Impact of Heavy Metal Toxicity to Plant Growth and Nodulation in Chickpea Grown Under Heavy Metal Stress

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ABSTRACT

The present research work carried out to assess by different heavy metals like (Zn, Ni, Pb, Cd and Cr) through the intake of locally grown, cereal crops from wastewater irrigated site. Heavy metal concentrations were several fold higher in all the collected samples from wastewater irrigated site when results compared to control. A pot study was conducted to investigate the toxic effects of certain heavy metals on the plant growth, dry matter and nodulation of chickpea (*Cicer arietinum* L.). Before the sowing, metal solution of different concentrations was added per pot. The plant growth and biomass production were adversely affected by heavy metals. Moreover, root nodulation was suppressed and number of nodules appreciably decreased. The study concludes among all the heavy metals tested, Cr proved to be the less toxic to the plants as resulting in the highest root and shoot length, dry matter, nodule number and nodule dry weight in the root. Heavy metal concentration effected in the order of Cd > Ni > Zn >Pb> Cr on chickpea plants.

Keywords: Cereal crop; Heavy metals; Wastewater; Root nodulation; Chickpea

1. INTRODUCTION

A great deal of research has been done on the toxic effects of industrial wastes which revealed the most abundant pollutants in the effluents of industries are noxious heavy metals. Wastewater contains substantial amounts of toxic heavy metals, which create problem (Pokhrel et al., 2009) and excessive accumulation of heavy metals in agricultural soils through wastewater irrigation may not only result in soil contamination, but also affect food quality and safety (Muchuweti et al., 2006).

Major water user industries are lock manufacturing factories, steel, paper, textile, chemical and petroleum refining. They account for nearly 80% of industrial wastewater. Aligarh city is famous for lock manufacturing factories. Hundreds of small and large scale factories are supposed to spill tremendous amount of heavy metals into the sewage in the form of industrial effluents (Malik and Ahmad, 1995). Contamination of soil in cultivated fields by industrial effluents loaded with toxic heavy metals has emerged as a new threat to agriculture. Most of the effluents and wastes contain heavy metals in an amount sufficient enough to cause toxicity to crop plants (Khan and Siddhu, 2006).With rapid increase in population and growth of industrialization, ground water quality is being increasingly threatened by agricultural chemicals and disposal of urban and industrial wastes. It has been estimated that once pollution enters the subsurface environment, it may remain concealed for many years, becoming dispersed over wide areas of ground water aquifer and rendering ground water supplies unsuitable for consumption and other uses. The rate of depletion of ground water levels and deterioration of ground water quality is of immediate concern in major cities and towns of the country (Jain et al., 2004; Ramasubramanian et al., 2004). Wastewater may contain various heavy metals including Zn, Cu, Pb, Mn, Ni, Cr and Cd, depending upon the type of activities, it is associated with. Continuous irrigation of agricultural land with industrial wastewater may cause heavy metal accumulation in the soil and vegetables (Sharma et al., 2007).

Contamination of soil in cultivated fields by industrial effluents loaded with toxic heavy metals has emerged as a new threat to agriculture. Most of the effluents and wastes contain heavy metals in an amount sufficient enough to cause toxicity to crop plants (Khan et al., 2006). Root nodulation was suppressed and number of functional nodules appreciably decreased (Khan and Khan, 2010).

The objective of this study is to examine the toxic effect of heavy metals on the plant and nodulation growth of chickpea plants to gain an insight on the loss of agricultural productivity of a very important cereal crop.

2. MATERIAL AND METHODS

2.1 Collection of Soil Samples

The study was conducted around Mathura road, situated at a suburban area in the Uttar Pradesh, district Aligarh, India, where soil samples were collected from here for our studies. The soil taken from the Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, U.P., India in the pots with treatment of heavy metals, some pots without metals were used as control.

2.2 Treatment Of Heavy Metals To The Soils

A pot experiment was conducted with chickpea (Cicer arietinum L.) as a test crop. Heavy metals in soil samples were determined by the method of McGrath and Cunliffe (1985), using flame Atomic Absorption Spectrophotometer. The metals determined included (mg kg^{-1}): Cd (13.24), Cr (30.26), Ni (162.76), Zn (220.04) and Pb (105.70). These metal concentrations were then used either alone or mixtures to evaluate their effects on chickpea. Seeds of chickpea (var. Avrodhi) were surface sterilized with 70% ethanol, 3% sodium hypochloride, rinsed with sterile water and dried (Vincent, 1970). The chloride salts of Cd, Cr, Ni, Pb and Zn were dissolved in distilled water and applied to moist soil before sowing the inoculated seeds in 25×22cm diameter clay pots. The effects of these metals were evaluated at half, normal and double doses (mg kg^{-1} soil): Cd at 6.62, 13.24 and 26.48, Cr at 15.13, 30.26 and 60.52 and Ni at 81.38, 162.76 and 325.52, Zn at 110.02, 220.04 and 440.08, and Pb at 52.85, 105.70 and 211.40.The effects of different mixtures were also evaluated (mg kg^{-1} soil): Cd with Cr (21.75, 43.50, 87), Ni with Cd (88, 176 and 352), Ni with Cr (96.51, 193.02 and 386.04), Ni with Cr and Cd (103.13, 206.26 and 412.52) Ni with Cr, Cd, Pb and Zn (266.00, 532.00 and 1064) (Table 1).

2.3 Experiment Design

Inoculated seeds of chickpea were sown in each pot. The soil in which the experiments have been conducted was a sandy clay loam and had received no exogenous input of metals. Heavy metal concentrations (Cd 13.24, Cr 30.26, Ni 162.76, Zn 220.04 and Pb 105.70 µg g^{-1} soil) and physiochemical properties, (pH 6.8, Organic carbon 0.81, NO_3-N 16.97, Organic matter 0.60%) in the contaminated soil. Each treatment was replicated six times and was arranged in a complete randomized design. One week after emergence, the seedlings were thinned to three in each pot. The pots were maintained in an open field. All plants in three pots were removed 135 days after seeding (DAS), and were used for nodulation studies. The roots were carefully washed and nodules produced on the roots systems of legume were detached, counted oven dried at 80^0C and weight plant growth, such as length of roots and shoots, dry weight of roots and shoots and total dry plant biomass of chickpea was recorded at each sampling dates. Plants at all the uprooted sampling intervals were oven dried at 80^0C to measure the total plant biomass (Ouzounidou et al., 1992).

2.4 Statistical Analysis

Since the experiment was conducted consecutively for 2 years under the identical environmental conditions using the same treatments, data of the measured parameters recorded for two years pooled together and subjected to analysis of variance (ANOVA) for three and two factor pot culture experiment i.e. inoculation and metal concentration, and critical difference (CD) was calculated at 1 and 5% probability level.

3. RESULTS

3.1 Analysis of Soil

The soil analysis of the Faculty of Agriculture sciences, Aligarh Muslim University, Aligarh showed that soil is alluvial sandy clay loam, having pH 7.2, organic matter 6.2g kg^{-1}, Khejdhal-N 0.75g kg^{-1}, olsen P 16mg kg^{-1}, cation exchange capacity 11.2cmol kg^{-1}, anion exchange capacity 5.1cmol kg^{-1} and water holding capacity 0.44mlg^{-1} in Table 2A.The physicochemical analysis of soil samples is presented in Table 2B. Test samples collected from Mathura road, Aligarh, the pH of agricultural soil irrigated with wastewater was 6.8. The soil of the sampling site is alluvial. The soil texture analysis showed that the soil is sandy clay loam having 0.81% organic carbon. Cation and anion exchange capacity (cmol kg^{-1}) 11.8 and 5.3, and water holding capacity 38ml kg^{-1} of the soil. Inorganic minerals such as PO_4-N, NO_3-N, Ca and Mg were present in variable concentrations in the test samples. The heavy metal concentration in the conventional cultivated soils of the Faculty of Agricultural Sciences (S2) was recorded as Ni (10.8mg kg^{-1} soil), Pb (8.12mg kg^{-1} soil), Zn (19.2mg kg^{-1} soil), Cr (6.3mg kg^{-1} soil), and cadmium (0.2mg kg^{-1} soil) (Table 2C). Heavy metal analysis in the soil of Mathura road, Aligarh (S1) was recorded for Cd (13.24), Cr (30.26), Ni (162.76), Zn (220.04) and Pb (105.70) (mg kg^{-1} soil) in contaminated site by Atomic Absorption Spectrophotometer (Table 2C).

3.2 Plant Growth

Toxicity of heavy metals on the growth of chickpea is presented in Table 3. The data indicated that the heavy metals were toxic to the growth of chickpea plants. Among all the single metal treatments Cd was found to be the most phytotoxic and Cr was the least phytotoxic and significantly ($P \leq 0.05$ and 0.01) reduced the plant growth. Maximum reduction of Cd in root length at 0.5x (6.62), 1.0x (13.24) and 2.0x (26.48)(mg kg^{-1} soil) after 60, 90 and 135 days was (65.43, 74.22 and 82.52%), (62.51, 69.93 and 79.14%) and (53.54, 66.48 and 74.28%),and similar reduction in shoot length was (69.07, 80.70 and 89.83%), (64.40, 75.09 and 85.33%) and (59.45, 71.46 and 80.39%) as compared to control (Table 3). Reduction in dry matter of roots with Cd after 60, 90 and 135 days was (82.40, 90.40 and 97.60%), (79.21, 89.11 and 94.56%) and (84.51, 91.77 and 94.68%), and reduction of shoot dry matter was (44.84, 53.06 and 54.33%), (45.73, 51.97, and 53.35%) and (48.04, 54.51 and 57.65%) as compared to control (Table 4). A minimum reduction in root length of Cr at concentration 0.5x (15.13), 1.0x (30.26) and 2.0x (60.52) (mg kg^{-1} soil) after 60, 90 and 135 days was (21.27, 48.78 and 65.04%), (17.01, 45.75 and 66.49%) and (12.98, 40.84 and 55.96%), and reduction in shoot length was (16.95, 34.57 and 46.11%), (19.57, 32.90 and 47.15%) and (22.18, 32.66 and 45.81%) as compared to control (Table 3). Reduction in dry root matter found to be lowest as after 60, 90 and 135 days were (10.40, 36 and 41.60%), (14.86, 45.04 and 59.90%) and (48.67, 65.61 and 77%), and dry shoot matter reduction was (4.34, 7.66 and 12.76%), (9.47, 14.09, and 17.78%) and (17.85, 23.14 and 26.27%) respectively as compared to control (Table 4).

Reduction in root length of chickpea with Zn inoculated soil, at concentration 0.5x (110.02), 1.0x (220.04) and 2.0x (440.08) (mg kg^{-1} soil) after 60, 90 and 135 days was (30.41, 54.24 and 71.04%), (22.18, 54.09 and 69.98%), and (20.91, 47.89 and 64.99%), and similarly reduction in shoot length was (30.29, 41.23 and 55.99%), (23.28, 41.01 and 52.60%)and (28.77, 38.60 and 51.65%) as compared to control (Table 3). Reduction in dry matter of root after 60, 90 and 135 days was (40.80, 50.40 and 57.60%), (51.48, 58.91 and 68.81%), (70.46, 73.36 and 79.90%), and shoot dry matter reduction was (22.95, 31.88 and 36.98%), (21.02, 32.10 and 38.33%) and (29.41, 37.06 and 43.14%) respectively as compared to control (Table4).

Reduction of root length with Ni inoculated soil at concentration 0.5x (81.38), 1.0x (162.76) and 2.0x (325.52) after 60, 90 and 135 days was (35.97, 59.46 and 70.49%), (29.25, 58.19 and 69.28%) and (23.74, 51.80 and 66.72%), and reduction in shoot length was (33.59, 43.21 and 69.37%), (29.59, 41.40 and 52.33%) and (31.73, 42.01 and 51.51%) in comparison to control (Table 3). Reduction in dry matter in root after 60, 90 and 135 days was (68.80, 78.40 and 85.60%), (74.75, 79.21 and 84.15%) and (82.57, 85.71 and 89.10%), and reduction in shoot dry weight was (28.06, 33.16 and 41.32%), (28.18, 34.41 and 41.11%) and (34.31, 38.43 and 45.29%) respectively as compared to control (Table 4).

The reduction of root length with Pb inoculated soil at concentration 0.5x (52.85), 1.0x (105.70) and 2.0x (211.40) (mg kg^{-1} soil) after 60, 90 and 135 days was (26.22, 50.80 and

70.21%), (21.02, 49.65 and 66.28%) and (16.82, 44.75 and 60.42%), and reduction in shoot length was (21.66, 37.88 and 55.05%), (18.77, 36.95 and 45.07%), and (25.87, 35.19 and 48.55%) as compared to control (Table 3). Reduction in dry matter of root after 60, 90 and 135 days was (50.40, 59.20 and 79.60%), (54.96, 59.91 and 68.82%) and (57.87, 70.70, 77.24%) and shoot dry matter reduction was (12.24, 18.62 and 26.27%), (14.55, 21.70 and 28.87%) and (22.16, 29.02 and 34.70) respectively compared to control (Table4).

The root shoot length and dry matter reduction significantly ($P \leq 0.05$) increased in the combination of metals. In combination of metals Ni+Cd the reduction in root length of chickpea at concentration 0.5x (88.00),1.0x (176.00) 2.0x (352.00) (mg kg^{-1} soil) after 60, 90 and 135 days was (49.16, 64.98 and 75.78%), (49.69, 59.59 and 73.06%) and (41.35, 53.30 and 68.36%), and reduction in shoot length was (60.79, 68.60, and 78.85%), (52.60, 65.02 and 73.87%) and (44.43, 59.79 and 70.84%) as compared to control (Table 3). Reduction in dry matter in root after 60, 90 and 135 days was (81.60, 90.40 and 96.80%), (79.21, 83.67 and 93.07%) and (84.02, 87.17 and 91.77%), and reduction in dry matter in the shoot was (54.84, 61.22 and 62.50%), (54.74, 59.35 and 60.74%), and (57.65, 60.40, 64.71%); respectively as compared to control (Table 4).

In combination of metals Ni+Cr, the reduction in root length of chickpea at concentration 0.5x (96.51), 1.0x (193.02), 2.0x (386.04) (mg kg^{-1} soil) after 60, 90 and 135 days was (42.92, 54.01 and 68.87%),(41.37, 53.88 and 60.53%) and (37.94, 49.11 and 59.13%), and shoot length was reduced (46.07, 64.82 and 72.71%), (39.42, 58.81 and 66.24%), and (42.69, 52.86 and 66.30%) respectively as compared to control (Table3). Reduction in dry matter in root at 60, 90 and 135 days was (56.80, 57.60 and 73.60%), (59.40, 63.86 and 73.77%) and (71.43, 74.58 and 78.94%), and reduction in dry matter of the shoot was (59.94, 66.07 and 69.89%), (58.66, 65.82 and 69.74%), and (61.76, 67.84 and 71.57%) in comparison to control (Table4).

In combination of Cr+Cd the reduction in root length of chickpea at concentration 0.5x (21.75), 1.0x (43.50) 2.0x (87.00) (mg kg^{-1} soil) after 60, 90 and 135 days was (59.91, 70.21 and 82.57%), (52.52, 64.97 and 79.22%) and (44.65, 61.68 and 74.18%), and reduction in shoot length was (55.94, 64.61 and 77.10%), (50.31, 60.30 and 73.32%) and (48.69, 57.31 and 67.57%) as compared to control (Table 3). Reduction in dry matter in root after 60, 90 and 135 days (56, 85.60, 93.60%), (59.91, 85.15 and 89.61%) and (74.82, 84.50 and 89.10%), and reduction in dry matter in the shoot was (63.77, 66.33 and 67.85%), (63.51, 68.13 and 68.36%) and (64.90, 67.45 and 70.78%) as compared to control (Table 4).

In combination of Ni+Cr+Cd the reduction in root length of chickpea at concentration 0.5x (103.13), 1.0x (206.26), 2.0x (412.52) (mg kg^{-1} soil) after 60, 90 and 135 days was (68.37, 82.07 and 90.81%), (65.71, 78.52 and 85.63%) and (60.49, 73.91 and 82.49%), and shoot length of the plant was reduced to (89.06, 91.68 and 96.05%), (83.69, 89.00 and 94.24%) and (79.37, 84.98 and 89.80%) as compared to control (Table3). Reduction in dry matter in root after 60, 90 and 135 days was (74.40, 92 and 97.60%), (70.79, 84.65 and 94.55%) and (81.84, 89.35 and 94.19%), and reduction in dry matter in the shoot was (66.81, 67.85 and 69.13%), (65.13, 67.67 and 69.51%) and (67.26, 70.20 and 71.76%) as compared to control (Table 4).

In combination of Ni+Cr+Cd+Pb+Zn the reduction in root of chickpea at concentration 0.5x (266), 1.0 (532) and 2.0x (1064) (mg kg^{-1} soil) after 60, 90 and 135 days was (68.60, 87.70 and 93.42%), (70.48, 82.35 and 88.71%), and (65.23, 75.62 and 84.17%) and reduction in shoot length was (73.57, 81.12 and 90.78%), (70.82, 79.32 and 85.11%) and (68.53, 76.92 and 82%) as compared to control (Table 3). Reduction in dry matter in root after 60, 90 and 135 days was (86.40, 94.40 and 99.20%), (85.14, 93.06 and 98.01%) and (86.68, 91.52 and 97.7%), and reduction in dry matter in the shoot was (71.17, 73.21 and 74.23%), (70.67, 74.59 and 76.67%), and (73.72, 76.27 and 79.21%) as compared to control (Table 4).

3.3 Symbiotic Traits

In leguminous plants, the dry weight of nodules varied inversely with the concentration of metals used, Cd is causing the greater effect compared to other metals used in the study. A maximum reduction in nodules number and nodules dry weight of the plant at three concentrations 0.5x (6.62), 1.0x (13.24) and 2.0x (26.48) (mg kg^{-1} soil) after 60, 90 and 135 days was (73.33, 88.93 and 95.60%), (67.17, 80.02 and 92.89%) and (57.30, 76.70 and 87.39%) nodule number, and nodules dry weight was (86, 86.14 and 87.39%), (79.55 79.98

and 82.23%), and (71.98, 77.19 and 85.31%) as compared to control (Table 5). A minimum reduction in nodule number of Cr at three concentrations 0.5x (15.13), 1.0x (30.26) and 2.0x (60.52) (mg kg^{-1} soil) after 60, 90 and 135 days was (46.67, 68.93 and 82.27%), (22.85, 62.88 and 77.15%) and (19.43, 50.48 and 70.87%), and minimum reduction of nodules dry weight was (32.76, 55.28 and 56.02%), (23.93, 45.83 and 46.12%) and (23.43, 36.40 and 43.15%) as compared to control (Table 5). Reduction in nodules per plant of chickpea when treated with Zn, at three concentrations 0.5x (110.02), 1.0x (220.04) and 2.0x (440.08) (mg kg^{-1} soil) after 60, 90 and 135 days was (44.47, 68.93 and 84.47%), (42.86, 65.71 and 77.15%) and (34, 57.30 and 70.87%), minimum reduction on nodules dry weight was (72.97, 78.95 and 89.43%), (52.50, 74.62 and 81.43%) and (41.16, 66.21 and 71.54%) as compared to control (Table5).

Reduction of nodules in Ni inoculated soil at three concentrations 0.5x (81.38), 1.0x (162.76) 2.0x (325.52) (mg kg^{-1} soil) after 60, 90 and 135 days was (64.47, 82.27 and 88.93%), (57.14, 72.87 and 85.73%) and (45.64, 69.91 and 79.61%), and minimum reduction of nodules dry weight was (78.79, 86.40 and 89.93%), (65.92, 75.49 and 82.23%) and (59.52, 65.60 and 77.68%) respectively as compared to control (Table 5).

Reduction in nodule number per plant of chickpea due to Pb at three concentrations 0.5x (52.85), 1.0x (105.70) and 2.0x (211.40) (mg kg^{-1} soil) after 60, 90 and 135 days was (33.33, 66.67 and 82.27%), (32.88, 61.41 and 78.57%) and (22.34, 55.34 and 74.77%), and minimum reduction in nodules dry weight was (54.46, 66.01 and 71.42%), (46.41, 53.66 and 60.77%) and (33.66, 40.98 and 50.10%) as compared to control (Table 5).

In combination of metals Ni+Cd the reduction in nodule per plant of chickpea at three concentrations 0.5x (88), 1.0x (176) 2.0x (352) (mg kg^{-1} soil) was after 60, 90 and 135 days (75.60, 84.46 and 97.80%), (70, 78.57 and 94.29%) and (69.91, 77.69 and 88.35%), and minimum reduction of nodules dry weight was (88.21, 89.60 and 96.64%), (84.26, 90.06 and 94.63%) and (70.98, 85.80 and 92.56%) respectively as compared to control (Table5).

In combination of metals Ni+Cr the reduction in nodule number per plant of chickpea at three concentrations 0.5x (96.51), 1.0x (193.02) 2.0x (386.04) (mg kg^{-1} soil) after 60, 90 and 135 days was (66.67, 82.27 and 95.60%), (62.88, 80.02 and 88.60%) and (63.12, 75.73 and 84.47%), and minimum reduction of nodules dry weight was (87.71, 88.53 and 93.28%), (74.11, 76.50 and 89.70%) and (66.15, 70.24 and 82.45%) respectively as compared to control (Table 5).

In combination of Cr+Cd the reduction in nodule number of chickpea at three concentrations 0.5x (21.75), 1.0x (43.50) 2.0x (87.00) (mg kg^{-1} soil) at 60, 90 and 135 days was (80, 95.33 and 97.80%), (74.28, 87.14 and 90.01%) and (68.95, 78.65 and 86.43%), and minimum reduction of nodules dry weight was (80.59, 85.26 and 94.68%), (75.34, 88.47 and 90.57%) and (71.48, 83.14 and 88.72%) as compared to control (Table 5).

In combination of Ni+Cr+Cd the reduction in nodule number of chickpea at three concentrations 0.5x (103.13), 1.0x (206.26), 2.0x (412.52) (mg kg^{-1} soil) after 60, 90 and 135 days were(87.26, 91.13 and 97.80%), (75.74, 88.60 and 94.30%) and (74.78, 84.47 and 92.25%),and minimum reduction of nodules dry weight was (90.33, 92.71 and 94.10%), (84.55, 88.98 and 89.48%) and (76.01 85.93, and 89.40%) respectively as compared to control (Table 5).

In combination of Ni+Cr+Cd+Pb+Zn the reduction in nodule number of chickpea at three concentrations 0.5x (266), 1.0 (532) 2.0x (1064) (mg kg^{-1} soil) at 60, 90 and 135 days was (84.46, 95.60 and 97.80%), (80.03, 90.02 and 97.17%) and (78.65, 86.42 and 93.20%), and minimum reduction of nodules dry weight was (96.40, 97.38 and 98.53%), (95.43, 96.66 and 97.61%) and (94.67, 95.91 and 97.40%) respectively as compared to control (Table 5).

4. DISCUSSION

Atomic Absorption analysis of agricultural soil irrigated with wastewater revealed the presence of several heavy metals, some of which are considered to be toxic to the biological systems (Majid, 2010). The levels of Cd were found to be highest in almost all the sampling sites. Cd is considered as a potential toxin that is principally dispersed in natural and agricultural environments through anthropogenic sources. The rhizospheric soil irrigated with industrial wastewater contained high levels of Cr, Zn, Ni, Cd, Cu and Pb as compared with the levels of these metals at the unpolluted site (control). This may be due to the presence of a large number of lock manufacturing and metal electroplating industries in the vicinity of the test sampling sites. The industrial effluents at Aligarh city actually contain quite large amount of heavy metal. High concentrations of heavy metals have been observed in soil samples as compared to wastewater samples from industrial effluents.

Heavy metals discharged from industrial operations and upon consequent accumulation in various ecological systems cause a massive threat to the varied agroecosystems (Cheung and Gu, 2007). When heavy metals accumulate in soil to an abnormal level, it causes dramatic changes in microbial composition and their activities (Khan et al., 2009; Krujatz et al., 2011), leading consequently to losses in soil fertility. As a result of depleted soil nutrient pools resulting from direct or indirect metal effect, the health of plants including legumes like greengram [Vigna radiata (L.) Wilczek] (Wani et al., 2007), pea (Pisum sativum L.) (Wani et al., 2008a) and chickpea (Cicer arietinum L.) (Wani et al., 2008b; Wani and Khan, 2010) growing in metal-enriched soil is adversely affected either due to nutrient deficiency or due to direct effects of toxicants.

Heavy metals such as Pb, Hg, Cd, Zn and Ni discharged from industries, their toxicity may affect the soil fertility, crop productivity and varies with the genotype and age of plants (Shaw and Rout, 2002), and also depends on the physiochemical properties of soil and root exudates. The concentrations of heavy metals in soil, affect the different metabolic activities of plants, leading to the decrease in overall growth of the plants including legumes (Das et al., 1997). With these considerations, the phytotoxic effects of three concentrations of Cd, Cr, Ni, Pb and Zn for chickpea were evaluated under pot house trials. These metals were used both alone and in combination for legume crop separately affects the overall growth of the chickpea plant (Table 3).

The variations in the uptake of metals by the legume plants could be due to several reasons. For instance, the smaller uptake of metals by plant tissues in amended soil could be due to the antagonistic effect of one metal on the other. A second possibility could be the existence of interaction of the root surface between metals for plant uptake. Lastly, there was probably a competition between metals for adsorption onto soil. A similar variation in the accumulation of metals in different legume plants is reported (Wang et al., 2002; Rodriguez et al., 2007).

In the present investigation, Cr was found to be the least but significantly phytotoxic metal as compared to other metals added separately to the soil. Low levels of the phytotoxicity of Cr have been attributed to its insolubility under most soil conditions (Ifigeneia, 2009; Abdul, 2010), and it did not affect the plant growth unless the concentrations were very large (Smith et al., 1992). The metal, however, caused toxicity to both the parts, suppression of root elongation and seedlings of Picea abies, celery and tomato plants due to various heavy metals on the extensiveness and proliferation of root and their subsequent effects on shoot growth Pb to suppression of dry matter production (Vanik et al., 2005). The presence of Cd in the soil decreases the growth of chickpea plants (Hasan et al., 2007), Cd at all levels tested was found to be the most toxic metal for the maize crop and caused the most severe reduction in the dry weight of shoot, root and seed yield (Abdul, 2010). Cd at all levels tested was found to be the most toxic metal which caused the most severe reduction in the dry weight of shoot, root followed in order by Ni and Zn (Table 4). Previous studies have also demonstrated a relatively higher phytotoxicity of Cd that of Zn (Kalyanaraman and Sivagurunathan, 1993). In general, the reduction in the dry weight of roots was more severe than the dry weight of shoots following treatment with heavy metals added separately or in combination (Table 4). This is supported by the findings of (Karataglis et al., 1991) who reported that the influence of relatively higher amounts of Zn, Pb, Ni, Cr and Cd in chickpea resulted in depressed shoot growth but the most evident symptoms were on roots and Abdul, 2010 find that metal accumulation by maize seeds was directly related to the applied heavy metal with greater concentrations of metals found in cases where metals were added individually rather than in combinations. Amendment of soil with the heavy metals at concentrations higher than the normal levels resulted in a striking decrease of root and shoot biomass expressed in terms of dry weight (Table 4). Biomass loss (fresh weight) under metal treatment has also been reported by many groups (Abdul, 2010).

In the present study, chickpea plant growth in sandy clay loam soil was treated separately with three concentrations of Zn, Ni, Pb, Cd and Cr had fewer nodules at 60, 90 and 135 DAS as compared to control (Table 5). The reduction in the number of nodules is possibly due to the direct toxic effect of these metals either on the root hairs or Rhizobia, as observed in Zn & Cd treated alfalfa plants (Ibekwe et al., 1996). The increasing concentrations of heavy metals like cadmium, zinc and lead significantly decreased nodule index: the number of nodules per gram of the total fresh biomass, at about 2.64mg Cdkg $^{-1}$, 300mg Znkg $^{-1}$ and 130mg Pbkg $^{-1}$. It was found that the nodulation index of white clover could serve as a suitable bioindicator of increased heavy metal toxicity in soil (Manier et al., 2009). Damage to the root system as a result of metal toxicity is supposed to be the reason for the lack of proper nodule formation, similar trends were also found in the present study (Table 5).

Cadmium even at considerably lower concentration was found toxic for the microsymbiont (Pereira et al., 2006; Younis, 2007) and (1) inhibited the nitrogenase activity; (2) affected the plant biomass production; (3) disrupted a nodule ultrastructure number of nodules and induced nodule senescence; (4) reduced dry matter accumulation in roots, shoot and leaf; and (5) adversely affected metabolic activities like photosynthesis of legumes (Wani et al., 2006; Noriega et al., 2007).

Table 1. Treatment of Heavy Metals Applied to the Soil

Metals applied	Dose rate (mg kg^{-1} soil)		
	Half (0.5x)	Normal (1.0x)	Double (2.0x)
Zn	110.02	220.04	440.08
Ni	81.38	162.76	325.52
Pb	52.85	105.70	211.40
Cd	6.62	13.24	26.48
Cr	15.13	30.26	60.52
Ni+Cd	88.00	176.00	352.00
Ni+Cr	96.51	193.02	386.04
Cr+Cd	21.75	43.50	87.00
Ni+Cr+Cd	103.13	206.26	412.52
Ni+Cr+Cd+Pb+Zn	266.00	532.00	1064
Control (Without metal)			

Table 2. Physicochemical Properties of the Contaminated Soil Used In This Study

Physicochemical characteristics	Soil (mg kg^{-1})
Texture	Sandy clay loam
Type	Alluvial
pH	6.8
Ca	288.53
Mg	188.06
NO_3-N	16.97
PO_4-N	10.37
Organic matter	0.60
% Organic carbon	0.81
Cation exchange capacity (CEC) (cmol kg^{-1})	11.8
Water holding capacity	44
Anion exchange capacity (AEC) (cmol kg^{-1})	5.3

Table 3. Root Length and Shoot Length Of Chickpea Plants As Influenced By Various Heavy Metals Added Alone Or In Combination To Soil

Metal applied	Dose rate (mg kg $^{-1}$ soil)	Length cm plant^{-1}					
		Root			Shoot		
		60D	90D	135D	60D	90D	135D
Zn	0.5x	12.50	18.95	23.22	16.25	22.11	25.28
	1.0x	08.22	11.18	15.30	13.70	17.00	21.79
	2.0x	05.20	07.31	10.28	10.26	13.66	17.16
Ni	0.5x	11.50	17.23	22.39	15.48	20.29	24.23
	1.0x	07.28	10.18	14.15	13.24	16.89	20.58
	2.0x	05.30	07.48	09.77	07.14	13.74	17.21
Pb	0.5x	13.25	19.23	24.42	18.26	23.41	26.31
	1.0x	08.82	12.26	16.22	14.48	18.17	23.00
	2.0x	05.35	08.21	11.62	10.71	15.83	18.26
Cd	0.5x	06.21	09.13	13.64	07.21	10.26	14.39
	1.0x	04.63	07.32	09.84	04.50	07.18	10.13
	2.0x	03.14	05.08	07.55	02.37	04.23	06.96
Cr	0.5x	14.14	20.21	25.55	19.36	23.18	27.62
	1.0x	09.20	13.21	17.37	15.25	19.34	23.90
	2.0x	06.28	08.16	12.93	12.56	15.23	19.23
Ni+Cd	0.5x	09.13	12.25	17.22	09.14	13.66	19.72
	1.0x	06.29	09.84	13.71	07.32	10.08	14.27
	2.0x	04.35	06.56	09.29	04.93	07.53	10.35
Ni+Cr	0.5x	10.25	14.25	18.22	12.57	17.46	20.34
	1.0x	08.26	11.23	14.94	08.20	11.87	16.73
	2.0x	05.59	09.61	12.00	06.36	09.73	11.96
Cr+Cd	0.5x	07.20	11.56	16.25	10.27	14.32	18.21
	1.0x	05.35	08.53	11.25	08.25	11.44	15.15
	2.0x	03.13	05.06	7.58	05.34	07.69	11.51
Ni+Cr+Cd	0.5x	05.68	08.35	11.60	2.55	04.70	07.32
	1.0x	03.22	05.23	07.66	01.94	03.17	05.33
	2.0	01.65	03.50	05.14	0.92	01.66	03.62
Ni+Cr+Cd+Pb+Zn	0.5x	05.64	07.19	10.21	06.16	08.41	11.17
	1.0x	02.21	04.30	07.16	04.40	05.96	08.19
	2.0x	1.18	2.75	04.65	02.15	04.29	06.39
	Control	17.96	24.35	29.36	23.31	28.82	35.49

	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	.069	.043	.038	.139	.120	.076	.240	.083	.052	.045	.166	.143	.090	.287
CD at 5%	.192	.121	.105	.385	.333	.211	.667	.323	.204	.177	.647	.560	.354	1.12

Metal applied	Dose rate (mg kg $^{-1}$	Length cm plant^{-1}													
		Root							Shoot						
CD at 1%		.270	.171	.148	.541	.469	.296	.938	.300	.189	.164	.600	.520	.328	1.04
CV		3.102							2.994						
a= Metal, b= Concentration, c= Days, ab= Metal x Concentration, ba=Concentration x Metal, ca = Days x Metal, abc= Metal x Concentration x Days, CD= Critical difference, CV= Coefficient of variation															

Table 4. Dry Matter (Root and Shoot Weight) Of Chick Pea Plant Exposed To Various Concentration Of Heavy Metals Added Either Separately Or In Combination

Metal applied	Dose rate (mgkg $^{-1}$ soil)	Dry weight (g plant^{-1})					
		Root			Shoot		
		60D	90D	135D	60D	90D	135D
Zn	0.5x	0.74	0.98	1.22	3.02	3.42	3.60
	1.0x	0.62	0.83	1.10	2.67	2.94	3.21
	2.0x	0.53	0.63	0.83	2.47	2.67	2.90
Ni	0.5x	0.39	0.51	0.72	2.82	3.11	3.35
	1.0x	0.27	0.42	0.59	2.62	2.84	3.14
	2.0x	0.18	0.32	0.45	2.30	2.55	2.79
Pb	0.5x	0.62	0.91	1.74	3.44	3.70	3.97
	1.0x	0.51	0.81	1.21	3.19	3.39	3.62
	2.0x	0.33	0.63	0.94	2.89	3.08	3.33
Cd	0.5x	0.22	0.42	0.64	2.17	2.35	2.65
	1.0x	0.12	0.22	0.34	1.84	2.08	2.32
	2.0x	0.03	0.11	0.22	1.79	2.02	2.16
Cr	0.5x	1.12	1.72	2.12	3.75	3.92	4.19
	1.0x	0.80	1.11	1.42	3.62	3.72	3.92
	2.0x	0.73	0.81	0.95	3.42	3.56	3.76
Ni+Cd	0.5x	0.23	0.42	0.66	1.77	1.96	2.16
	1.0x	0.12	0.33	0.53	1.52	1.76	2.02
	2.0x	0.04	0.14	0.34	1.47	1.70	1.80
Ni+Cr	0.5x	0.54	0.82	1.18	1.57	1.79	1.95
	1.0x	0.53	0.73	1.05	1.33	1.48	1.64
	2.0x	0.33	0.53	0.87	1.18	1.31	1.45
Cr+Cd	0.5x	0.55	0.81	1.04	1.42	1.58	1.79
	1.0x	0.18	0.30	0.64	1.32	1.38	1.66
	2.0x	0.08	0.21	0.45	1.26	1.37	1.49
Ni+Cr+Cd	0.5x	0.32	0.59	0.75	1.34	1.51	1.67
	1.0x	0.10	0.31	0.44	1.26	1.40	1.52
	2.0x	0.03	0.11	0.24	1.21	1.32	1.44
Ni+Cr+Cd +Pb+Zn	0.5x	0.17	0.30	0.55	1.13	1.27	1.34
	1.0x	0.07	0.14	0.35	1.05	1.10	1.21

Metal applied	Dose rate (mgkg $^{-1}$ soil)	Dry weight (g plant^{-1})					
		Root			Shoot		
		60D	90D	135D	60D	90D	135D
	2.0x	0.01	0.04	0.10	1.01	1.01	1.06
	Control	1.25	2.02	4.13	3.92	4.33	5.10

	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	.015	.009	.008	.030	.026	.016	.052	.017	.011	.009	.035	.030	.019	.061
CD at 5%	.042	.026	.023	.084	.072	.046	.145	.048	.030	.026	.097	.084	.053	.168
CD at 1%	.059	.037	.032	.118	.102	.064	.205	.068	.043	.037	.137	.118	.075	.237
CV	8.766							3.771						

a=Metal, b=Concentration, c=Days, ab=Metal x Concentration, ba=Concentration x Metal, ca=Days x Metal, abc=Metal x Concentration x Days, CD=Critical difference, CV=Coefficient of variation

Table 5. Nodule Number And Nodule Dry Weight Of Chickpea Exposed To Various Concentration Of Heavy Metals Added Either Or Alone Or In Combination

Metal applied	Dose rate (m kg^{-1} soil)	Nodulation plant^{-1}					
		Nodule no.plant^{-1}			Nodule dry weight mg g^{-1}		
		60D	90D	135D	60D	90D	135D
Zn	0.5x	8.33	13.33	22.66	3.30	6.55	9.49
	1.0x	4.66	8.00	14.66	2.57	3.50	5.45
	2.0x	2.33	5.33	10.00	1.29	2.56	4.59
Ni	0.5x	5.33	10.00	18.66	2.59	4.70	6.53
	1.0x	2.66	6.33	10.33	1.66	3.38	5.55
	2.0x	1.66	3.33	7.00	1.23	2.45	3.60
Pb	0.5x	10.00	15.66	26.66	5.56	7.39	10.70
	1.0x	5.00	9.00	15.33	4.15	6.39	9.52
	2.0x	2.66	5.00	8.66	3.49	5.41	8.05
Cd	0.5x	4.00	7.66	14.66	1.71	2.82	4.52
	1.0x	1.66	4.66	8.00	1.69	2.76	3.68
	2.0x	0.66	1.66	4.33	1.54	2.45	2.37
Cr	0.5x	8.00	18.00	27.66	8.21	10.49	12.35
	1.0x	4.66	8.66	17.00	5.46	7.47	10.26
	2.0x	2.66	5.33	10.00	5.37	7.43	9.17
Ni+Cd	0.5x	3.66	7.00	10.33	1.44	2.17	4.68
	1.0x	2.33	5.00	7.66	1.27	1.37	2.29
	2.0x	0.33	1.33	4.00	0.41	0.74	1.20

Metal applied	Dose rate ($m\ kg^{-1}$ soil)	Nodulation plant^{-1}					
		Nodule no.plant^{-1}			Nodule dry weight mg g^{-1}		
		60D	90D	135D	60D	90D	135D
Ni+Cr	0.5x	5.00	8.66	12.66	1.50	3.57	5.46
	1.0x	2.66	4.66	8.33	1.40	3.24	4.80
	2.0x	0.66	2.66	5.33	0.82	1.42	2.83
Cr+Cd	0.5x	3.00	6.00	10.66	2.37	3.40	4.60
	1.0x	0.70	3.00	7.33	1.80	1.59	2.72
	2.0x	0.33	2.33	4.66	0.65	1.30	1.82
Ni+Cr+Cd	0.5x	2.66	5.66	8.66	1.18	2.13	3.87
	1.0x	1.33	2.66	5.33	0.89	1.52	2.27
	2.0x	0.33	1.33	2.66	0.72	1.45	1.71
Ni+Cr+Cd +Pb+Zn	0.5x	2.33	4.66	7.33	0.44	0.63	0.86
	1.0x	0.66	2.33	4.66	0.32	0.46	0.66
	2.0x	0.33	0.66	2.33	0.18	0.33	0.42
	Control	15.00	23.33	34.33	12.21	13.79	16.13

	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	.177	.112	.097	.355	.308	.194	.616	.0548	.034	.030	.109	.094	.060	.189
CD at 5%	.493	.311	.270	.986	.854	.540	1.708	.151	.096	.083	.303	.263	.166	.526
CD at 1%	.693	.438	.379	1.386	1.200	.759	2.401	.213	.135	.117	.427	.369	.234	.739
CV	9.754							3.147						

a= Metal, b= Concentration, c= Days, ab= Metal x Concentration, ba=Concentration x Metal, ca = Days x Metal, abc= Metal x Concentration x Days, CD= Critical difference, CV= Coefficient of variation

5. CONCLUSION

In chickpea, growth of root and shoot, dry matter were adversely affected by heavy metals. Moreover, root nodulation was suppressed and number of nodules appreciably decreased. Among all the heavy metals tested Cr proved to be the less toxic and Cd proved highest toxic as comparable to other metals.The farmers may be suggested to use fresh groundwater and avoid industrial wastewater, when planting legumes and other crops in fields, industrial wastewater decreased the total yield of the crop and causes different types of diseases in human beings.

ACKNOWLEDGMENTS

Financial assistance provided by University Grant Commission (UGC), New Delhi, India, is highly acknowledged.

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