (Table 1). The percent nitrogen in shoot of chickpea was reduced at three concentrations 0.5x (6.62mg/kg soil) 1.0x (13.24mg/kg soil) and 2.0x (26.48mg/kg soil) was at 60 days, (79.86, 89.65 and 93.99%), at 90 days

(73.36, 82.45 and 90.76%), at 135 days (67.45, 80.38 and 88.65%) respectively as compared to control (Table 1).

Chromium was found to be the least phytotoxic and significantly at ($P \leq 0.05$ and 0.01) among all the single metal treatments increased the percentage of nitrogen of root and shoot at all the three concentration among the all dose. A minimum reduction in root of chickpea at three concentrations 0.5x (15.13mg/kg soil), 1.0x (30.26mg/kg soil) and 2.0x (60.52mg/kg soil) after 60 days (38.94, 67.63 and 78.73%), after 90 days (26.80, 51.62 and 67.40%), and after 135 days (29.04, 49.46 and 64.88%) as compared to control (Table 1). Reduction in percent nitrogen of shoot varied inversely with the amount of metals added, chromium causing the least effect in comparison to other single metals added. Among all the single metal treatments chromium was found to be the least phytotoxic and reduced the percent nitrogen of shoot of the crop chickpea at 60 days was found to be (36.49, 60.40 and 73.17%), at 90 days 29.28, 48.25 and 63.88%, at 135 days 32.29, 47.42 and 64.02% in shoot as compared to control (Table 1). Reduction in percent nitrogen of root of plant of chickpea at zinc, at three concentrations 0.5x (110.02mg/kg) at 1.0x (220.04mg/kg soil) and at 2.0x (440.08mg/kg soil) after 60 days was found to be as (65.21, 85.42 and 89.03%), after 90 days as (49.86, 75.18 and 82.79%), after 135 days as (41.66, 67.71 and 80.26%) respectively as compared to control (Table 1). Reduction in percent nitrogen of shoot after 60 days was recorded as (48.79, 73.66 and 86.67%), after 90 days (40.04, 64.27 and 77.98%), and after 135 days (38.73, 60.25 and 71.91%) respectively in shoot as compared to control show in table (1).

Reduction percent of N of in the percent in the nickel inoculated plants in root of chickpea at 0.5x (81.38mg/kg soil), 1.0x (162.76mg/kg soil) 2.0x (325.52mg/kg soil) after 60 days was found to be (72.22, 90.48 and 93.89%), at 90 days (60.55, 84.55 and 92.66%) at 135 days (56.85, 76.67 and 86.49%) respectively as compared to control (Table 1). Reduction in percent nitrogen of shoot at 60 days was (67.22, 79.86 and 87.91%), at 90 days (59.65, 71.79 and 85.65%), at 135 days (53.96, 69.09 and 82.73%) respectively compared to control (Table 1). Due to lead at three concentrations 0.5x (52.85mg/kg soil), 1.0x (105.70mg/kg soil), and 2.0x (211.40mg/kg soil) reduction in nitrogen percent of root of plant of chickpea was recorded as at 60 days (44.58, 72.55 and 82.14%), at 90 days (32.05, 58.90 and 74.30%), at 135 days (33.06, 56.43 and 72.34%) respectively as compared to control (Table 1). Reduction in nitrogen percent of shoot after 60 days (40.70, 6171 and 73.85%), after 90 days (34.74, 46.87 and 68.74%) and after 135 days 35.51, 50.74 and 63.92% respectively in shoot as compared to control (Table 1).

In combination of metals Ni+Cd the reduction in percent nitrogen of root of plant of chickpea at concentration 0.5x (88mg/kg soil), 1.0x (176.00mg/kg soil) 2.0x (352mg/kg soil) was recorded after 60 days was recorded as (65.20, 84.77 and 89.62%), after 90 days (54.36, 76.49 and 87.56%), after 135 days (55.62, 70.27 and 82.25%) respectively as compared to control show in table (1). Reduction in percent nitrogen of shoot after 60 days was recorded as (66.91, 80.42 and 91.63%), at 90 days 59.26, 74.79 and 84.42%, at 135 days 55.41, 70.85 and 82.20% respectively as compared to control (Table 1). Among all the combination of metal treatments Ni+Cr was found to be the least phytotoxic and significantly ($P \leq 0.05$ and 0.01) percent of nitrogen in root and shoot all the three concentration among the all doses. In combination of metals Ni+Cr the reduction in percent of nitrogen plant root of chickpea at three concentrations 0.5x (96.51mg/kg soil) 1.0x (193.02mg/kg soil) 2.0x (386.04mg/kg soil) at 60 days was calculated as (72.62, 85.69 and 87.92%), at 90 days (54.90, 76.27 and 81.04%), at 135 days (49.43, 68.17 and 79.65%) respectively as compared to control (Table 1).

Reduction in percent nitrogen of shoot at 60 days was calculated as (67.84, 79.98 and 88.47%), at 90 days (54.54, 4.74 and 84.32%), at 135 days (50.15, 67.67 and 81.31%) respectively as compared to control table (1). Reduction in percent nitrogen of root and shoot was least with this combination in comparison to other combination of metals. In combination of Cr+Cd the reduction in percent of nitrogen in root of chickpea at concentration 0.5x (38.75 mg/kg soil) (21.75mg/kg soil), 1.0x (43.50mg/kg soil), 2.0x (87.00mg/kg soil) at 60 days was recorded as days (71.37, 85.36 and 89.23%), at 90 days (54.46, 75.23 and 87.18%), at 135 days (55.32, 71.54 and 83.86%) respectively as compared to control (Table 1). Reduction in percent nitrogen of shoot at 60 days was recorded as (67.41, 86.49 and 87.91%), at 90 days (54.93, 78.67 and 83.83%), at 135 days (55.94, 71.70 and 81.10%) respectively as compared to control (Table 1).

The nitrogen content of root and shoot of chickpea was reduced even further when cadmium was used with combination of nickel and chromium. In combination of Ni+Cr+Cd the reduction in of chickpea at concentration 0.5x (103.13mg/kg soil) 1.0x (206.26mg/kg soil), 2.0x (412.52mg/kg soil) was recorded at 60 days as (79.13, 87.79 and 93.76%), at 90 days (65.37, 81.10 and 90.46%), at 135 days (64.80, 74.98 and 86.88%) respectively as compared to control table (1). The percent of nitrogen of chickpea plant was reduced even further when cadmium was used with combination of nickel and chromium. Reduction in percent nitrogen of shoot at 60 days was recorded as (73.97, 86.86 and 93.30%), at 90 days (67.42, 80.49 and 88.99%), at 135 days (63.14, 77.38 and 85.02%) respectively as compared to control (Table 1).

Among all the combination of metal treatments Ni+Cr+Cd+Pb+Zn was found to be the most phytotoxic and significantly ($P \leq 0.05$ and 0.01) reduced the percent of nitrogen of chickpea at all the three concentrations among the all dose. When cadmium was applied along with nickel, chromium, lead and zinc it declined the nitrogen content root and shoot of the plant. In combination of Ni+Cr+Cd+Pb+Zn the reduction of percent of nitrogen of root at concentration 0.5x (266mg/kg soil), 1.0x (532mg/kg soil), 2.0x (1064mg/kg soil) at 60 days was recorded as (85.29, 91.07 and 94.94%), at 90 days (76.16, 87.01 and 92.66%), at 135 days (68.13, 83.28, 91.58%) as compared to control (Table 1).

Reduction in percent nitrogen of shoot at 60 days was found to be (73.97, 86.86 and 93.30%), at 90 days (67.42, 80.49 and 88.99%), at 135 days (66.86, 76.86 and 87.85%) respectively as compared to control (Table 1).

Chlorophyll content

The effect of single heavy metal and heavy metal mixtures on chlorophyll content declined with increasing concentration of metals. A gradual and significant reduction in the total chlorophyll content occurred in the plants treated with cadmium at three different concentrations at 0.5x (6.62mg/kg soil) 44.08%, at 1.0x (13.24mg/kg soil) 48.39% and at 2.0x (26.48mg/kg soil) 51.61% after 90 days as compared to control, (Table 2).

Table.1 Effect of various concentration of heavy metals added either or separately or in combination on Nitrogen content of chickpea inoculated but metal free control

Metals	**Dose rate ($mg\ kg^{-1}$ soil)**	**Nitrogen content ($mg\ g^{-1}$)**					
		Root			**Shoot**		
		60D	**90D**	**135D**	**60D**	**90D**	**135D**
Zn	**0.5x**	5.30	9.15	15.25	8.28	12.20	17.34
	1.0x	2.22	4.53	8.44	4.25	07.27	11.25
	2.0x	1.67	3.14	5.16	02.15	04.48	07.95
Ni	**0.5x**	4.23	7.20	11.28	05.29	08.21	13.03
	1.0x	1.45	2.82	6.10	03.25	05.74	08.75
	2.0x	0.93	1.34	3.53	01.95	02.92	04.89
Pb	**0.5x**	8.44	12.40	17.50	09.57	13.28	18.25
	1.0x	4.18	7.50	11.39	06.18	10.81	13.94
	2.0x	2.72	4.69	7.23	04.22	06.36	10.21
Cd	**0.5x**	2.35	4.34	8.23	03.25	05.42	09.21
	1.0x	1.25	2.22	4.85	01.67	03.57	05.55
	2.0x	0.89	1.46	2.33	00.97	01.88	03.21
Cr	**0.5x**	9.30	13.36	18.55	10.25	14.39	19.16
	1.0x	4.93	8.83	13.21	06.39	10.53	14.88
	2.0x	3.24	5.95	9.18	04.33	07.35	10.18
Ni+Cd	**0.5x**	5.30	8.33	11.60	05.34	08.29	12.62
	1.0x	2.32	4.29	7.77	03.16	05.13	08.25
	2.0x	1.58	2.27	4.64	01.35	03.17	05.04
Ni+Cr	**0.5x**	4.17	8.23	13.22	05.19	09.25	14.11
	1.0x	2.18	4.33	8.32	03.23	05.14	09.15
	2.0x	1.84	3.46	5.32	01.86	03.19	05.29
Cr+Cd	**0.5x**	4.36	8.31	11.68	05.26	09.17	12.47
	1.0x	2.23	4.52	7.44	02.18	04.34	08.01
	2.0x	1.64	2.34	4.22	01.95	03.29	05.35
Ni+Cr+Cd	**0.5x**	3.18	6.32	9.20	04.20	06.63	10.43
	1.0x	1.86	3.45	6.54	02.12	03.97	06.40
	2.0x	0.95	1.74	3.43	01.08	02.24	04.24
Ni+Cr+Cd+Pb+Zn	**0.5x**	2.24	4.35	8.33	03.35	06.25	09.38
	1.0x	1.36	2.37	4.37	02.35	03.75	06.55
	2.0x	0.77	1.34	2.20	01.06	02.12	03.44
	Control	15.23	18.25	26.14	16.14	20.35	28.30

	a	b	c	a×b	b×a	c×a	a×b×c	a	b	c	a×b	b×a	c×a	a×b×c
SE±m	.018	.011	.009	.036	.031	.019	.062	.046	.029	025	093	.081	.051	.162
CD at 5%	.116	.071	.060	2.11	.201	.127	.402	.129	.082	.071	.259	.224	.142	.449
CD at 1%	.070	.044	.038	.141	.122	.077	.245	.081	.054	.046	.159	.136	.089	.290
CV	1.198							2.712						

a= Metal, b= Concentration, c= Days, ab= Metal x Concentration, ba=Concentration x Metal, ca = Days x Metal, abc= Metal x Concentration x Days

Values are mean of three replicates where each replicate constituted three plants/pot

In this table, value 0.5x of metal concentration indicate half, 1.0x indicate normal and 2.0x indicate double values of metal, symbol indicates, Zn for zinc, Ni for nickel, Pb for lead, Cd for cadmium and Cr for chromium

Table.2 Effect of heavy metals on chlorophyll, leghaemoglobin and seed yield of chickpea inoculated but metal free control

Metal applied	Dose rate (mgkg^{-1} soil)	Chlorophyll content (mg g^{-1}) 90 DAS	Leghaemoglobin content {mmol (g f.m.$^{-1}$)} 90 DAS	Seed yield g plant^{-1}
Zn	**0.5x**	0.86	0.24	2.46
	1.0x	0.76	0.15	1.75
	2.0x	0.70	0.10	1.70
Ni	**0.5x**	0.73	0.16	2.09
	1.0x	0.62	0.10	1.80
	2.0x	0.60	0.06	1.34
Pb	**0.5x**	0.80	0.33	3.10
	1.0x	0.71	0.25	2.30
	2.0x	0.68	0.15	1.92
Cd	**0.5x**	0.52	0.10	1.09
	1.0x	0.48	0.06	0.89
	2.0x	0.45	0.03	0.74
Cr	**0.5x**	0.86	0.26	4.09
	1.0x	0.84	0.20	3.61
	2.0x	0.82	0.13	2.79
Ni+Cd	**0.5x**	0.41	0.09	1.13
	1.0x	0.39	0.06	0.83
	2.0x	0.37	0.04	0.70
Ni+Cr	**0.5x**	0.36	0.11	2.17
	1.0x	0.35	0.08	1.74
	2.0x	0.29	0.05	1.54
Cr+Cd	**0.5x**	0.55	0.08	1.45
	1.0x	0.51	0.06	1.20
	2.0x	0.46	0.04	0.48
Ni+Cr+Cd	**0.5x**	0.30	0.06	0.96
	1.0x	0.28	0.04	0.64
	2.0x	0.27	0.02	0.50
Ni+Cr+Cd+Pb+Zn	**0.5x**	0.29	0.05	0.96
	1.0x	0.26	0.03	0.64
	2.0x	0.23	0.01	0.33
	Control	0.93	0.41	7.23
SE±m		0.982	0.892	0.589
CD at 5%		0.277	0.252	0.166
CD at 1%		0.369	0.335	0.221
CV		3.148	12.96	5.829

Values are mean of three replicates where each replicate constituted three plants/pot
In this table, value 0.5x of metal concentration indicate half, 1.0x indicate normal and 2.0x indicate double values of metal, symbol indicates, Zn for zinc, Ni for nickel, Pb for lead, Cd for cadmium and Cr for chromium

Cadmium was comparatively more inhibitory than other single metal for chlorophyll. Among the single metal treatment cadmium showed a profound toxic effect on symbiosis and reduced the chlorophyll content in the leaves of chickpea plant. Chromium was found to be the least phytotoxic and significantly ($P \leq 0.05$ and 0.01) reduced the chlorophyll content among all the single metal treatments and decreased the chlorophyll at all the three concentrations among the all dose. In contrast the chlorophyll content increased significantly at concentration 0.5x, 1.0x and 2.0x concentrations of metal. The minimum reduction of chlorophyll at concentration 5.0x (15.13mg/kg soil) was recorded as after 90 days 7.52%, at 1.0x (30.26mg/kg soil) was 9.67% and at 2.0x (60.52mg/kg soil) was 11.83%, respectively over control.

Leghaemoglobin content

Cadmium was comparatively more inhibitory than other single metal treatment. A gradual and significant reduction in the leghaemoglobin content occurred in the plants treated with cadmium three concentration of metal, at 0.5x (6.62mg/kg soil) 75.61%, at 1.0x (13.24mg/kg soil) 85.36% and 2.0x (26.48mg/kg soil) 92.68% after 90 days in comparison to control (Table 2).

Chromium was found to be the least phytotoxic and significantly ($P \leq 0.05$ and 0.01) reduced the leghaemoglobin content among all the single metal treatments and decreased the leghaemoglobin at all the three concentrations among the all dose. In contrast the leghaemoglobin content increased significantly at 0.5x, 1.0x and 2.0x concentrations of metal. The minimum reduction of leghaemoglobin at concentration 5.0x (15.13mg/kg soil) was recorded as after 90 days 36.58%, at 1.0x (30.26mg/kg soil) was 51.22% and at 2.0x (60.52mg/kg soil) was 68.29%, respectively over control (Table 2).

Reduction in leghemoglobin of plant of chickpea at zinc, at concentration 0.5x (110.02mg/kg) was 41.46%, at 1.0x (220.04mg/kg soil), was 63.41%, and at 2.0x (440.08mg/kg soil) it was 75.61% in comparison to control (Table 2). Reduction of leghaemoglobin in nickel inoculated of plant of chickpea at concentration 0.5x (81.38mg/kg soil), was 60.97%, at 1.0x (162.76mg/kg soil) was 75.61% and at 2.0x (325.52mg/kg soil) was 85.36% in comparison to control (Table 2). Due to lead at three concentrations 0.5x, 1.0x and 2.0x leghaemoglobin reduced at increasing concentration. At 0.5x (52.85mg/kg soil) it was, 19.51%, at 1.0x (105.70mg/kg soil), it was 39.02% and at 2.0x (211.40mg/kg soil) it was 63.41% in comparison to control (Table 2). Among the combination of metals treatments Ni+Cd the reduction of leghaemoglobin in chickpea plant at concentration 0.5x (88mg/kg soil) was 78.05% at, 1.0x (176.00mg/kg soil) was 85.37% and at 2.0x (352mg/kg soil) was 90.24% in comparison to control (Table 2). Among all the combination of metal treatments Ni+Cr was found to be the least phytotoxic and significantly ($P \leq 0.05$ and 0.01) decreased nodules and the leghaemoglobin of chickpea plant at all the three concentrations among the all doses. In combination of metals Ni+Cr the reduction in leghaemoglobin of chickpea at concentration 0.5x (96.51mg/kg soil) was recorded as 73.17%, at 1.0x (193.02mg/kg soil) as 80.49% and at 2.0x (386.04mg/kg soil) as 87.80% in comparison to control (Table 2). In combination of Cr+Cd the reduction in leghaemoglobin of chickpea at concentration 0.5x (21.75mg/kg soil) was 80.49%, at 1.0x (43.50mg/kg soil) was 85.36% and at 2.0x (87.00mg/kg soil) it was 90.24% in comparison to control table (2). The leghaemoglobin content of chickpea was reduced even further when cadmium was used with combination of nickel and chromium. In combination of Ni+Cr+Cd the reduction in of chickpea at concentration 0.5x (103.13mg/kg soil) was 85.36%, at 1.0x (206.26mg/kg soil) was 90.25% and at 2.0x (412.52mg/kg soil) was 95.12% in comparison to control (Table 2). Among all the combination of metal treatments Ni+Cr+Cd+Pb+Zn was found to be the most phytotoxic and significantly (P≤0.05 and 0.01) reduced the leghaemoglobin of chickpea at all the three concentrations among the all dose. In combination of Ni+Cr+Cd+Pb+Zn the reduction leghaemoglobin was recorded at concentration 0.5x (266mg/kg soil) as 87.80%, at 1.0x (532mg/kg soil) as 92.69% and at 2.0x (1064mg/kg soil) as 97.56% in comparison to control (Table 2).

Seed yield

Due to the effect of heavy metal treatments the seed yield varied considerably (Table 2). A gradual and significant reduction in the seed yield of content the plants treated with cadmium three concentration of metal at 0.5x (6.62mg/kg soil) was recorded as 84.92%, at 1.0x (13.24mg/kg soil) as 87.70%, and at 2.0x (26.48mg/kg soil)was found to be, 89.76% respectively in comparison to control table table (2). Chromium was found to be the least phytotoxic and significantly at (P≤0.05 and 0.01) among all the single metal treatments increased the seed yield at all the three concentration among the all dose. In contrast the seed yield increased significantly at 0.5x, 1.0x and 2.0x concentrations of metal. The minimum reduction at 0.5x (15.13mg/kg soil) 43.43%, at 1.0x (30.26mg/kg soil) 50.07% and at 2.0x (60.52mg/kg soil) 61.41%, respectively over control show in table table (2) Reduction in seed yield of plant of chickpea at zinc, at three concentrations at 0.5x (110.02mg/kg), was found to be 65.97%, at 1.0x (220.04mg/kg soil), was 75.79%, and at 2.0x (440.08mg/kg soil) was 76.49% in comparison to control table (2). Reduction in seed yield of plant of chickpea with nickel at three concentrations at 0.5x (81.38mg/kg soil) was 71.10, %, at 1.0x (162.76mg/kg soil) was 75.10% and at 2.0x (325.52 mg/kg soil) was 81.47% in comparison to control table (2). Due to lead at three concentrations 0.5x, 1.0x and 2.0x seed yield reduced at increasing concentration. At 0.5x (52.85mg/kg soil), 57.12%, at 1.0x (105.70mg/kg soil), it was 68.19% and at 2.0x (211.40mg/kg soil) was 73.44% reduction in seed yield of plant of chickpea in comparison to control table (2). Among the composite metal treatments Ni+Cd the reduction of seed yield in chickpea plant at concentration 0.5x (88mg/kg soil) was 84.37% at, 1.0x (176.00mg/kg soil) 88.52% and at 2.0x (352mg/kg soil) 90.32% in comparison to control table (2). Among all the combination of metal treatments Ni+Cr was found to be the least phytotoxic and significantly at (P≤0.05 and 0.01) decreased seed yield of chickpea plant at all the three concentrations. In combination of metals Ni+Cr the reduction in seed yield of plant of chickpea at three concentrations at 0.5x (96.51mg/kg soil) 69.99%, at 1.0x (193.02mg/kg soil) 75.93% and at 2.0x (386.04mg/kg soil) 78.70% in comparison to control table (2). In combination of Cr+Cd the reduction in seed yield of chickpea at concentration 0.5x (21.75mg/kg soil) was recorded as 79.94%, at 1.0x (43.50mg/kg soil) as 83.41% and at 2.0x (87.00mg/kg soil) as 93.36% in comparison to control table (2). The seed yield of chickpea was reduced even further when cadmium was used with combination of nickel and chromium. In combination of Ni+Cr+Cd the reduction in seed yield of chickpea at concentration 0.5x (103.13mg/kg soil) was 86.72%, at 1.0x (206.26mg/kg soil) was 91.15% and at 2.0x (412.52mg/kg soil) it was 93.08% in comparison to control table (2). Among all the combination of metal treatments Ni+Cr+Cd+Pb+Zn was found to be the most phytotoxic and significantly (P≤0.05 and 0.01) reduced the seed yield of chickpea at all the three concentrations among the all dose. When cadmium was applied along with nickel, chromium, lead and zinc it declined the seed yield of the plant. In combination of Ni+Cr+Cd+Pb+Zn the reduction seed yield of chickpea at concentration 0.5x (266mg/kg soil) was recorded as 86.72%, at 1.0x (532mg/kg soil) as 91.15% and at 2.0x (1064mg/kg soil) as 95.43% as compared to control table (2).

The nodulation of chickpea plants through their host specific rhizobial partner is an important aspect of legume-*Rhizobium* symbiosis that provide nitrogen to the legume plants. The effect of metals on symbiosis varied greatly with the types and concentration of metals as well as age of plants, grown either in the absence & presence of metals. The proper development of function of nodules & N_2 fixation appear to be hindered by the metal application (Broos *et al.,* 2004; 2005). Legume plants grown in metal amended soil had considerably a lower nitrogen content in roots and shoots, compared to control plants. The nitrogen content was lessening roots, compared to shoots. Similar observations were also observed by (Chaudri *et al.,* 2000) for field grown pea raised in soil amended with zinc and chromium. In contrast, plant grown in the presence of bio inoculated considerably increased the nitrogen content under the stress of each metal. In the present study chickpea plants grow in sandy clay loam soil were treated separately with three concentration of Zn, Ni, Pb, Cd and Cr (chickpea) had fewer nodules at 60, 80 and 110, DAS (For chickpea) compared to control. However, there are several possible explanations for these effects–a) one or more of the metals present might have prevented the formation of effectively N_2 fixing nodules by active *Rhizobium* strains present in the soil (Giller *et al.,* 1993) or b) the metal application might have resulted in the elimination of effective *Rhizobium* strains from the soil (Broos *et al.,* 2004) as a result, indirectly affected the N_2 fixation (Chaudri *et al.,* 2000). (Giller *et al.,* 1998) also concluded that clover *Rhizobia* are more sensitive to the toxic effects of heavy metals than are their host plant. The toxic effect of heavy metals on N_2 fixation is according to Giller *et al.,* 1998, due to toxicity to *Rhizobia* in the soil, which results in their gradual extinction. The important role of the legheamoglobin in the nodule suggests that changes in its concentration could affect the entire system of nitrogen fixation. The leghaemoglobin, an iron containing protein which binds to oxygen is an indicator of nodule activity. The leghaemoglobin facilitates the oxygen diffusion throughout the interior of the nodule, while bacteroids require oxygen to maintain metabolic function. In this experiment, the nodules on the root system of chickpea plant raised in soil treated with metals had considerably a lower concentration of leghaemoglobin. Levels of leghaemoglobin in multiple combinations were significantly decreased compared either with un-treated control or single metal treatments. Since chromium for chickpea had least effect, it was expected that nodules in the presence of these metals could contain leghaemoglobin at levels greater than the control. This finding thus suggested that the leghaemoglobin was not the target of the leghaemoglobin at levels greater than the control. The effect of cadmium on the seeds (*Cicer arietinum* L.) decreases the legmaemoglobin content with increasing concentration of cadmium (Hasan *et al.,* 2007). This finding thus suggested that the leghaemoglobin was not the target of the Cr for chickpea. Comparable observations on the effect of Cd, Ni, Cu, Zn on soybean nodules has been reported, (Stephen & Giliden saul, 1978). Similarly, adverse effect of heavy metals on the synthesis of leghaemoglobin and reduction in the nitrogenase activity is reported (Skujins *et al.*, 1986). Chlorophyll is the most important photosynthetic pigment which plays an important role in converting light energy into chemical energy chlorophyll molecule, has a cyclic tetrapyrolic structure (Porpyrin) with an isocyclic ring containing ring containing a magnesium atom at its centre and a phytol chain attached to it. Bearing the significant of this pigment in mind, the effect of different metals on the chlorophyll content of fresh foliage of chickpea grown in metal treated soil was determined at different stages of legume growth. From these investigations, it was evident that the photosynthetic apparatus were very sensitive to the toxicity to heavy metals. However, the metal induced changes in chlorophyll synthesis in two legumes were types and metal concentration and age legume genotype dependent. It is generally believed that the heavy metals react with the photosynthetic apparatus at various levels of organization and architecture leading to (i) accumulation of metals in leaves (ii) metal interaction with cytosolic enzymes and organics (iii) alternation of the functions of chloroplast membrane and (iv) supra molecular level action particularly on PS1, PS2 membrane acyl liquids and carrier proteins in vascular tissues (Prasad, 1999). In these studies, Cd might have affected the biosynthesis of chlorophyll more in mature leaves having properly organized inner membrane than in younger developing leaves. Similar reduction in chlorophyll content following metal application for different plant species has been reported (Mysliwa-Kurdzies & Strzatka, 2002a; Bibi & Husaain, 2005). In addition, the reduction in the chlorophyll content following the heavy metal applications could be due to the inhibition of the whole photosynthetic electron transport chain, as also observed in other higher plants (Yruela *et al.*, 1993). Indeed the metals lead to the formation of hydrogen peroxide and superoxide radical, whose production might have declined the photosynthetic rates and accounted for decrease in chlorophyll biosynthesis. A similar reduction in chlorophyll content in *Phavolus aureus* exposed to Cd has been reported due to the generation of $^1O_2$ (Shaw and Rout 2000). Additionally, the enzymes of photosynthetic carbon reduction (PCR) cycle, such as Rubisco, 3-PGA kinase, NADP, NAD-Glyceraldehyde-3-P-dehydrogenase and aldose have also been found to be inversely affected by Cd and Ni (Sheoran *et al.*, 1990a). In comparison, the other metals (e.g. Pb and Cr for chickpea and Pigeonpea) stimulated the synthesis of chlorophyll content, as also reported by Tripathi *et al.*, (2005), who showed that the bioinoculant *Pseudomonas putida* KNP4 improved the chlorophyll content of mungbean, when they were grown in Cd amended soil. The biosynthesis of chlorophyll affects the overall growth of legume including the symbiotic properties of legume and a strong correlation between chlorophyll and leghaemoglobin was observed *in vitro*.

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