Results
4.1 Total heavy metal concentration in soils

Heavy metal in polluted soils of Mathura Road and Exhibition ground, Aligarh and non-polluted soils of Faculty of Agricultural Sciences, AMU, Aligarh was determined by atomic absorption spectrophotometer. The heavy metal concentrations in polluted soils of Mathura Road (S1) were (mg/kg soil): Cd 11.5; Cr 67.5; Ni 290.1; Zn 4890; Cu 669.1 and Pb 195. The heavy metals determined in contaminated soils of Exhibition ground (S2) included (mg/kg soil): Cd 9.8; Cr 64.2; Ni 334; Zn 3550; Cu 535 and Pb 191. In comparison, the heavy metal concentration of the conventional cultivated soils of Faculty of Agricultural Sciences (S3) were Ni 10.8 mg/kg, Pb 8.1 mg/kg, Zn 19.2 mg/kg, Cr 6.3 mg/kg, Cu 12.2 mg/kg and Cd 0.2 mg/kg (Table 11).

4.2 Microbial diversity of polluted and non-polluted soils

The rhizospheric soils of chickpea, greengram and brinjal (S1); chickpea, greengram, lentil and pea (S2) and mustard and tomato (S3) were subjected to microbial analysis (Table 12). The viable counts of bacteria, fungi and phosphate solubilizing microorganisms (PSM) differed among rhizospheric soils. Generally, the microbial populations were less in polluted soils (S1 and S2) compared to non-polluted soils (S3). The bacterial populations in the rhizosphere of chickpea, greengram and brinjal (S1) were 265 x 10^3, 309 x 10^3 and 377 x 10^3 CFU/g soil, respectively. The metal polluted soils of chickpea, greengram, lentil and pea (S2) showed bacterial population of 380 x 10^3, 320 x 10^3, 302 x 10^3 and 298 x 10^3 CFU/g soil, respectively. In contrast, the rhizospheric soils of mustard and tomato (S3) had a viable bacterial count of 840 x 10^3 and 752 x 10^3 CFU/g soil, respectively. The fungal population in all the rhizospheric soils ranged between 11 x 10^4 (S2) to 25 x 10^4 CFU/g soil (S3). In general, the phosphate solubilizing bacteria (PSB) were found to more than the phosphate solubilizing fungi (PSF) in both polluted (S1 and S2) and non-polluted (S3) soils of Aligarh (Table 17). Among all the rhizosphere soils, population of PSB (6 x 10^5 CFU/g soil) and PSF (3 x 10^5 CFU/g soil) was greater in the rhizosphere of mustard (Plate IC). Neither PSB nor PSF were recovered from lentil rhizosphere (S2) while PSF were not detected in the rhizospheric soils of greengram, grown in both S1 and S2 soils.

4.3 Characterization of nitrogen fixing and phosphate solubilizing bacteria

In the present study, a total of 50 strains each of *Mesorhizobium*, *Bradyrhizobium* and *Rhizobium* were isolated from the nodules of chickpea, greengram, lentil and pea crop using
yeast extract mannitol agar plates. Moreover, 50 strains of phosphate solubilizing bacteria were isolated from the rhizospheric soils of mustard and tomato. Of these bacterial strains, 20% of Mesorhizobium spp. (chickpea), 28% each of Bradyrhizobium spp. (greengram) and Rhizobium spp. (pea), 30% of Rhizobium spp. (lentil) and 20% of phosphate solubilizers were selected for assaying the plant growth promoting activities. The isolated bacterial cultures showed a variable morphological and biochemical characteristics (Table 13). Generally, the rhizobial strains were Gram negative while phosphate solubilizers were both Gram positive and negative. Rhizobial strains in general were positive to all the biochemical reactions except voge proskauer, indole, gelatin and methyl red test. Among the phosphate solubilizers, Gram negative strains of phosphate solubilizing bacteria showed negative activity towards methyl red, voge proskauer, starch hydrolysis, triple sugar iron agar, mannitol and sucrose utilization.

4.4 Functional diversity among plant growth promoting rhizobacteria

Of the total 200 nitrogen fixers and 50 phosphate solubilizers, a total of 53 nitrogen fixers and 10 phosphate solubilizers were screened for their multiple plant growth promoting (PGP) traits. The mesorhizobial strains were grouped into four PGP groups (Table 15). The PGP group I included 30% of strains which showed four PGP traits (i.e. production of ammonia, hydrogen cyanide, siderophore and indole acetic acid) followed by PGP group II, which had 40% of strains positive to ammonia, HCN and IAA. In PGP group III, 20% of strains exhibited a positive reaction to ammonia and HCN, while PGP group IV contained only one strain (Mesorhizobium RC10) and displayed the synthesis of indole acetic acid only. Similarly, Rhizobium strains isolated from pea nodules were grouped into four PGP groups (Table 16). The PGP group I contained two isolates (Rhizobium RP5 and RP7) and displayed four PGP traits (i.e. ammonia, HCN, siderophore and IAA production). This was followed by group II, which had only one strain (RP3) and was positive for ammonia, siderophore and indole acetic acid. The group III contained 22% of the strains which were found to be positive for ammonia, hydrogen cyanide and indole acetic acid. A total of 57% of the strains of group IV were positive for ammonia and indole acetic acid. Bradyrhizobium strains were grouped into two PGP groups (Table 17). The PGP group I contained 21% of isolates and showed ammonia, HCN, siderophore and IAA synthesis (four PGP traits). This was followed by group II which included 79% of strains, positive for ammonia and indole acetic acid. Rhizobium strains isolated from lentil nodules were grouped into four PGP groups (Table 18). The PGP group I
contained four (26.7%) isolates and showed four PGP traits (ammonia, HCN, siderophore and IAA) while the group II included only one strain (*Rhizobium RL3*) which was positive for ammonia, siderophore and indole acetic acid. The group III contained 6.7% of the strains which were positive to ammonia, hydrogen cyanide and indole acetic acid. The group IV contained 60% of the strains which were positive for ammonia and indole acetic acid only. Similarly, phosphate solubilizing bacterial strains were grouped into four PGP groups (Table 19). The PGP group I contained three (30%) isolates with five PGP traits (ammonia, HCN, siderophore, IAA and phosphate solubilization) while group II had only five strains which were positive for ammonia, siderophore, indole acetic acid and phosphate solubilization. The group III contained 10% of the strains which were found to be positive for ammonia, hydrogen cyanide, indole acetic acid and phosphate solubilization. Strain PSB9 of group IV was found to be positive for ammonia, indole acetic acid and phosphate solubilization.

### 4.5 Tolerance of plant growth promoting rhizobacteria to metals and antibiotics

The selected plant growth promoting rhizobacterial strains were tested for their ability to tolerate various concentrations of heavy metals like cadmium, chromium, nickel, lead, zinc and copper using agar plate dilution method. Generally, the PGPR strains showed a varied level of tolerance to heavy metals. Among the *Mesorhizobium* strains, strain RC3 showed highest tolerance to most of the metals (Fig. 11). Strain RC3 tolerated a concentration of 400, 500, 500, 1500, 1500 and 400 |µg/ml| of cadmium, chromium, nickel, lead, zinc and copper, respectively, amended in agar plates whereas strain RC4 showed a tolerance level of 400, 400, 400, 1400, 1400 and 300 |µg/ml| to cadmium, chromium, nickel, lead, zinc and copper, respectively, added to solid plates. In contrast, strain RP5 (Fig. 12), RM8 (Fig. 13) and RL 9 (Fig. 14) of *Rhizobium* spp. (pea), *Bradyrhizobium* spp. and *Rhizobium* spp. (lentil), respectively, showed highest tolerance to most of the metals. Of these, strain RP5 showed a higher tolerance to cadmium (250 |µg/ml|), chromium (350 |µg/ml|), nickel (350 |µg/ml|), lead (1200 |µg/ml|), zinc (1500 |µg/ml|) and copper (200 |µg/ml|). Out of the 15 strains of *Rhizobium* isolated from lentil nodules, strain RL9 tolerated cadmium, chromium, nickel, lead, zinc and copper to a level of 300, 400, 500, 1400, 1000 and 300 |µg/ml|, respectively (Fig. 14), while strain RM8 showed a high tolerance of 75 |µg/ml| to cadmium, 200 |µg/ml| to chromium, 300 |µg/ml| to nickel, 1300 |µg/ml| to lead, 1500 |µg/ml| to zinc and 100 |µg/ml| to copper (Fig. 13). In comparison, among the phosphate solubilizers, the *Bacillus* spp. PSB1, PSB7 and PSB 10, tolerated most of the tested metals (Fig. 79).
Bacillus PSB1 showed a higher tolerance to cadmium, nickel and copper (400 μg/ml for each metal), chromium (500 μg/ml) and 1400 μg/ml each to lead and zinc, strain PSB7 showed a higher tolerance to cadmium and nickel (300 μg/ml for each metal), chromium and copper (400 μg/ml for each metal), 1600 μg/ml to lead and 1400 μg/ml to zinc whereas PSB10 displayed a higher tolerance of 300 μg/ml each to cadmium and copper, 550 μg/ml to chromium, 400 μg/ml to nickel, 1600 μg/ml to lead and 1400 μg/ml to zinc.

The reaction to antibiotics among metal tolerant rhizobacterial strains differed considerably (Table 14). Among *Mesorhizobium* spp., 33% strains were resistant to both nitrofurantoin and methicillin while 33% *Rhizohium* spp. isolated from lentil nodules showed resistance towards nalidixic acid and ampicillin. Among the bradyrhizobial isolates, only one isolate (RM8) was resistant to ampicillin. In comparison, none of the strains of *Rhizobium*, isolated from pea nodules were resistant to any antibiotic tested. Among the phosphate solubilizers, *Bacillus* PSB7 showed resistance to nalidixic acid (30 μg/disc) (Table 14).

**4.6 Bioassay of plant growth promoting activities**

The plant growth promoting (PGP) substances like IAA, phosphate solubilization, siderophore, hydrogen cyanide and ammonia synthesized by the selected PGPR strains were assayed both qualitatively and quantitatively under *in vitro* experiments and are explained in the following section.

**4.6.1 Indole acetic acid**

The production of IAA by the selected bacterial strains was assayed in LB broth supplemented with different concentrations of tryptophan and is given in Table 20-24. The *Mesorhizobium* spp. exhibited a substantial production of IAA after 24 h of incubation (Table 20). Moreover, the data revealed a concentration dependent increase in IAA, the maximum being 34.5, 30.6, 27.9, 26.5, 23.5 and 10.9 μg of IAA/ml in LB broth supplemented with 100, 80, 60, 40, 20 and 0 (without tryptophan) μg tryptophan /ml, respectively, by strain RC3. This was followed by strain RC4, which produced a maximum amount of 34.2, 29, 27.3, 26.1, 23.1 and 10.4 μg IAA/ml in LB broth supplemented with 100, 80, 60, 40, 20 and 0 μg tryptophan /ml, respectively. While comparing the effect of various concentrations of tryptophan on IAA production by the mesorhizobial strains, 100 μg/ ml tryptophan showed a significant (P ≤ 0.05) increase of 229 and 18% in IAA over 20 and 80 μg/ ml tryptophan, respectively, by the strain RC4. Among the *Rhizobium* (pea) isolates, strain RP5 produced a maximum amount of 20.9,
17.9, 11.8, 9.7, 5.8 and 3.9 µg IAA/ml in LB broth supplemented with 100, 80, 60, 40, 20 and 0 µg tryptophan /ml, respectively (Table 21). This was followed by strain RP7 which produced a maximum amount of 22.7, 18.8, 12.3, 7.9, 5.6 and 3.8 µg IAA/ml in LB broth supplemented with 100, 80, 60, 40, 20 and 0 mg tryptophan /ml, respectively. Among the various concentrations of tryptophan, 100 µg/ml tryptophan increased the IAA synthesis significantly (P ≤ 0.05) by 305 and 21% over 20 and 80 µg/ml tryptophan, respectively, by the strain RP7. *Bradyrhizobium* strains used in this study also produced a significant amount of IAA, the maximum being 13.3, 10.2, 7.3, 6.2, 4.7 and 3.6 µg of IAA when strain RM8 was grown in LB broth having 100, 80, 60, 40, 20 and 0 µg/ml of tryptophan, respectively (Table 22) which was followed by strain RM2 that produced a substantial amount of 12.5, 9.5, 7.2, 5.8, 3.2 and 2.4 µg IAA/ml in LB broth supplemented with 100, 80, 60, 40, 20 and 0 µg tryptophan /ml, respectively. A significant increase of 291 and 32% in IAA by strain RM2 was observed at 100 µg/ml tryptophan compared to those observed with 20 and 80 µg/ml tryptophan, respectively. In comparison, a maximum amount of 28, 21, 15, 13, 10 and 5 µg IAA/ml was synthesized by RL11, when grown in LB broth amended with 100, 80, 60, 40, 20 and 0 µg tryptophan /ml, respectively, which was followed by 33, 23, 15.2, 9.2, 6.4 and 5 µg IAA/ml (at 100, 80, 60, 40, 20 and 0 µg tryptophan /ml, respectively) by strain RL9 (Table 23). Like other rhizobial strains, strain RL9 and RL11 showed a maximum synthesis of IAA with 100 µg/ml tryptophan and increased it by 416 and 460% over 20 µg/ml tryptophan, respectively. The IAA production by phosphate solubilizing bacteria was also assayed in this study (Table 24). The IAA production increased progressively with increasing concentrations of tryptophan added to LB broth; the maximum being 19.3, 17.7 and 17.4 µg/ml (at 100 µg/ml of tryptophan), which was followed by 15.7, 10.8 and 17 (at 60 µg/ml of tryptophan) and 11.3, 5.7 and 11.7 µg ml⁻¹ of IAA (at 20 µg/ml of tryptophan) by *Bacillus sp.* PSB 1, PSB 7 and PSB 10, respectively. The IAA production by the *Bacillus* PSB1, PSB7 and PSB10 enhanced considerably by 23, 64 and 35% at 100 µg/ml tryptophan, over 60 µg/ml of tryptophan. Similarly, *Pseudomonas* PSB9 produced a considerable amount of IAA at all the tested concentrations of tryptophan, added to LB broth.

**4.6.2 Bioassay of siderophore**

Another important trait of plant growth promoting rhizobacteria is the production of siderophores that may indirectly affect the growth of plants. In the present investigation, the
PGPR strains were tested for both qualitative and quantitative production of siderophores using CAS agar (Plate 1B) and ethyl acetate extraction method. On CAS agar plates, 30% of the Mesorhizobium strains produced siderophore. Of these strains, RC1, RC3 and RC4 displayed 7, 11 and 9 mm colored zone on CAS plates after four days of incubation. Further, the ethyl acetate extraction from culture supernatant of Mesorhizobium strain RC1 yielded 15.5 and 20.5 mg/ml salicylate (SA) and 2,3-dihydroxy benzoic acid (DHBA), strain RC3 produced 17 and 24.5 mg/ml of SA and DHBA and strain RC4 yielded 16.5 and 24 mg/ml SA and DHBA, respectively (Table 20). Among the Rhizobium species isolated from pea nodules, 21% of the strains produced siderophore where strain RP3, RP5 and RP7 demonstrated 13, 11 and 15 mm colored zone on CAS plates after four days of incubation (Table 21). Further, the strains produced 24.2 and 30 (strain RP3), 24.2 and 21.2 (RP5) and 34.2 and 35.2 (RP7) mg/ml SA and DHBA, respectively. Strains RM1, RM2 and RM8 of Bradyrhizobium species showed 8, 9 and 12 mm colored zone, respectively, on CAS plates after four days of incubation and produced 15.5 and 14.1 (strain RM1), 15.8 and 15 (RM2) and 17.4 and 16.3 (RM8) mg/ml SA and DHBA, respectively (Table 22). In comparison, among the Rhizobium species isolated from lentil nodules, 33% of the rhizobial isolates produced siderophore both on CAS agar plates and in liquid culture medium (Table 23). Among these strains, strain RL2, RL9 and RL11 displayed a colored zone of 11, 12 and 10 mm size respectively, on CAS plates and yielded 15 and 13 (RL2), 15 and 18.3 (RL9) and 14 and 12 mg/ml SA and DHBA (RL11), respectively. In this study, the phosphate solubilizing bacteria (Bacillus and Pseudomonas) were also analyzed for siderophore production (Table 24). A total of 70% strains of selected phosphate solubilizing groups displayed the siderophore production on CAS agar plates and liquid culture medium. Among the phosphate solubilizers, strain PSB1, PSB7 and PSB10 of Bacillus spp. produced 13, 11 and 15 mm colored zone on CAS plates. The Bacillus spp. showed a substantial production of 13 and 16.5 mg/ml of SA and DHBA by PSB 1, 12.6 and 10 mg/ml of SA and DHBA by PSB 7 and 13.5 and 14.5 mg/ml of SA and DHBA by PSB 10, respectively (Table 24). In general, Pseudomonas did not show production of siderophores either on CAS agar plates or in liquid culture medium.

4.6.3 Phosphate solubilization on solid and liquid medium
The plant growth promoting rhizobacteria were further evaluated for their phosphate solubilizing potential, both on solid and liquid Pikovskaya medium. In the present study, about
20% of the PGPR strains showed the phosphate solubilizing activity, as detected by the formation of clear halo around their growth (Plate 1C). Among the phosphate solubilizing PGPR strains, strain PSB1, PSB 7 and PSB10 of *Bacillus* spp. produced the largest zone of P solubilization on solid Pikovskaya medium (Fig. 16) and the solubilization index (SI) ranged between 1.3 (PSB 7) and 1.36 (PSB 10) (Fig. 18). Further, a considerable amount of tri-calcium phosphate (TCP) was solubilized in liquid broth by PSB 1 (375 µg/ml), PSB 7 (340 µg/ml) and PSB 10 (379 µg/ml), respectively (Fig. 17). The solubilization of TCP was coupled with decrease in pH values (from 7 to 5) that remained identical for all the three strains (Fig. 19). Similarly, *Pseudomonas* PSB9 showed solubilization of TCP both on solid and liquid Pikovskaya medium (Fig. 16 and 17).

### 4.6.4 In vitro assay of ammonia and HCN

The plant growth promoting rhizobacterial strains were tested further for the synthesis of ammonia and hydrogen cyanide using peptone water and HCN induction medium, respectively. Generally, all PGPR strains including nitrogen fixers and phosphate solubilizers were found positive for ammonia while > 81% strains were found to be positive for HCN (Plate 1A).

### 4.7 Chromium reduction studies

A total of 15 metal tolerant PGPR strains that included *Mesorhizobium* (N= 3), *Rhizobium* specific to pea (N= 3), *Bradyrhizobium* (N= 3), *Rhizobium* specific to lentil (N= 3) and phosphate solubilizers (N=3) were tested for evaluation of their chromium reducing ability under *in vitro* conditions. This study was carried out to assess the (i) effect of different pH values on the reduction of Cr (VI) and (ii) the effect of chromate concentration on chromium (VI) reduction.

#### 4.7.1 Effect of pH on reduction of hexavalent chromium

The effect of different pH values on the reduction of chromium (VI) was variable both for phosphate solubilizers (Fig. 20) and nitrogen fixers (Fig. 24). Maximum reduction (87%) of chromium (VI) was observed at pH 7 by *Bacillus* spp. PSB 10, which was followed by PSB 1 (83%) and PSB7 (74%). Similarly, PGPR isolates *Bacillus* PSB 10, PSB1 and PSB7 reduced the chromium considerably at pH 6 (71, 67 and 58%) and at pH 8 (68, 65 and 56%), respectively, at a concentration of 100 µg Cr/ ml after 120 h of incubation. A maximum decrease in reduction of chromium (VI) was found to be 159, 24, 28 and 137% at pH 5, 6, 8 and 9 in comparison to the reduction observed at pH 7 by *Bacillus* sp. PSB 1. A maximum
decrease in chromium (VI) reduction by *Bacillus* sp. PSB 7 was found to be 147, 28, 32 and 131% in comparison to the reduction at pH 7 whereas a maximum decrease in reduction of Cr (VI) was found to be 118, 23, 28 and 129% at pH 5, 6, 8 and 9 in comparison to the reduction at pH 7 by *Bacillus* spp. PSB 10. Similarly, the maximum reduction (90%) of Cr (VI) was observed at pH 7 by *Mesorhizobium* RC3 which was followed by RC1 (84%) and RC4 (83%). The rhizobial isolates RC3, RC1 and RC4 reduced chromium (VI) considerably at pH 6 (by 75, 70 and 68%, respectively) and at pH 8 (by 73, 68 and 66 %, respectively), at 100 μg/ml Cr (VI), after 120 h of incubation. A maximum decrease in reduction of Cr (VI) was found to be 104, 20, 23 and 114% at pH 5, 6, 8 and 9 in comparison to the reduction recorded at pH 7 by RC3 and 110, 20, 24 and 121%) at pH 5, 6, 8 and 9 in comparison to the reduction observed at pH 7 by RC1. While, a maximum decrease in reduction of chromium (VI) by RC4 was determined as 113, 22, 26 and 131% at pH 5, 6, 8 and 9 in comparison to the reduction determined at pH 7.

### 4.7.2 Effect of chromate concentration

In this study, the chromium reducing ability of PGPR strains were assessed using nutrient broth (for phosphaste solubilizers) and YEM broth (for nitrogen fixers) supplemented with 50, 100 and 150 μg/ml K₂Cr₂O₇ in order to determine the effect of chromium (VI) reducing ability of the selected cultures under *in vitro* conditions. The time for total reduction of chromium (VI) increased with increase in the concentration of chromium (VI). During this study, the complete reduction of chromium (VI) occurred after 100 h by *Bacillus* spp. PSB 1 (Fig. 21), 120 h by PSB 7 (Fig 22) and 100 h by PSB 10 (Fig 23) at 50 μg/ml of chromium. A maximum increase of 21 and 75 % (by *Bacillus* sp. PSB 1), 35 and 89 % (by *Bacillus* spp. PSB 7) and 15 and 67% (by *Bacillus* spp. PSB 10) in chromium (VI) reduction was observed at 50 μg/ml chromium (VI) compared to 100 and 150 μg/ml. For nitrogen fixers, the complete reduction of chromium (VI) occurred after 120 h by *Mesorhizobium* RC1 (Fig. 25), RC 3 (Fig. 26) and RC4 (Fig 27) at 50 μg/ml Cr (VI). A maximum increase of 11 and 43 % (by RC3), 19 % and 49 % (by RC1) and 20 and 45% (by RC4) in Cr (VI) reduction was recorded at 50 μg ml⁻¹ chromium (VI) compared to 100 and 150 μg Cr/ml. The complete reduction of Cr (VI) did not occur at concentrations higher than 50 μg/ ml even after 120 h but the extent of Cr (VI) reduction at these concentrations was considerably higher for nitrogen fixers (> 60 %) and phosphate solubilizers (> 50%).

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4.8 Metal solubilization

In this study, phosphate solubilizers (*Bacillus* and *Pseudomonas*) were grown in nutrient broth, supplemented with 300 μg/ml each of lead chloride and zinc oxide and incubated for 5 days. Among the test isolates, *Bacillus* sp. PSB 1 solubilized maximum amounts of both zinc oxide (102.6 μg/ml) and lead chloride (229.9 μg/ml) which was followed by the isolate PSB 10 (98.4 and 171.8 μg/ml of ZnO and PbCl₂, respectively) and *Bacillus* PSB 7 (88.5 and 97.5 μg/ml of ZnO and PbCl₂ respectively). Moreover, *Pseudomonas* and rhizobial strains were also tested for their metal solubilizing potential but none of these strains could solubilize any of the two metals in this study (Table 30).

4.9 Growth behaviour of Plant growth promoting rhizobacteria under metal stress

In this study, a total of five PGPR strains belonging to *Bacillus* (PSB10) and nitrogen fixers (*Mesorhizobium* RC3, *Rhizobium* RP5, *Bradyrhizobium* RM8 and *Rhizobium* RL9) were grown in nutrient and YEM broth amended with different concentrations of chromium (VI), nickel, lead and zinc, in order to evaluate the toxicity of these metals to their growth. The growth response of *Bacillus* sp. PSB10 varied considerably with different concentrations of hexavalent chromium, nickel, lead and zinc (Fig. 28) in liquid culture. During the initial 72 h of growth, the maximum population was observed at all the three concentrations tested. A maximum population of 10.20, 10.19, 10.25 and 10.19 log CFU/ml was observed in liquid culture medium at 50 μg ml⁻¹ of hexavalent chromium and nickel and 300 μg/ml of lead and zinc respectively, after 72 h of incubation, compared to control. The bacterial populations at 150 μg/ml nickel decreased by 6% compared to 50 μg/ml nickel while 900 μg/ml of lead and zinc decreased the bacterial population by 5% each compared to 300 μg/ml of lead and zinc after 72 h of incubation. Similarly, the growth response of *Mesorhizobium* RC3 towards different concentrations of chromate, nickel, lead and zinc in liquid culture varied considerably (Fig. 29). During the initial 48 h of growth, the maximum rhizobial population was observed at all the three concentrations of metals tested. A maximum population of 10.46, 10.13, 10.22 and 9.94 log CFU/ml was observed at 50 μg ml⁻¹ of Cr (VI) and nickel and 300 μg ml⁻¹ of lead and zinc respectively, after 48 h of incubation, compared to control (Fig. 29). The population decreased by 3 and 6% at 150 μg/ml chromium (VI) and nickel compared to 50 μg/ml chromium (VI) and nickel while 900 μg/ml of lead and zinc decreased the population each by 6% compared to 300 μg/ml of lead and zinc after 48 h of incubation.
The effect of various concentrations of chromium (VI), nickel, lead and zinc on growth of *Rhizobium* sp. RP5 was variable (Fig. 30). A maximum increase in population of rhizobial strain following chromium (VI), nickel, lead and zinc application was observed after 36 h of growth in YEM medium. A maximum population of 9.81, 9.99, 10.12 and 9.62 log CFU/ml was observed in liquid culture medium at 50 μg ml⁻¹ of Cr (VI) and nickel and 300 μg/ml of lead and zinc respectively, after 36 h of incubation. Similarly, *Bradyrhizobium* strain RM8 grown in YEM broth amended with metals showed a variable growth pattern. A maximum rhizobial population in YEM broth was observed with the three concentrations of each metal after 48 h of incubation. A maximum population of 9.99, 9.68, 10.09 and 10.22 log CFU/ml was observed in liquid culture medium at 50 μg/ml of Cr (VI) and nickel and 300 μg/ml of lead and zinc respectively, after 48 h of incubation, compared to control (Fig. 31). The population decreased by 8 and 6% compared at 150 μg/ml Cr (VI) and nickel compared to 50 μg/ml chromium (VI) and nickel while 900 μg/ml of lead and zinc decreased the population by 8 and 9% compared to 300 μg/ml of lead and zinc after 36 h of incubation. Likewise, *Rhizobium* strain RL9, had the maximum population of 9.86, 9.99, 10.16 and 9.89 log CFU/ml when grown in YEM broth with 50 μg/ml of Cr (VI), nickel, lead and zinc respectively, after 36 h of incubation, compared to control (Fig. 32). The rhizobial growth at 150 μg/ml of Cr (VI), nickel, lead and zinc decreased by 6, 4, 4 and 7% compared to those observed at 50 μg/ml Cr, Ni, Pb and Zn after 36 h of incubation.

4.10 Plant growth promoting activities under metal stress

Metal tolerant bacterial strains were evaluated further for their PGP activities in their respective medium supplemented with different concentrations of selected metals. The bacterial strains showing higher tolerance to metals and exhibiting substantial production of plant growth promoting substances *in vitro* were included in this study.

4.10.1 Indole acetic acid production under metal stress

A total of 15 PGPR strains belonging to *Mesorhizobium* (N= 3), *Rhizobium* specific to pea (N= 3), *Bradyrhizobium* (N= 3), *Rhizobium* specific to lentil (N= 3) and phosphate solubilizers (N=3) were used in this study. The effect of three concentrations each of chromium, nickel, lead and zinc on IAA production by *Mesorhizobium* spp. was determined in LB broth supplemented with 20, 60 and 100 μg/ml of tryptophan (Table 25). Metal tolerant strains of *Mesorhizobium* spp. used in this study produced a substantial amount of plant...
growth promoting substances both in the absence and presence of metals (Table 25). The data revealed a concentration dependent increase in IAA, the maximum being 34.7 μg/ml of IAA when strain RC3 was grown in LB broth having 100 μg/ml of tryptophan and supplemented with 50 μg Cr/ml. While comparing the effect of various concentrations of tryptophan in LB broth supplemented with fixed amount of chromium (50 μg/ml) on IAA production by the strain RC3, 100 μg/ml tryptophan showed a significant increase of 46 and 22% over 20 and 60 μg/ml tryptophan, respectively. Nickel at 50 μg/ml and lead and zinc at 600 μg/ml produced a maximum amount of 34.4, 32.8 and 32.6 μg/ml of IAA, respectively in LB broth medium supplemented with 100 μg tryptophan/ml and showed a significant (P ≤ 0.05) increase of 60, 37 and 36%, respectively, over 20 μg/ml of tryptophan. The amounts of IAA produced by the rhizobial strain decreased progressively with increase in metal concentrations. Rhizobium strains specific to pea used in this study produced a substantial amount of plant growth promoting substances both in the absence and presence of metals (Table 26). A maximum amount of 20.2 and 20.9 μg/ml of IAA by strain RP5 was produced with 100 μg/ml tryptophan supplemented with 50 μg/ml each of chromium and nickel respectively. Chromium and nickel (50 μg/ml each) when added to 100 μg/ml tryptophan increased the IAA synthesis by strain RP5 by 261 and 273% respectively over 20 μg/ml tryptophan. Lead and zinc at 600 μg/ml produced a maximum amount of 20.3 and 21.1 μg/ml of IAA, respectively in LB broth supplemented with 100 μg tryptophan/ml and showed a significant (P ≤ 0.05) increase of 263 and 284% respectively over 20 μg/ml tryptophan.

Among the bradyrhizobium strains, RM8 in general displayed a maximum of 13.3 μg/ml IAA with 100 μg/ml of tryptophan but devoid of any metal. The effect of three concentrations each of chromium, nickel, lead and zinc on IAA production however, differed considerably with change in the concentrations of each metal (Table 27). Nickel at 50 μg/ml produced a maximum amount of 13.6 μg/ml of IAA at 100 μg tryptophan/ml and showed a significant increase of 183% and 81% over 20 and 60 μg/ml tryptophan, respectively. In comparison nickel at 50, lead at 600 and zinc at 300 μg/ml produced a maximum amount of 13.6, 13.6 and 13.5 of IAA at 100 μg/ml tryptophan and significantly increased the IAA production by 183, 152 and 181% over 20 μg/ml tryptophan, respectively. Generally, the production of IAA by the bradyrhizobial strain decreased progressively with increase in metal concentration but did not differ significantly among treatments. Rhizobium strains (RL9)
specific to lentil produced a maximum amount of 33.5 µg/ml IAA when grown in LB broth having 100 µg/ml tryptophan and supplemented with 50 µg Cr/ml and 100 µg tryptophan/ml and showed a significant increase of 365 and 116% over 20 and 60 µg/ml tryptophan, respectively (Table 28). Nickel and lead at 50 µg ml⁻¹ and zinc at 100 µg/ml produced a maximum amount of 36, 35.5 and 39 µg/ml of IAA, respectively, with 100 µg tryptophan/ml and significantly (P ≤ 0.05) increased the IAA by 437, 438 and 388%, respectively over 20 µg/ml tryptophan. Generally, the production of IAA by the metal tolerant rhizobial strains was greater at the lowest rates of each metal compared to control (without metal) but the amount of IAA produced at the highest tested rates of each metal was lower compared to control. In general, the heavy metals did not affect the synthesis of IAA by the rhizobial strains negatively, though it decreased marginally with increasing concentrations of heavy metals.

Metal tolerant strains of phosphate solubilizing bacteria were also tested for IAA production under metal stressed environment (Table 29). The synthesis of IAA by the P solubilizers increased consistently with increasing concentrations of tryptophan both in the presence and absence of heavy metals, but decreased progressively with increasing levels of heavy metals (Table 29). The IAA production by the three P solubilizing isolates (Bacillus PSB1, PSB7 and PSB10) under metal stress did not differ significantly. Among the three concentrations of each metal, hexavalent chromium and nickel at 50 µg/ml and lead and zinc at 300 µg/ml showed an increase of 15, 5, 16 and 16% by Bacillus strain PSB1, 14, 4, 10 and 11% by Bacillus strain PSB7 and 5, 3, 4 and 7% by Bacillus strain PSB10 in IAA, respectively, compared to those observed for 150 µg/ml each of hexavalent chromium and nickel and 900 µg/ml of lead and zinc. Like nitrogen fixers, the amounts of IAA produced by the three strains of Bacillus spp. were higher at the lower rates of each metal, which decreased progressively with increase in metal concentration at 20, 60 and 100 µg/ml of tryptophan.

4.10.2 Bioassay of siderophore under metal stress

In the present investigation, production of siderophores by the metal tolerant strains of PGPR was also determined on CAS agar plates supplemented with or without hexavalent chromium, nickel, lead and zinc (Table 25-29). Generally, the PGPR strains showed siderophore activity on metal amended CAS agar plates. Among the rhizobial isolates, Mesorhizobium strains
RC1, RC3 and RC4 produced a 7, 11 and 9 mm colored zone on CAS plates supplemented with 50 μg/ml of chromium (VI) and nickel and 300 μg/ml of lead and zinc. The size of siderophore zone produced on CAS agar plates decreased with increasing concentrations of each metal, maximum being 14% each for chromium and nickel at 150 and lead and zinc at 900 μg/ml by strain RC1 and 11% each for chromium and nickel at 150 and lead and zinc at 300 μg/ml by strain RC4, compared to 50 μg/ml of chromium and nickel and 300 μg/ml of lead and zinc, respectively. No reduction in zone size at any concentration of the tested metals was observed for strain RC3 (Table 25). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strains yielded a maximum amount of 15.5 and 20.5 mg/l SA and DHBA by RC1, 17 and 24.5 mg/l of SA and DHBA by RC3 and 16.5 and 24 mg/l SA and DHBA by RC4, respectively, grown in the Modi medium (Table 25). When, 50 μg/ml of chromium (VI) and nickel and 300 μg/ml of lead and zinc was also added to Modi medium, the strains RC1, RC3 and RC4 slightly increased the SA and DHBA compared to control. The amount of SA and DHBA in the supernatant of mesorhizobial strains decreased consistently with increasing dose of each metal. On CAS agar plates, supplemented with Cr (VI), Ni, Pb and Zn, *Rhizobium* strains RP3, RP5 and RP7 produced a 13, 11 and 15 mm colored zone in the absence and presence of chromium (VI), nickel, lead and zinc except 150 μg/ml of chromium (VI), 900 μg/ml of nickel and 300 μg/ml of lead and zinc except 150 μg/ml of chromium and nickel, 900 μg/ml of lead and zinc which inhibited the siderophore activity (Table 26). The reduction in zone size with increase in metal concentration varied between 8 (by RP3 at 150 μg/ml of chromium and nickel and 300 μg/ml of lead and zinc) to 13% (by RP7 at 150 μg/ml of chromium and nickel) after four days of incubation, in comparison to control. Further, the ethyl acetate extraction from culture supernatant of strains grown in the presence of chromium and nickel at 50 μg/ml and lead and zinc at 300 μg/ml slightly increased the SA and DHBA, in comparison to control. Moreover, chromium and nickel at 150 μg/ml and lead and zinc at 900 μg/ml respectively, slightly reduced the siderophore activity.

On CAS agar plates supplemented with selected heavy metals, the bradyrhizobial strains RM1, RM2 and RM8 produced 8, 9 and 12 mm colored zone both in the absence and presence of each metal except 100 and 150 μg/ml of chromium and nickel which displayed a 13 and 25% decrease while 900 μg/ml each of lead and zinc decreased the zone size by 13%
(by strain RM1); 150 \mu g/ml of nickel reduced it by 17% and 900 \mu g/ml of lead showed a reduction of 8% in zone size by strain RM8 after four days of incubation. Bradyrhizobial strain grown with chromium and nickel at 50 \mu g/ml and zinc and lead at 300 \mu g/ml either did not affect or slightly increased the SA and DHBA in comparison to control (Table 27). In comparison, the higher concentrations of Cr (VI) and Ni (150 \mu g/ml) and Pb and Zn (900 \mu g/ml) though decreased the SA and DHBA production by strain RM1, RM2 and RM8, but the variation among treatments was non-significant. The Rhizobium strains RL2, RL9 and RL11 specific to lentil plants produced 11, 12 and 10 mm colored zone on CAS plates in the presence of 50 \mu g/ml of chromium (VI), nickel, lead and zinc except 150 \mu g/ml chromium, 100 and 150 \mu g/ml of nickel and zinc and 150 \mu g/ml of lead, which displayed a 9% decrease in zone size by RL2; 100 and 150 \mu g/ml of chromium, nickel and zinc which showed a reduction of 10% in zone size by RL11. No reduction in zone size was observed at any concentration of selected heavy metals for strain RL9. Moreover, the ethyl acetate extraction of Rhizobium yielded a maximum amount of 15 and 13 mg/l SA and DHBA by RL2, 15 and 18.3 mg/l SA and DHBA by RL9 and 14 and 12 mg/l SA and DHBA by RL11, respectively, grown in the Modi medium devoid of each metal (Table 28). Chromium (VI) and Ni at 50 \mu g/ml and Pb and Zn at 300 \mu g/ml (except Ni at 50 \mu g/ml for SA in case of strain RL2) marginally increased the SA and DHBA by RL2, RL9 and RL11 compared to control. The amount of SA and DHBA in the supernatant of rhizobial strains specific to lentil decreased consistently with increase in each metal concentration.

On CAS agar plates, Bacillus stains showed the siderophore activity both in the absence and presence of chromium, nickel, lead and zinc (Table 29). Among the isolates, Bacillus spp. PSB 1, PSB 7 and PSB 10 produced a 13, 11 and 15 mm colored zone on CAS plates both in the absence and presence of chromium, nickel, lead and zinc except PSB 1 at 150 \mu g/ml of nickel and 900 \mu g/ml of lead and zinc which displayed a 8% decrease in zone size, PSB 7 at 150 \mu g/ml of chromium, 100 and 150 \mu g/ml of nickel, 600 and 900 \mu g/ml of lead and zinc displayed a 9% decrease and PSB10 at 600 and 900 \mu g/ml of zinc decreased siderophore activity by 7% respectively, when grown on CAS plates. The ethyl acetate extraction from culture supernatant yielded 13 and 16.5 mg/l of SA and DHBA by Bacillus spp. PSB 1, 12.6 and 10 mg/l of SA and DHBA by Bacillus spp. PSB 7 and 13.5 and 14.5 mg/l of SA and DHBA by Bacillus spp. PSB 10, respectively (Table 34). In contrast, chromium and nickel at
50 and lead and zinc at 300 μg/ml (except zinc at 300 μg/mL in case of PSB7) marginally increased the SA and DHBA by *Bacillus* PSB 1, PSB 7 and PSB 10 compared to control.

### 4.10.3 *In vitro* assay of ammonia and HCN under metal stress

All the bacterial strains were further tested for HCN and ammonia production under *in vitro* conditions in the presence of different concentrations of selected heavy metals. In the presence of three concentrations of Cr (VI), Ni, Pb and Zn, the HCN and ammonia was produced by *Mesorhizobium* (Table 25), *Rhizobium* specific to pea (Table 26), *Bradyrhizobium* (Table 27), *Rhizobium* specific to lentil (Table 28) and phosphate solubilizing strains of *Bacillus* (Table 29) were found positive for both HCN and ammonia.

### 4.10.4 Phosphate solubilization as influenced by heavy metals

The phosphate solubilizing potentials of the PGPR strains in the presence of varying concentrations of Cr (VI), Ni, Pb and Zn was assayed both qualitatively and quantitatively using solid and liquid Pikovakaya medium (Table 30). In this study, the phosphate solubilizing bacteria only belonging to genera *Bacillus* were used. About 20% of the *Bacillus* strains showed the phosphate solubilizing activity, as detected by the formation of clear halo around their growth. Among the *Bacillus* strains, *Bacillus* PSB1, PSB 7 and *Bacillus* PSB10 produced a largest zone of P solubilization on solid Pikovskaya medium (Table 30) devoid of chromium, nickel, lead and zinc (plate) whose solubilization index (SI) ranged between 1.3 (*Bacillus* PSB 7) and 1.36 (*Bacillus* PSB 10). In contrast, the zone of solubilization by PSB1 decreased by 25% with 150 μg/ml of chromium, 900 μg/ml of lead and zinc and by 50% with 150 μg/ml of nickel; 33 % with chromium and nickel at 150 μg/ml and 900 μg/ml of lead and zinc by PSB 7 and 20% with chromium and nickel at 150 μg/ml and lead and zinc at 900 μg/ml by PSB10, respectively when cultures were grown in Pikovskaya medium compared to control. Further, a considerable amount of tri-calcium phosphate was solubilized in liquid broth by *Bacillus* PSB 1 (375 μg/ml), *Bacillus* PSB 7 (340 μg/ml) and *Bacillus* PSB 10 (379 μg/ml). In general, the amount of P solubilized decreased consistently with increase in the concentration of chromium, nickel, lead and zinc. A maximum decrease in P solubilization was 17% by PSB 1 at 150 μg/ml of chromium and nickel and 15 and 18% at 900 μg/ml of lead and zinc, respectively; decrease in solubilization at 150 of chromium and 900 μg/ml of zinc was 15%, 18% at 150 μg/ml of nickel and 13% at 900 μg/ml of lead by *Bacillus* PSB7 while for *Bacillus* PSB 10,
the decrease in P solubilization was 9% at 150 of chromium and 900 \( \mu g/ml \) of zinc, 12% at 150 \( \mu g/ml \) of nickel and 10% at 900 \( \mu g/ml \) of lead, compared to control (Table 30).

4.11 Metal solubilization

Among the test isolates, *Bacillus* sp. PSB 1 solubilized maximum amounts of both ZnO (102.6 \( \mu g/ml \)) and PbCl\(_2\) (229.9 \( \mu g/ml \)) which was followed by the isolate PSB 10 (98.4 and 171.8 \( \mu g/ml \) of ZnO and PbCl\(_2\), respectively) and *Bacillus* PSB7 (88.5 and 97.5 \( \mu g/ml \) of ZnO and PbCl\(_2\), respectively) when grown in the absence of chromium, nickel, lead and zinc (Table 30). In comparison, maximum solubilization of ZnO and PbCl\(_2\) by *Bacillus* PSB1 was observed at 50 \( \mu g/ml \) of chromium and nickel, 600 \( \mu g/ml \) of lead (solubilization of ZnO) and 300 \( \mu g/ml \) of lead (solubilization of PbCl\(_2\)) and 300 \( \mu g/ml \) of zinc (solubilization of PbCl\(_2\)). *Bacillus* PSB7 showed maximum metal solubilization activity at 50 \( \mu g/ml \) of chromium and nickel and 600 \( \mu g/ml \) of lead (solubilization of ZnO) and 300 \( \mu g/ml \) of lead (solubilization of PbCl\(_2\)) and 300 \( \mu g/ml \) of zinc (solubilization of ZnO) and 600 \( \mu g/ml \) of zinc (solubilization of PbCl\(_2\)).

However, the three concentrations of four metals did not show any significant reduction in metal solubilization by any of the three *Bacillus* strains, compared to control (Table 30).

4.12 Heavy Metal Toxicity To Legumes

4.12.1 Chickpea

4.12.1.1 Plant growth

The effect of heavy metals on chickpea crop, grown in unsterilized pot soil was variable and metal concentration dependent (Plate 2). Among the single metal treatments, cadmium was found to be the most phytotoxic and significantly (\( P < 0.05 \)) reduced the plant growth at all the three concentrations. A maximum reduction of 60, 25 and 28% in root length, 43, 17 and 32% in shoot length, 66, 46 and 43% in dry root weight, 16, 39 and 34% in dry shoot weight (Table 31) and 43, 14 and 36% in total dry matter production (Table 32) at 60, 90 and 135 DAS, respectively, was observed with cadmium at 24 mg kg\(^{-1}\) soil, which was followed by the application of zinc (9780 mg/kg soil) to soils that substantially reduced the measured parameters. Chromium at 34 mg/kg increased the total biomass by 5% at 60 DAS; at 68 mg/kg increased the biomass by 48% (90 DAS) and at 136 mg/kg increased the total dry weight of...
chickpea by 22% (at 135 DAS), compared to control. Generally, a dose dependent decrease in dry matter production was observed at all the stages of growth following metal treatment to soil. Lead at 97.5 mg/kg increased the dry matter accumulation by 42 (90 DAS) and 23% (135 DAS) while 2445 mg/kg of zinc and 669 mg/kg of copper added to soil, increased the dry biomass by 23% at 90 DAS, compared to control. Among the dual metal combinations, chromium with nickel (34+145 mg/kg soil) had the largest stimulatory effect on chickpea plants which showed an increase of 27% in total dry matter accumulation at 90 DAS, compared to control but was less than those observed for either chromium or nickel, applied at the same dose rates (Table 32). The plant growth was reduced even further when cadmium was used in combination with chromium, nickel and lead. Combination treatment of cadmium with nickel (24 mg Cd and 580 mg Ni/kg) decreased the root length, shoot length, root dry mass, shoot dry mass (Table 31) and total dry biomass production (Table 37) significantly (P ≤ 0.05) by 70, 53, 72, 31 and 54% at 60 DAS, 32, 25, 59, 24 and 11% at 90 DAS and 36, 41, 52, 40 and 43% at 135 DAS, respectively, compared with the control plant. When cadmium (24 mg/kg) was applied along with chromium (136 mg/kg) and nickel (580 mg/kg), declined the dry matter by 58, 53 and 59% at 60, 90 and 135 DAS, over control (Table 32). The multiple metal application of lead + zinc + copper (390 + 9780 + 1383 mg/kg soil), showed an increase of 5 and 2% at 60 DAS and 10% each at 135 DAS, respectively, in dry matter production over combination of Cd + Cr (24 + 136 mg/kg soil) and cadmium + nickel (24 + 580 mg/kg soil), respectively. In general, the plant growth increased with plant age but decreased with increasing concentration of each metal, used either singly or in combination treatments. Generally, the combination of two or three metals showed enhanced phytotoxic effect relative to the single metal application treatments.

4.12.1.2 Symbiotic traits and flowering

The nodulation response to the three rates of heavy metals at 60, 90 and 135 DAS differed among treatments (Table 32). Generally, the large sized nodules were produced on the main roots while the small size nodules were scattered all through the adventitious roots (Plate 9C). Comparison between the metal free control and each metal treatment revealed an increase of 23 (at 34 mg Cr/kg and 136 mg Cr/kg) to 54% (at 68 mg Cr/kg) in the number of nodules per plant at 60 DAS and 22 (34 mg Cr/kg) to 44% (at 136 mg Cr/kg) at 90 DAS, compared to control. Similarly, lead at 97.5 mg/kg soil, significantly increased the number of nodules per
plant at 90 DAS at 90 DAS by 18 and 70% over chromium (136 mg/kg) and control (27 nodules/plant), respectively. The number of nodules per plant decreased considerably with cadmium, nickel, zinc and copper at both the stages of plant growth and were greatly influenced by the concentration of metals applied. Among the single metal treatments, cadmium showed a profound toxic effect on symbiosis and reduced the number of nodules per plant by 54 (at 6 mg/kg), 62 (at 12 mg/kg) and 69% (at 24 mg/kg) at 60 DAS, while at 90 DAS, it reduced the number of nodules per plant by 7, 15 and 22% at the same rate of application, respectively. Similarly, the dual metal treatments, except the mixture of chromium + lead, decreased the number of nodules per plant significantly. Among the dual metal application treatments, cadmium with chromium (at 24 + 136 mg/kg soil) and cadmium with nickel (136 + 580 mg/kg soil), resulted in the largest adverse effect as did the mixtures of cadmium + chromium + nickel (24 + 136 + 580 mg/kg soil), reduced the number of nodules by 77% at 60 DAS and by 26, 33 and 52%, at 90 DAS, respectively, compared to 13 and 27 nodules/plant observed at 60 and 90 DAS in control treatment. In contrast, Cr with Pb (at 34 + 97.5 mg/kg) enhanced the number of nodules per plant by 8 and 19% at 60 and 90 DAS, respectively while 136 and 390 mg/kg of chromium and lead respectively increased the number of nodules per plant by 7% only at 90 DAS, compared to control plants. In comparison, the triple metal treatment showed greatest adverse effect on nodulation compared with either the control plants or dual metal treatments. The reduction in nodulation was accompanied by a significant decrease in dry mass of nodules. Generally, the metal impact was more profound at double the normal concentration of the dual or tripple metal combinations, compared with the lower rates tested in this study. The nodule numbers was positively correlated with nodule mass at 60 DAS ($r = 0.14$) and at 90 DAS ($r = 0.20$) with all the metals and all the concentrations.

Flowering in chickpea was delayed significantly (P ≤ 0.05) at the highest metal concentrations tested, relative to the control plant. Among the single metal treatments, cadmium, zinc and copper showed greatest adverse effect at half (0.5 x), normal (1 x) and double (2 x) the normal concentration and delayed the flowering significantly compared with the control plants. In comparison, the half and normal concentration of chromium, nickel and lead did not delay the flowering in chickpea plants significantly. A maximum delay in flowering occurred at all the tested concentrations of cadmium with nickel which was followed by cadmium with chromium. Among the multiple metal combinations, cadmium + chromium +
nickel significantly delayed the flowering by 73 (6 + 34 + 145 mg/kg soil), 74 (12 + 68 + 290 mg/kg soil) and 77 days (24 + 136 + 580 mg/kg soil), compared with the 65 days observed for control plant (Table 32).

4.12.1.3 Chlorophyll and leghaemoglobin content

The effect of sole heavy metals and heavy metal mixtures on chlorophyll content of foliage and leghaemoglobin at 60 and 90 DAS consistently declined with increasing rates of metals, but was only significant at the three rates of cadmium, normal rates of zinc (4890 mg/kg soil) and double the normal rates of nickel (580 mg/kg soil), zinc (9780 mg/kg soil) and copper (1338 mg/kg soil) (Table 33). Among the single metal, cadmium was found to be the most toxic and reduced the chlorophyll content at 24 mg/kg soil by 56 and 47% at 60 and 90 DAS, respectively, compared to control (0.91 and 0.99 mg/g at 60 and 90 DAS, respectively). In contrast, the chlorophyll content in foliage of chickpea plants increased by 7% at 390 mg/kg of lead at 60 DAS compared to control. Generally, the chlorophyll contents in fresh foliage increased progressively with increasing concentration of lead at 60 DAS which decreased consistently with increasing dose of lead at 90 DAS, compared to control. However, a 9% increase in chlorophyll content was observed at 90 DAS compared to those recorded at 60 DAS for control treatments. Among the composite metal treatments, cadmium with nickel at 6 + 145, 12 + 290 and 24 + 580 mg/kg soil showed the greatest adverse effect on chlorophyll content and decreased it by 49, 62 and 63% respectively at 60 DAS and by 43, 49 and 53% at 90 DAS, respectively, compared to control. The mixtures of cadmium + chromium + nickel (24 + 136 + 580 mg/kg soil), reduced the chlorophyll contents in the foliage by 71% at 60 DAS and by 59% at 90 DAS, relative to the control. Cadmium at 24 mg/kg soil reduced the leghaemoglobin by 42 and 40% at 60 and 90 DAS, respectively, compared to control. In contrast, the leghaemoglobin content increased significantly at 136 mg/kg of chromium by 17 % at 60 DAS and 20% at 90 DAS, respectively over control. Among the composite metal treatments, cadmium with nickel at 6 + 145, 12 + 290 and 24 + 580 mg/kg soil showed the greatest adverse effect on the synthesis of leghaemoglobin and decreased it by 25, 33 and 58% respectively, at 60 DAS and by 33, 40 and 53% at 90 DAS, respectively, compared to control (0.12 and 0.15 m mol/g.f.m at 60 and 90 DAS, respectively). The mixtures of cadmium + chromium + nickel (24 + 136 + 580 mg/kg soil), reduced the leghaemoglobin contents by 67% at 60 DAS and by 60% at 90 DAS, compared to the control (Table 33).
4.12.1.4 N content, seed yield and grain protein

The effect of metal treatments on the N contents of roots and shoots at 60, 90 and 135 DAS differed among treatments (Table 33). The maximum reduction in root N content occurred at all the three concentrations of cadmium, 145 mg/kg of nickel, 2445 mg/kg of zinc and 669 and 1338 mg/kg of copper, relative to the control. Lead at 195 mg/kg soil increased the root N by 16 and 12% at 90 and 135 DAS respectively, while lead at 390 mg/kg soil significantly increased the root N content by 10% at 60 DAS while chromium at 136 mg/kg soil increased the root N content by 4, 9 and 4% at 60, 90 and 135 DAS, compared to control. On the contrary, cadmium at 24 mg/kg soil statistically (0.5 x) reduced the root N content by 33, 22 and 29% at 60, 90 and 135 DAS, respectively, relative to the control. The N content in roots increased consistently with increasing rates of combination of chromium + lead, chromium + zinc and nickel + lead at 60 DAS only, compared to those observed for control plants (28.7 mg/g at 60 DAS). Generally, the maximum reduction in N content was observed with dual or multiple metal application treatments relative to the control. Among the dual metal treatments, cadmium + nickel at 24 + 580 mg/kg soil significantly (0.5 x) decreased the root N content by 39% at 60 DAS and cadmium + lead at 24 + 390 mg/kg soil decreased the root N content by 43% at 90 DAS and 40% at 135 DAS, respectively, compared to control. In comparison, the multiple metal combination of cadmium + chromium + nickel at 24 + 136 + 580 mg/kg soil reduced the root N content by 41, 58 and 46% at 60, 90 and 135 DAS, respectively, compared to control plants. Similarly, the maximum reduction in N content in shoots occurred at double the normal concentration of all metal treatments. The toxicity of the metals on shoot N content increased with increasing rates of all metals, except the three concentrations of lead which either did not increase or decrease the shoot N at any of the stages of plant growth. The mixtures of chromium + lead, though consistently increased the N contents with increasing rates at 60, but was comparatively less than the control, compared to control. The N content of the plant roots was more severely affected than the N content of the shoots, at all the concentrations of the metals used. Generally, the root and shoot N decreased with plant age but the N contents in shoots was higher than roots at 60 (by 12%), 90 (by 47%) and 135 DAS (by 41%) in untreated control (Table 33).

The effect of heavy metals on seed yield varied considerably among treatments (Table 33). Seed yield decreased consistently for each metals, used either singly or in combination but
was only significantly \((P \leq 0.05)\) reduced at double the normal concentration of all metals (except chromium and lead) and half \((0.5 \times)\) and normal \((1 \times)\) concentration of cadmium, zinc and copper. Among the single metal treatments, chromium, and lead consistently and significantly increased the grain yield, relative to the control plants. The average maximum increase of 12.9\% and 9\% was observed with lead at 97.5 and 195 mg/kg respectively, which was followed by an increase of 11\% and 9\% by chromium at 34 and 68 mg/kg respectively, compared with those obtained for metal free but inoculated control \((5.4 \, g/plant)\). Among the dual metal treatments, cadmium with nickel had the highest adverse effect on grain yield and decreased it significantly by 17 \((6 + 145 \, mg/kg)\), 20 \((12 + 290 \, mg/kg)\) and 28\% \((24 + 580 \, mg/kg)\). In comparison, the combination of chromium with nickel and chromium with lead marginally increased the seed yield at half and normal concentrations, compared with the control treatment. Grain yield decreased with multiple metal application that ranged between 19 \((6 + 34 + 145 \, mg/kg \) of Cd with Cr and Ni to 33\% \((24 + 136 + 580\) of cadmium with chromium and nickel) and 11 \((97.5 + 2445 + 334.5 \, mg/kg \) of lead, zinc and copper) to 26\% \((390 + 9780 + 1338 \, mg/kg \) of lead, zinc and copper), compared to control. While comparing the sum of mean values of three rates of each metal treatment, the order of toxicity on seed mass increased in the following order: lead > chromium > nickel > copper > zinc > cadmium.

The effect of three doses of single, double and triple metal treatments on grain protein \((GP)\) was variable \((Table 33)\). The average maximum GP \((256 \, mg/g)\) was obtained at 390 mg kg\(^{-1}\) of lead and was significantly \((P \leq 0.05)\) greater than those obtained for inoculated but metal free control \((242 \, mg/g)\). In comparison, double the normal concentration of all metal treatments significantly decreased the grain protein. Cadmium, zinc and copper, when used alone, significantly decreased the protein contents in grains at half \((0.5 \times)\) and normal concentrations \((1 \times)\). Among the double metal treatments, the mixture of cadmium + nickel declined the grain protein by 10\% at 6 + 145 mg/kg of cadmium + nickel and 14\% at 12 + 290 mg kg\(^{-1}\) of cadmium + nickel, respectively, relative to the control. Among all metal treatments, the mixtures of cadmium + chromium + nickel and lead + zinc + copper resulted in the highest decrease in grain protein at double the normal concentrations, compared with the control. The decrease in GP ranged between 25 \((390 + 9780 + 1338 \, mg/kg \) of Pb + Zn + Cu) and 27\% \((24 + 136 + 580 \, mg/kg \) of Cd + Cr + Ni), compared with the control.
4.12.1.5 Phyto-accumulation of heavy metals

The uptake of metals by the roots and shoots at 60, 90 and 135 DAS and grains at 135 DAS of chickpea plants differed again considerably. The accumulation of metals in roots, shoots and grains were influenced greatly by the concentration of each metal tested. A higher amount of metal was found in plant parts (e.g. roots, shoots and grains) when these metals were applied to non-sterilized sandy clay loam soil individually compared with the levels obtained for double or triple metal treatment. Metal uptake by the roots, shoots and grains was found to be directly related to the heavy metal applied. Among the single metal treatments, the concentration of cadmium (Fig. 33), Cr (Fig. 34), Ni (Fig. 35), Pb (Fig. 36), Zn (Fig. 37) and Cu (Fig. 38) was higher in roots of chickpea plants raised in soil treated with 24, 136, 580, 390, 9780 and 1338 mg/kg of Cd, Cr, Ni, Pb, Zn and Cu respectively, compared to shoots and grains measured at 60, 90 and 135 DAS, respectively. A greater uptake of zinc was observed in roots (3104 µg/g), shoots (2070 µg/g) and grains (935 µg/g) at 135 DAS when 9780 mg/kg of zinc was added to soil, compared to other single metal treatments. In comparison, the concentration of cadmium was maximum i.e. 2.5, 4.7 and 9.2 µg/g in roots and 0.8, 1.5 and 1.8 µg/g in shoots at 60 (Fig. 39), 90 (Fig. 40) and 135 DAS (Fig. 41) at 24 + 136 mg/kg of Cd + Cr. Similarly, the concentration of Cd and Ni was higher in roots and shoots at 60 (Fig. 42), 90 (Fig. 43) and roots, shoots and grains at 135 DAS (Fig. 44) when soil was treated with 24 + 580 mg/kg of Cd and Ni. A similar pattern for metal uptake by plant organs was observed for Cd with Pb (Fig. 45, 46 and 47), Cr with Ni (Fig. 48, 49, 50), Cr with Pb (Fig. 51, 52, 53), Cr with Zn (Fig. 54, 55, 56), Ni with Pb (Fig. 57, 58, 59), Ni with Zn (Fig. 60, 61, 62) and Pb + Zn + Cu (Fig. 63, 64, 65). The uptake of metals by roots, shoots and grains were positively correlated. The accumulation of nickel, zinc and copper in roots, shoots and grains of chickpea plants following double and triple metal application differed considerably. Generally, the phytoaccumulation of heavy metals was higher in roots, compared with the shoots and grains at all the rates of metals, applied in double or triple application treatments. The uptake of Cd by root and shoot at 60 DAS was positively correlated at 0.5x (r = 0.36), 1x (r = 0.35) and 2x (r = 0.41). Similarly the Cr uptake by roots and shoots was positively correlated at 0.5x (r= 0.42), 1x (r= 0.40) and 2x (r= 0.41) and the uptake of Ni by roots and shoots at 60 DAS was also correlated at 0.5x (r= 0.36), 1x (r= 0.31) and 2x (r= 0.38).
4.12.2 Greengram

4.12.2.1 Plant growth

4.12.2.1.1 Root and shoot length

The effect of three concentrations of cadmium (6, 12 and 24 mg/kg soil), chromium (34, 68 and 136 mg/kg soil) and copper (334.5, 669, 1338 mg/kg soil), applied separately and in combinations, on greengram plants differed among treatments (Plate 5). Generally, the length of plants decreased consistently with increasing rates of metals (except chromium) but increased progressively with plant age. Among the single metal treatments, cadmium was found as the most phytotoxic and reduced the root length significantly ($P < 0.05$) by 32, 32 and 37% at 50 days after sowing (DAS) and by 36, 41 and 41 % at 80 DAS with 6, 12 and 24 mg/kg soil, respectively, followed by copper, which reduced the root length by 5, 21 and 26% at 50 DAS and 9, 18 and 27% at 80 DAS, respectively, at the same dose rates compared to control. Comparison between the three dose rates of cadmium revealed a significant decrease of 8 (at 50 DAS) and 7% (at 80 DAS) in length of roots per plant at 24 mg Cd/kg soil, compared to those observed at 6 mg/kg soil. Similarly, copper at 1338 mg/kg soil reduced the length of roots per plant by 22 and 26 % (at 50 DAS) and 20 and 27% (at 80 DAS) compared to 334.5 mg/kg of copper applied to soils and untreated control, respectively. In contrast, chromium at 34 and 68 mg/kg soil increased the root length by 11 and 37% at 50 DAS and 9 and 32% at 80 DAS, respectively, above the control. A maximum increase of 24 and 21% in root length was observed at 50 and 80 DAS, respectively, when soil was treated with 68 mg Cr/kg soil compared to 34 mg Cr/kg soil. The root length was reduced even further when cadmium was used in combination with chromium or copper at all the three concentrations. Cadmium with Cu (at 24 and 1338 mg/kg soil) was the most phytotoxic combination and reduced the root length by 47 (at 50 DAS) and 50 % (80 DAS), compared to control plants.

Cadmium at 6,12 and 24 mg/kg soil showed the greatest toxicity and decreased the shoot length significantly ($P \leq 0.05$) by 12, 24 and 29% (at 50 DAS) and 10, 15 and 25 % (at 80 DAS), followed by copper, which reduced the shoot length by 6, 12 and 18% at 50 DAS and 5, 15 and 25% at 80 DAS, respectively, compared to control. While comparing the effect of three concentrations of cadmium, a significant decrease of 20 (at 50 DAS) and 17% (at 80 DAS) in length of shoots per plant was recorded at 24 mg Cd/kg soil, compared to those observed for 6...
mg Cd/kg soil. Similarly, copper at 1338 mg/kg soil reduced the length of shoots per plant by 13 and 18% (at 50 DAS) and 21 and 25% (at 80 DAS) compared to 334.5 mg/kg of copper and metal free control. The shoot length reduced even further when cadmium was used in combination with chromium and copper at all the three concentrations, the maximum being 41 and 35 % when cadmium was used with copper at 24 and 1338 mg/kg soil at 50 and 80 DAS, respectively, compared to control plants. In comparison, 34 and 68 mg Cr/kg soil increased the shoot length by 6 and 53% (at 50 DAS) and 30 and 40% (at 80 DAS), respectively, relative to the control. The shoot length was augmented by 10% at 80 DAS, even, with 136 mg/kg of chromium, compared to control. A maximum increase of 44 and 8% in shoot length was observed at 50 and 80 DAS, respectively, when soil was treated with 68 mg/kg soil compared to 34 mg Cr/kg soil (Table 34).

4.12.2.1.2 Dry biomass production

The phytotoxicity of cadmium, chromium and copper to dry biomass production by plant organs (roots and shoots) and total dry matter accumulation in greengram plants consistently decreased with increasing concentration of metals except chromium, applied separately or as mixture with copper (Table 34). Of the single metal treatments, cadmium at 6, 12 and 24 mg/kg soil decreased the dry matter accumulation in roots by 20, 25 and 40% at 50 DAS and 17, 29 and 33% at 80 DAS, respectively, compared to plants grown in metal free soils. This was followed by copper which reduced the dry matter accumulation in roots by 10 and 15% at 50 DAS and 8 and 17% at 80 DAS at 669 mg/kg and 1338 mg/kg soil, compared to control. Among the three dose rates of cadmium, a significant decrease of 25 (at 50 DAS) and 20% (at 80 DAS) in dry matter accumulation in roots was determined at 24 mg Cd/kg soil, compared to those observed at 6 mg/kg soil. Similarly, copper at 1338 mg/kg soil strongly inhibited the dry matter accumulation in roots and reduced it by 15 (at 50 DAS) and 17% (at 80 DAS) compared to 334.5 mg/kg of copper applied to soils. In contrast, chromium at 34, 68 and 136 mg/kg soil increased the root dry weight by 65, 80 and 85% at 50 DAS and 46, 54 and 75% at 80 DAS, respectively, above the control. A maximum increase of 9 and 6% in dry matter accumulation in roots was observed at 50 and 80 DAS, respectively, when soil was treated with 68 mg/kg soil compared to 34 mg Cr/kg soil. Similarly, the dry matter accumulation in shoots decreased progressively with the increase in the concentration of all metals except chromium, which consistently increased the dry mass of shoots, as observed for roots. Cadmium at 6, 12 and 24
mg/kg soil decreased the shoot dry weight by 18, 22 and 26% at 50 DAS and 11, 15 and 19% at 80 DAS, compared to control plants. This was followed by copper which reduced the dry matter accumulation in shoots by 18% at 50 DAS and 20% at 80 DAS with 1338 mg/kg soil, compared to control. In contrast, chromium at 34, 68 and 136 mg/kg soil increased the shoot dry weight by 63, 100 and 133% at 50 DAS and 61, 95 and 144% at 80 DAS, respectively, above the control. A trend similar to those observed for increase/decrease in roots at higher concentration of each combination of metal compared to the lower tested rates of each metal was also observed for dry matter accumulation in shoots at both the growth stages.

Similarly, cadmium was found as the most phytotoxic metal and reduced the total dry matter accumulation significantly (P ≤ 0.05) by 18, 22 and 27 % (at 50 DAS) and 13, 17 and 21% (at 80 DAS), at 6,12 and 24 mg kg⁻¹ soil, respectively compared to control (273 and 290 mg/plant at 50 and 80 DAS). This was followed by copper which decreased the total dry matter by 18% at 50 DAS and 20% at 80 DAS at 1338 mg/kg soil, compared to control. Comparison between the three dose rates of cadmium revealed a significant decrease of 12 (at 50 DAS) and 11% (at 80 DAS) in total dry matter at 24 mg Cd/kg soil, compared to those observed for 6 mg/kg soil. Similarly, copper at 1338 mg/kg soil reduced the total dry matter accumulation by 15 (at 50 DAS) and 18% (at 80 DAS) compared to 334.5 mg/kg of copper applied to soils. In contrast, chromium at 34, 68 and 136 mg/kg soil increased the total dry matter production 0.6, 1 and 1.3 fold (at 50 DAS) and 0.6, 0.9 and 1.4 times (at 80 DAS), respectively, relative to the control. A maximum increase of 43 and 49% in total dry matter accumulation was observed at 50 and 80 DAS, respectively, when soil was treated with 136 mg/kg soil compared to 34 mg Cr/kg soil. The total dry matter accumulation was reduced even further when cadmium was used in combination with chromium or copper at all the three concentrations. The reduction in dry biomass of greengram following mixtures of metals ranged between 24 (Cd with Cr at 6 and 34 mg/kg soil) to 41% (Cd with Cu at 24 and 1338 mg/kg), above the control at 50 and 80 DAS, respectively. In contrast, the combination of Cr and Cu (136 and 1338 mg/kg soil) increased the dry matter by 31 and 26%, at 50 and 80 DAS respectively, relative to the control.

4.12.2.2 Symbiotic traits

4.12.2.2.1 Nodule numbers

Nodulation response to the three concentrations of cadmium, chromium and copper at 50 and 80 days after sowing varied considerably (Plate 9B). Comparison between the metal free
control and each metal treatment, revealed a significant increase in the number of nodules per plant following 34, 68 and 136 mg Cr/kg soil at pod fill stage (50 DAS) and at harvest (80 DAS), while cadmium at 6, 12 and 24 mg/kg soil and 334.5, 669 and 1338 mg Cu/kg soil reduced the number of nodules considerably (Table 34). Among the single metal treatments, cadmium and copper at 24 and 1338 mg/kg soil declined the number of nodules per plant by 38 and 23% at pod fill stage and 36 and 27% at harvest, respectively, compared to control (13 and 11 at 50 and 80 DAS, respectively). Among the three dose rates of cadmium, a significant decrease of 27 (at 50 DAS) and 30% (at 80 DAS) in nodule numbers per plant was observed at 24 mg Cd/kg soil, compared to those recorded at 6 mg/kg soil. Similarly, copper at 1338 mg/kg soil reduced the number of nodules by 17 (at 50 DAS) and 27% (at 80 DAS) compared to 334.5 mg/kg of copper applied to soils. In contrast, chromium at 136 mg Cr/kg soil significantly (P < 0.05) increased the number of nodules by 100%, each at pod filling and at harvest stage, in comparison to control. A maximum increase of 29% in number of nodules per plant was observed at 80 DAS when soil was treated with 136 mg/kg soil compared to 34 mg Cr/kg soil. Similarly, the mixture of heavy metals at all concentrations except chromium applied with copper (at 34 and 334.5 mg/kg soil) decreased the number of nodules per plant at pod fill stage, compared to control. Among the metal combinations, when cadmium was used with copper at 24 and 1338 mg/kg soil showed a largest adverse effect and significantly (P < 0.05) reduced the number of nodules per plant at pod fill stage and at harvest by 62 and 64%, respectively, above the control. The reduction in nodulation was accompanied by significant decrease in dry matter accumulation in nodules as well (Table 34). Among the single metal treatments, cadmium at 24 mg/kg soil decreased the dry weight of nodules both at pod fill and harvest stage by 33 and 42%, respectively, compared to control. This was followed by copper which reduced the total nodule matter by 11 and 22% at 50 DAS and 25 and 42% at 80 DAS at 669 and 1338 mg/kg soil respectively, compared to control. In comparison, chromium at 136 mg Cr/kg soil significantly (P < 0.05) increased the dry matter accumulation in nodules by 233 and 108% at pod filling stage and at harvest, respectively, compared to the control. The combination treatments of cadmium and copper (at 24 and 1338 mg/kg soil) declined the dry mass of nodules by 44 and 50% at pod fill (50 DAS) and harvest (80 DAS) stage, respectively, over control. A trend similar to those observed for increase/decrease at higher concentration of each metal compared
to the lower tested rates of each metal was also observed for nodule dry mass at both the
growth stages of greengram plants.

4.12.2.3 Chlorophyll and leghaemoglobin content

The effect of three concentrations of Cd, Cr and Cu on chlorophyll and leghaemoglobin content
in foliage and nodules, measured at pod fill stage (50 DAS) is presented in Table 35. The
chlorophyll content in fresh foliage consistently declined with increasing rates of metals but
was significant (P ≤ 0.05) only at 12 and 24 mg/kg of cadmium and 1338 mg/kg of copper
(Table 35). Of the single metal treatments, cadmium at 24 mg/kg declined the chlorophyll
content by 10 and 20%, compared to 6 mg Cd/kg and control (0.83 mg/g), respectively.
Conversely, chromium at 34 and 68 mg/kg, increased the chlorophyll content marginally,
compared to control. The chlorophyll content was reduced even further when cadmium was
used in combination with chromium and copper at all the three tested concentrations, the
maximum being 26%, when cadmium was used with copper (at 24 and 1338 mg/kg), compared
to control plants. The nodules collected from the root system of greengram plants, raised in soil
treated with cadmium and copper had considerably a lower concentration of leghaemoglobin.
Cadmium at 24 and copper at 1338 mg/kg soil showed 0.05 and 0.06 m mol/gfm of
leghaemoglobin and decreased it by 38 and 25% respectively, compared to control (0.08 m
mol/gfm). Conversely, the leghaemoglobin content in fresh nodules at 50 DAS was increased
by 50 % at 136 mg Cr/kg soil. A maximum increase of 9% in leghaemoglobin content was
observed at 50 DAS when soil was treated with 136 mg/kg soil compared to 34 mg Cr/kg soil.
Levels of leghaemoglobin content in combined metal treatments were significantly decreased
compared to single metal treatment and control (Table 35). A maximum reduction of 50 % in
leghaemoglobin content in nodules was observed with 12 and 669 and 24 and 1338 mg kg⁻¹ soil
of cadmium – copper (0.04 m mol/gfm).

4.12.2.4 N content

The effects of three concentrations of cadmium, chromium and copper on N content in roots
and shoots at 50 and 80 days after seeding was variable (Table 35). The average maximum
decline in root N at 50 and 80 DAS following single metal occurred at 24 mg Cd/kg (35 and 30
mg N/g, respectively) and at 1338 mg Cu/kg (36 and 32 mg N/g, respectively) (Table 35).The
root N were decreased significantly (P ≤ 0.05) by 22 and 25% respectively, at 50 and 80 DAS,
respectively by cadmium (24 mg/kg soil) and 20% each at 50 and 80 DAS by copper (1338
mg/kg soil), above the control. Comparison between the three dose rates of cadmium revealed a significant decrease of 13% (at 50 DAS) and 14% (at 80 DAS) in root N content at 24 mg Cd/kg soil, compared to those observed at 6 mg/kg soil. Similarly, copper at 1338 mg/kg soil reduced the root N content by 14% each at 50 (36 mg/g) and 80 (32 mg/g), compared to 334.5 mg/kg of copper (42 and 37 mg/g at 50 and 80 DAS) applied to soils. In comparison, chromium progressively enhanced the root N by 29, 33 and 42% (at 50 DAS) and 33, 38 and 48% (at 80 DAS) at 34, 68 and 136 mg/kg soil, compared to control. A maximum concentration of N content in roots was observed as 64 (an increase of 10%) and 59 mg/g (an increase of 11%) at 50 and 80 DAS respectively, when soil was treated with 136 mg/kg soil compared to 34 mg Cr/kg soil. Among the dual metal treatments, cadmium with copper (at 24 and 1338 mg/kg soil) significantly reduced the N content by 29 and 30% at 50 and 80 DAS, respectively, compared to the control. A trend similar to root N was observed for shoot N with three metals and their combinations. The average maximum increase in shoot N content with Cr ranged between 22% (34 mg Cr/kg soil) to 31% (136 mg Cr/kg soil) at 50 DAS and 6% (34 mg Cr/kg soil) to 18% (136 mg Cr/kg soil) at 80 DAS, compared to control. A trend similar to those observed for increase/decrease at higher concentration of each metal compared to the lower tested rates of each metal was also observed for shoot N content at both the growth stages of greengram. The N content of the roots was more severely affected than the shoot N at all the concentrations of tested metals, but N concentration in roots and shoots in general were less at 80 DAS compared to 50 DAS.

4.12.2.5 Seed yield and grain protein

The effect of heavy metals on seed yield was variable (Table 35). Seed yield decreased consistently for each metal with increasing concentration, used either separately (except the three concentrations of chromium) or in combination. The average maximum increase of 62 and 74% was observed with chromium at 136 mg kg⁻¹ soil in comparison to 34 mg Cr/kg soil (4.5 g/plant) and control (4.2 g/plant), respectively. In contrast, cadmium at 24 mg kg⁻¹ soil significantly (P ≤ 0.05) decreased the seed yield by 29 and 40%, compared to 6 mg Cd/kg (3.5 g/plant) and the control respectively, which was followed by a significant decrease of 18 and 26% when 1338 mg Cu/kg soil was applied to soils, compared to 334.5 mg Cu/kg soil (3.8 g/plant) and control, respectively. The average reduction in seed yield among combination treatments ranged between 17 (at 34 and 334.5 mg/kg of Cr and Cu respectively) and 60% (at
24 and 1338 mg/kg of cadmium and copper respectively), relative to the control. While comparing the sum of mean values of each metal treatment, the order of toxicity to seed mass decreased in the following order: cadmium < copper < chromium.

The effect of heavy metals on grain protein differed among metal treatments (Table 35). Chromium in general, consistently increased the grain protein with increasing concentrations. The average maximum grain protein (283 mg/g) was observed with 136 mg Cr/kg which was greater by 5 and 11 % than observed for 34 mg Cr/kg (270 mg/g) and control (256 mg/g). In comparison, other metals used either alone or in combination decreased the grain protein consistently with increase in concentration relative to the control. Cadmium at 24 mg/kg and copper at 1338 mg/kg soil decreased the grain protein by 8 and 6%, respectively, compared to control. Among the dual metal combination treatments, cadmium with copper declined the grain protein by 7 (at 6 and 334.5 mg/kg soil), 8 (12 and 669 mg/kg soil) and 10 % (at 24 and 1338 mg/kg soil), respectively, relative to the control. The combinations of metals in general had the greatest toxic effect on grain protein compared to single metal treatments.

4.12.2.6 Metal uptake by plant organs
The concentration of cadmium, chromium and copper in plant tissues (e.g. roots and shoots) at 50 and 80 days and grains at harvest (80 DAS) varied among treatments. Generally, the concentration of metals, in roots and shoots and grains were influenced greatly by the concentration of each metal tested. A higher amount of cadmium (Fig. 66), chromium (Fig. 67) and copper (Fig. 68) in roots, shoots and grains were observed when these metals were used individually compared with dual metal application. The greengram plants showed a maximum accumulation of cadmium at 50 and 80 days after seeding in roots (2 and 3.1 μg/g), shoots (0.72 and 0.84 μg/g) and grains (0.35 μg/g) at 24 mg/kg soil (Fig. 66). In comparison, the concentration of chromium at 50 and 80 DAS was higher in roots (29.9 and 32.2 μg/g), shoots (10.5 and 15.5 μg/g) and grains (4.5 μg/g) at 136 mg/kg soil (Fig. 67). The concentration of copper at 50 and 80 DAS was higher in roots (60.1 and 64.5 μg/g), shoots (26.2 and 28.2 μg/g) and grains (15.7) at 1338 mg/kg soil (Fig. 68). Following the dual metal treatments, the concentration of cadmium, chromium and copper in plant tissues and grains were in general reduced marginally at 24 and 136 mg/kg of cadmium with chromium (Fig. 69 and 70), 24 and 1338 mg/kg of cadmium with copper (Fig. 71 and 72) and 136 and 1338 mg/kg of chromium with copper (Fig. 73 and 74). The concentration of chromium was maximum both at 24 and
136 mg/kg of Cd with Cr and 136 and 1338 mg/kg of Cr with Cu at both 50 and 80 DAS in roots (29 and 31 µg/g), shoots (10 and 15 µg/g) and grains (4.3 µg/g) compared to other treatments. The phyto-accumulation of metals was higher in roots compared to shoots or grains at all rates of metals, applied singly or in dual treatments.

4.12.3 Lentil

4.12.3.1 Length of plant organs

The effect of three concentrations of cadmium, chromium and copper on lentil plants differed among treatments (Plate 4). Cadmium at 24 mg/kg soil was the most toxic and decreased the root length significantly (P ≤ 0.05) by 24 and 33% (at 90 DAS) and 23 and 31% (at 120 DAS), respectively, compared to 6 mg/kg and control. This was followed by copper which reduced the root length by 22 and 25% (at 90 DAS) and 18 and 21% (at 120 DAS), at 1338 mg/kg soil, compared to 334.5 mg Cu/kg soil and control (Table 36). In contrast, chromium at 34 mg/kg soil showed a stimulatory effect on the root development when lentil plants were uprooted 90 and 120 days after seeding, above the control. The root length was reduced even further when cadmium was used in combination with copper and chromium. Among the mixture of metals, cadmium with copper at 24 mg/kg and 1338 mg/kg soil, reduced the root length by 46 and 38% at 90 (13 cm length) and 120 DAS (18 cm length), respectively, compared to control (24 and 29 cm length at 90 and 120 DAS, respectively). Generally, the length of roots increased considerably with age of plants for all the treatments but decreased progressively with increasing concentrations of metals. Like the effect of cadmium on roots, it also had the similar toxic effect on shoot growth and reduced its length significantly (P ≤ 0.05) by 21 and 29% (at 90 DAS) and 14 and 27% (at 120 DAS) respectively, at 24 mg/kg soil, compared to 6 mg/kg soil and control. This was followed by copper which reduced the shoot length by 20 and 24% (at 90 DAS) and 13 and 23% (at 120 DAS), at 1338 mg/kg soil, compared to 334.5 mg Cu/kg soil and control, respectively. In contrast, chromium at 34 mg/kg of soil enhanced the shoot length by 4 and 7% after 90 and 120 DAS respectively, above the control. Generally, when Cd was used along with Cu and Cr showed a substantial decrease in length of the measured organs of lentil plants. The dual combination of Cd with Cu at 24 mg/kg and 1338 mg/kg soil declined the shoot length by 43 and 46% at 90 and 120 DAS, respectively, compared to control. Like the
effects of metals on root length, the shoot length also increased with plant age but was affected negatively with increase in concentration of metals, used either alone or as mixture (Table 36).

4.12.3.2 Dry matter accumulation in roots and shoots
The dry matter accumulation in roots and shoots decreased with the increase in the concentration of the metals (Table 36). Among the single metal treatments, cadmium at 24 mg kg⁻¹ soil decreased the root dry weight by 9% at 120 DAS (68 mg/plant), while at the same rate, cadmium increased the root dry weight marginally at 90 DAS, compared to control (60 and 75 mg/plant at 90 and 120 DAS, respectively). While comparing the effect of three dose rates of cadmium on root biomass, a decrease of 7 (at 90 DAS) and 9% (at 120 DAS) was observed when 24 mg Cd/kg was added to soil, compared to those observed with 6 mg/kg soil. In contrast, copper and chromium at all the three concentrations except copper at 1338 mg/kg soil at 120 DAS either showed no adverse effect or stimulated the dry matter production by roots, compared to untreated control. Similarly, copper at 1338 mg/kg soil and chromium at 136 mg/kg soil reduced the root dry weight each by 8 (at 90 DAS) and 9% (at 120 DAS) respectively, compared to 334.5 mg/kg of copper and 34 mg/kg of chromium applied to soils.

The dry matter accumulation in roots was reduced when the three concentrations of cadmium was used in combination with copper or chromium. Cadmium with copper (at 24 and 1338 mg/kg soil) decreased the root dry weight by 8 (90 DAS) and 11% (120 DAS), compared to control. The dry matter accumulation in shoots following 24 mg Cd/kg soil at 90 DAS (150 mg/plant) increased by 20% while the same concentration of cadmium decreased shoot biomass by 8% at 120 DAS, compared to control plants. Comparison between the three dose rates of cadmium revealed a significant decrease of 4% each at 90 and 120 DAS in shoot dry weight at 24 mg Cd/kg soil, compared to those observed at 6 mg/kg soil. The mixture of cadmium and copper (at 24 and 1338 mg/kg soil) decreased the shoot dry weight by 11% at 120 DAS while the same combination treatment increased the shoot dry weight by 14% at 90 DAS, compared to control. Generally, the dry matter accumulation in roots and shoots increased with the age of plants grown either in treated or un-amended sandy clay loam soil.

4.12.3.3 Total biomass and symbiotic traits
The biomass production by lentil plants when grown in soils treated differently with metals varied considerably (Table 36). The total phytomass in the present study decreased with increase in the concentration of metals, used either alone or when they were applied
simultaneously to the soils. Like other legumes, the total dry matter production of lentil plants also increased with plant age but decreased substantially with increasing rates of each single or combined metal treatment. While comparing the effect of concentration of each metal on dry matter accumulation, cadmium at 24 mg/kg soil displayed the highest phytotoxic effect and reduced the dry biomass of plants by 6 and 12% at 120 DAS, relative to 6 mg Cd/kg soil and the metal free control, which was followed by a 6% decrease at the same dose of cadmium at 90 DAS, compared to 6 mg Cd/kg soil. Chromium or copper when applied with cadmium also had a toxic effect on the dry mass production of lentil plants. A maximum decrease of 16% was observed for 24 and 1338 mg/kg of cadmium-copper at 120 DAS, which was followed by the combination of cadmium-chromium (24 and 136 mg/kg soil) that reduced the total biomass by 13%, compared to control. Cadmium with copper (at 24 and 1338 mg/kg soil) enhanced the total biomass marginally at 90 DAS, compared to control.

Nodulation response to each metal treatment at 90 and 120 DAS varied considerably (Plate 9D). Generally the three concentrations of each metal (except 34 mg/kg of chromium) used either alone or as mixture decreased the number of nodules per plant, compared to untreated control. For instance, 24 mg Cd/kg soil decreased the number of nodules by 36 and 46 (at 90 DAS) and 55 and 60% (at 120 DAS), respectively, compared to 6 mg Cd/kg soil and control (Table 36). In contrast, the number of nodules produced on the root system of lentil plants increased significantly (P < 0.05) by 12% at 90 DAS (15 nodules/plant) with 34 mg Cr/kg. Moreover, a significant decrease of 27 (at 90 DAS) and 38% (at 120 DAS) in nodule numbers per plant was observed at 136 mg Cd/kg soil, compared to those observed at 34 mg/kg soil. Similarly, mixtures of metals at all levels decreased the number of nodules per plant compared to control plants. For example, cadmium (24 mg/kg) with copper (1338 mg/kg) showed the largest adverse effect and significantly (P < 0.05) reduced the number of nodules per plant by 62 and 70%, at 90 and 120 DAS respectively, above the control.

The reduction in nodulation was accompanied by significant decrease in dry mass of nodules (Table 36). The nodule dry mass recorded at 90 DAS (7 mg/plant) and 120 DAS (8 mg/plant) in plants grown with 24 mg Cd/kg soil decreased by 22 and 53% (90 DAS) and 20 and 58% (120 DAS), compared to 6 mg Cd/kg soil and control. Similarly, mixtures of metals at all concentrations decreased the dry nodule mass compared to control. Among the metal combinations, cadmium (24 mg/kg) with copper (1338 mg/kg) showed the highest toxic effects.
and significantly reduced the dry nodule mass by 25 and 60 (at 90 DAS) and 22 and 63 % (at 120 DAS) respectively, compared to 6 mg/kg of cadmium with 334.5 mg/kg of copper and control. In general, there was a decrease in the number of nodules while nodule mass increased with the plant age for each treatment.

4.12.3.4 Chlorophyll and leghaemoglobin content

The effect of heavy metals on chlorophyll content in fresh foliage at 90 DAS varied among treatments (Table 37). A significant reduction of 23 and 33% in chlorophyll content of lentil foliage was observed when plants were raised in soil treated with 24 mg Cg/kg, compared to those observed at 6 mg Cd/kg soil and control plants (0.30 mg/g). Conversely, chromium at 68 and 136 mg/kg, increased the chlorophyll content by 90 and 80% respectively, compared to the control. Further, 68 and 136 mg Cr/kg soil increased the chlorophyll content by 36 and 29%, respectively, compared to 34 mg Cr/kg soil. The chlorophyll content was reduced substantially when cadmium was used in combination with chromium and copper at all the three concentrations. The combination treatment of cadmium with copper at 24 and 1338 mg/kg decreased the chlorophyll content by 47%, which was followed by a decrease of 40% at 24 and 136 mg/kg soil of Cd with Cr, over control plants.

The nodules on the root system of lentil plants raised in soil amended with cadmium in general, had considerably a lower concentration of leghaemoglobin and decreased it by 25 and 40% at 24 mg Cd/kg soil, compared to 6 mg/kg soil and control (0.10 m mol/g f.m) (Table 37). In contrast, the leghaemoglobin content was enhanced by 20 % at 34 mg Cr/kg soil, compared to control, which progressively decreased with increasing rates of metals. Comparison between the three dose rates of chromium revealed a statistically significant (P < 0.05) decrease of 25% in leghaemoglobin content at 136 mg Cr/kg soil, compared to those observed at 34 mg/kg soil.

In general, the leghaemoglobin in nodules of combined metal treatments was significantly (P ≤ 0.05) decreased; the maximum reduction being 60% with cadmium -copper (at 24 and 1338 mg/kg soil), compared to control plants.

4.12.3.5 Nitrogen content

The average maximum decline in root N occurred at 24 mg Cd/kg that reduced the root N by 5 and 6 (at 90 DAS) and 5 and 8% (at 120 DAS) respectively, compared to 6 mg Cd/kg soil and control (14.2 and 13.8 mg/g at 90 and 120 DAS, respectively) (Table 37). In comparison, chromium enhanced the root N marginally at 90 and 120 DAS at 34 mg/kg soil, which
consistently decreased with increase in the concentration of metals and plant age. While comparing the effect of three dose rates of chromium, 136 mg Cr/kg decreased the root N by 5% each at 90 and 120 DAS, compared to those observed at 34 mg Cr/kg soil. Among the dual metal treatments, cadmium (24 mg/kg soil) when applied with copper (1338 mg/kg soil) reduced the N content by 11 and 14% after 90 and 120 DAS, respectively, relative to the control. A trend similar to root N was observed for shoot N with three metals and their combinations. Cadmium at 24 mg/kg had greater effect at 90 DAS and decreased the shoot N by 5 and 7%, compared to 6 mg Cd/kg soil and control respectively. Conversely, the lower rate of chromium marginally increased the shoot N content at both the sampling days, compared to control. The dual combinations of cadmium (24 mg/kg soil) and copper (1338 mg/kg soil) significantly (P < 0.05) reduced the shoot N content by 11 and 6% after 90 and 120 DAS, respectively, compared to the control. Generally, the N content of roots was severely affected than the shoot N with all levels of metals and plant age.

**4.12.3.6 Seed yield and grain protein**

Seed yield decreased consistently with increase in the rates of the metals, used either separately or in combination, compared to control (Table 37). Cadmium at 24 mg/kg decreased the seed yield by 12 and 17%, compared to 6 mg Cd/kg soil and control plants (100 mg/plant) respectively. On the contrary, chromium at 34 mg/kg had the greatest stimulatory effect and increased the seed yield by 4% compared to control while 136 mg Cr/kg soil reduced the seed yield by 12%, compared to 34 mg Cr/kg soil. The combination treatment of all the metals in general, inhibited the seed yield at all the concentrations, compared to control. The combination of cadmium (24 mg/kg) with copper (1338 mg/kg soil) decreased the seed yield by 29%, which was followed by 21% decrease in grain yield at 24 and 136 mg/kg of cadmium and chromium, compared to control treatment.

Like the effect of metals on seed yield, grain protein also decreased with elevated concentrations of metals (Table 37). Though, cadmium was found as the most toxic metal but marginally decreased the grain protein by 3 and 5% at 24 mg/kg, compared to 6 mg Cd/kg soil and control. Among other metals, chromium at 34 mg/kg increased the grain protein marginally compared to control. Comparison between the three dose rates of chromium revealed a decrease of 4% in grain protein at 136 mg Cr/kg soil, compared to those observed at 34 mg/kg soil. The combination treatment of cadmium with copper at 24 and 1338 mg/kg decreased the
grain protein by 9% above the control treatment (240 mg/g). The multiple treatment of Cd with Cr and Cu decreased the grain protein at all the concentrations.

4.12.3.7 Glutathione reductase activity

The glutathione reductase (GR) activity under metal stress, increased considerably with increase in the concentration of cadmium, chromium and copper (Table 38). In this experiment, the maximum increase in GR activity was observed for cadmium at 24 mg/ kg which increased the GR activity of roots by 282 and 280% 90 and 120 days after sowing, compared to those observed for control at 90 (17 n mol/mg protein) and 120 DAS (15 n mol/mg protein), respectively. In comparison, the same concentration of cadmium increased the GR activity in nodules by 300 and 308% after 90 and 120 DAS, respectively, compared to control. Copper at 1338 mg/kg soil, increased the GR activity of roots and nodules by 100 and 93% (at 90 DAS) and 40 and 54% (at 120 DAS) respectively, compared to control. While comparing the effect of three dose rates of cadmium on GR activity, a significant increase of 117 and 115% (at 90 DAS) and 119 and 141% (at 120 DAS) in GR activity of roots and nodules, respectively, was observed for 24 mg Cd/kg soil, compared to those determined at 6 mg/kg soil. Similarly, copper at 1338 mg/kg soil increased the GR activity of roots and nodules by 36 and 17% (at 90 DAS) and 24 and 25% (at 120 DAS) compared to 334.5 mg/kg of copper applied to soils. In combination treatments, the maximum increase in GR activity in roots was observed with cadmium and copper (24 and 1338 mg/kg) which increased the GR activity by 335 and 336% after 90 and 120 DAS, respectively, relative to the control. Similarly, the GR activity in nodules increased by 327 (at 90 DAS) and 338% (at 120 DAS) at 24 mg Cd/kg and 1338 mg Cu/kg soil compared to control plants. Generally, the GR activity was recorded more in roots compared to those observed for nodules. The combined application of metals showed the greatest GR activity in both roots and nodules, compared to single metal application, which decreased consistently with increase in plant age.

4.12.3.8 Phytoaccumulation of heavy metals

The accumulation of cadmium, chromium and copper in roots and shoots (at 90 and 120 DAS) and grains (at 120 DAS) differed among treatments and a dose dependent decrease in metals was observed. A higher amount of cadmium (Fig. 75), chromium (Fig. 76) and copper (Fig. 77) in roots, shoots and grains, were observed when these metals were used individually compared with dual metal application. The lentil plants showed a maximum accumulation of cadmium in
roots (1.9 and 2.8 μg/g) and shoots (0.5 and 0.8 μg/g) after 90 and 120 DAS, respectively, and grains (0.3 μg/g) at 120 DAS with 24 mg kg⁻¹ soil (Fig. 75). In comparison, the concentration of chromium recorded in roots was (23.7 and 30.9 μg/g) and shoots (14.5 and 20.6 μg/g) at 90 and 120 DAS, respectively, and grains (5.8 μg/g) after 120 DAS, at 136 mg/kg soil (Fig. 76). The concentration of copper was higher in roots (72.1 and 82 μg/g) and shoots (38.3 and 42.2 μg/g) at 90 and 120 DAS, respectively, and grains (10.5 μg/g) after 120 DAS at 1338 mg/kg soil (Fig. 77). The concentration of cadmium, chromium and copper in plant organs were however, reduced marginally when 24 mg kg⁻¹ of cadmium was applied with 136 mg/kg of chromium (Fig. 78 and 79) or 1338 mg/kg of copper (Fig. 80 and 81) and when 136 mg/kg of chromium was used with 1338 mg/kg of copper (Fig. 82 and 83). Generally, the accumulation of heavy metals was higher in roots compared with the shoots or grains at all levels of metals. The simultaneous application of metals in general, reduced the uptake of metals by plant organs.

4.12.4 Pea

4.12.4.1 Plant growth

4.12.4.1.1 Length of plant organs

The effect of three concentrations of cadmium, chromium and copper on pea organs measured at 90 and 120 days after sowing differed among treatments (Plate 3). Among the single metal treatments, copper at 1338 mg/kg soil was the most toxic and reduced the root length significantly (P ≤ 0.05) by 21 and 32% (at 90 DAS) and 23 and 32% (120 DAS), compared to 334.5 mg Cu/kg and control. On the other hand, all the three concentrations of cadmium and chromium except 136 mg Cr/kg (at 90 and 120 DAS) increased the root length, above the control. A maximum increase of 9% (at 90 DAS) and 12% (at 120 DAS) in root length, was recorded at 12 mg Cd/kg soil, compared to control. While chromium at 68 mg/kg enhanced the root length by 12% at 120 DAS, in comparison to control. Chromium at 34 and 68 mg/kg, increased the root length by 23 and 22%, respectively, at 120 DAS, compared to those recorded at 90 DAS. The reduction in root length of pea following mixtures of metals ranged between 23 (chromium with copper at 34 and 334.5 mg/kg soil) to 36% (chromium with copper at 136 and 1338 mg/kg) at 90 DAS while at 120 DAS, the reduction was between 20 (chromium with copper at 34 and 334.5 mg/kg soil) to 36% (chromium with copper at 136 and 1338 mg/kg), above the control. Similarly, the three concentrations of cadmium and chromium
except chromium at 136 mg/kg (at 120 DAS) increased the shoot length at the measured stages of plant growth, above the control. Copper at 1338 mg/kg soil had the most toxic effect and reduced the shoot length by 16 and 22% (at 90 DAS) and 14 and 23% (at 120 DAS) respectively, compared to 334.5 mg Cu/kg soil and control. In comparison, cadmium at 12 mg/kg soil, increased the shoot length the maximum being 15% each (at 90 and 120 DAS), compared to control. The reduction in shoot length of pea following mixtures of metals ranged between 15 (chromium with copper at 34 and 334.5 mg/kg soil) to 27% (chromium with copper at 136 and 1338 mg/kg), at 90 DAS and 23 (chromium with copper at 34 and 334.5 mg/kg soil) to 30% (chromium with copper at 136 and 1338 mg/kg) at 120 DAS, above the control. The increase in shoot length at 120 DAS was 15% greater than those observed at 90 DAS (47 cm) at 12 mg Cd/kg. In contrast, the mixture of cadmium (6 mg/kg soil) and chromium (34 mg/kg soil) increased the length marginally at both the stages of plant growth, relative to the control.

4.12.4.1.2 Root, shoot and whole biomass

The dry weight of roots decreased with increase in the concentration of metals (Table 39). Among the single metal treatments, copper at 1338 mg kg$^{-1}$ soil decreased the root dry weight by 20 and 25% (at 90 DAS) and 23 and 29% (120 DAS), respectively, compared to 334.5 mg/kg soil and control plants. In contrast, cadmium at 12 mg/kg soil increased the root dry weight by 35% (90 DAS) and 30% (120 DAS) respectively, compared to control. Among the dual metal treatments, cadmium with copper (24 and 1338 mg kg$^{-1}$ soil) decreased the root dry weight by 33 and 35%, at 90 and 120 DAS, respectively, compared to control. The increase in root dry weight at 120 DAS was 24% greater than those observed at 90 DAS at 12 mg Cd/kg. Copper at 1338 mg/kg soil decreased the shoot dry weight by 12 and 16% (at 90 DAS) and 9 and 15% (at 120 DAS), respectively, compared to 334.5 mg Cu/kg soil and control plants. Conversely, cadmium at 24 mg/kg soil increased the shoot dry weight by 7 and 57% (at 90 DAS) and 3 and 52% (at 120 DAS) respectively, compared to 6 mg Cd/kg soil and control. Among dual metal treatments, cadmium and copper (at 24 and 1338 mg/kg soil) decreased the shoot dry weight by 17 and 14%, at 90 and 120 DAS, respectively, compared to control. The increase in shoot dry weight at 120 DAS was 21% greater than those observed at 90 DAS following 12 mg Cd/kg soil.

The effect of three concentrations of cadmium, chromium and copper on dry matter accumulation in whole plants was variable (Table 39). Among the single metal treatments,
copper at 1338 mg/kg soil was the most toxic and reduced the total dry matter significantly (P ≤ 0.05) by 14 and 18% (at 90 DAS) and 12 and 17% (at 120 DAS) respectively, compared to 334.5 mg Cu/kg soil and control. In contrast, the three concentrations of cadmium and chromium increased the dry matter, above the control, the maximum being 60 and 40% at 90 DAS and 59 and 36% at 120 DAS following 12 mg Cd/kg and 68 mg Cr/kg soil, respectively, compared to control. Comparison between the three dose rates of cadmium revealed an increase of 10% each at 90 and 120 DAS in total dry weight at 12 mg Cd/kg soil, compared to those observed at 6 mg/kg soil. Similarly, chromium at 68 mg/kg soil increased the whole biomass by 7% each at 90 DAS and 120 DAS, compared to 34 mg Cu/kg of soil. The dry matter accumulation was reduced even further when copper was used in combination with cadmium and chromium. The reduction in dry biomass of pea following mixtures of metals ranged between 6 and 7% (chromium with copper at 34 and 334.5 mg/kg soil) to 16 and 18% (chromium with copper at 136 and 1338 mg/kg), at 90 and 120 DAS, respectively above the control. In contrast, the mixture of cadmium (24 mg/kg soil) and chromium (136 mg/kg soil) increased the dry matter by 16 and 13% at 90 and 120 DAS, respectively, relative to the control. The increase in total dry biomass of pea plants at 120 DAS was 20% greater than those observed at 90 DAS when soil was treated with 12 mg/kg of Cd.

4.12.4.2 Symbiotic traits

Nodulation response to each metal at 90 and 120 DAS varied considerably (Plate 9A). Comparison between control and metal treatments, revealed a significant increase in the number of nodules per plant following cadmium and chromium application to soil (Table 39). Generally, the application of copper to sandy clay loam soil reduced the number of nodules. Among the single metal treatments, 1338 mg Cu/kg soil decreased the number of nodules by 16 and 25% (at 90 DAS) and 17 and 22 % (at 120 DAS) respectively, compared to 334.5 mg Cu/kg soil and control. In contrast, the number of nodules increased significantly (P ≤ 0.05) by 53% (at 90 DAS) and 72% (at 120 DAS) with 24 mg Cd/kg, compared to control and by 31% (at 90 DAS) and 50% (at 120 DAS) with 136 mg Cr/kg soil respectively, compared to control (75 and 83 nodules/plant at 90 and 120 DAS, respectively). Similarly, mixtures of metals at all levels except the three dose rates of cadmium with chromium decreased the number of nodules relative to the control. Among the metal combinations, cadmium (24 mg/kg) with copper (1338 mg/kg) showed the largest adverse effect and significantly reduced the number of nodules by
33% each at 90 and 120 DAS respectively, above the control. Cadmium with chromium (6+ 34 mg/kg) significantly enhanced the number of nodules per plant by 37 and 47% at 90 and 120 DAS, respectively, compared to control plants. The reduction in nodulation was accompanied by significant decrease in dry mass of nodules following metal application or metal free control (Table 39). Of the sole metal application, 1338 mg Cu/kg soil decreased the nodule dry mass by 17 and 22% (at 90 DAS) and 23 and 24% (at 120 DAS) respectively, compared to 334.5 mg Cu/kg and control. While the nodule dry mass increased significantly by 51% (at 90 DAS) and 55% (at 120 DAS), respectively, with 24 mg Cd/kg compared to control and 30% (at 90 DAS) and 34% (at 120 DAS) at 136 mg Cr/kg soil, compared to control respectively. Similarly, all rates of mixtures of metals except the three concentrations of cadmium with chromium decreased the dry nodule mass compared to control. Among the metal combinations, Cd (24 mg/kg) with Cu (1338 mg/kg) showed the largest toxic effects and significantly (P < 0.05) reduced the dry matter accumulation in nodules by 27 and 25% at 90 and 120 DAS respectively, above the control (Table 39). Generally, the number of nodules on root system of pea plants grown in metal treated soils decreased with plant age while nodule mass increased with increasing age of plants.

4.12.4.3 Chlorophyll, nitrogen and leghaemoglobin content

The chlorophyll content in fresh foliage of pea plants determined at 90 DAS varied among treatments (Table 40). Cadmium at 24 mg kg⁻¹ declined the chlorophyll content by 13 and 17% compared to 6 mg/kg soil and control plants (0.84 mg/g) respectively. In comparison, the three concentrations of chromium marginally increased the chlorophyll content. The chlorophyll content was reduced even further when cadmium was used in combination with chromium and copper at all the three concentrations. The mixture of cadmium with copper (at 24 and 1338 mg/kg) decreased the chlorophyll content by 12 and 21% compared to 6 and 334.5 mg cadmium-copper/kg and control, respectively.

The average maximum decline in root N occurred at 1338 mg Cu/kg (Table 40) that significantly reduced the root N by 15 and 20% (at 90 DAS) and 14 and 17% (at 120 DAS), in comparison to 334.5 mg Cu/kg soil and control, respectively. Cadmium at 12 mg kg⁻¹ soil however, enhanced the root N by 5 and 14% (at 90 DAS) and 9 and 17% (at 120 DAS), compared to 6 mg Cd/kg soil and control, respectively. Among the dual metal treatments, cadmium (24 mg/kg soil) when applied with copper (1338 mg/kg soil) significantly (P ≤ 0.05)
reduced the root N content by 26% and 20% 90 and 120 days after sowing, respectively, compared to the control. Generally, the accumulation of N was more in roots at 90 DAS which progressively decreased with increase in plant age for all the treatments; the maximum being 16% (cadmium alone at 6 mg/kg soil) to 17% (cadmium-copper at 6 and 334.5 mg/kg soil) at 120 DAS compared to those observed for 90 DAS. A trend similar to root N was observed for shoot N with three metals and their combinations. The average maximum increase in shoot N content at 24 mg Cd/kg was 28% (at 90 DAS) and 29% (at 120 DAS), compared to control. Among the dual metal treatments, cadmium (24 mg/kg soil) when applied with copper (1338 mg/kg soil) significantly reduced the N content by 13 and 21% 90 and 120 days after sowing respectively, compared to the control. The N content of roots was severely affected than shoot N with all levels of metals and followed a trend similar to those for roots in terms of percent increase or decrease with plant age.

The nodules on the root system of pea plants raised in soil amended with 1338 mg Cu/kg soil had considerably a lower concentration of leghaemoglobin (0.08 m mol/g f m). Copper at 1338 mg/kg soil decreased the leghaemoglobin measured at 90 DAS by 27 and 33%, compared to 334.5 mg Cu/kg soil and control, respectively (Table 40). In contrast, the leghaemoglobin content was increased by 14 and 33% at 12 mg Cd/kg soil, compared to 6 mg Cd/kg soil and control, respectively. In general, the leghaemoglobin in nodules of combined metal treatments were significantly decreased compared to control. A maximum reduction of 33% in leghaemoglobin was observed with Cd -Cu (at 24 and 1338 mg/kg soil), relative to the control which was followed by 25% at 136 and 1338 mg Cr-Cu/kg soil.

4.12.4.4 Seed yield and grain protein

Seed yield decreased progressively with increase in concentration of copper used either separately or in combination (Table 40). Copper at 1338 mg Cu/kg soil significantly decreased the seed yield by 12 and 15%, relative to 334.5 mg Cu/kg soil and control respectively. The average maximum increase of 13 and 8% was observed with Cd at 24 mg kg⁻¹ soil and chromium at 136 mg/kg soil respectively, compared to control. A maximum increase of 7 and 6% in seed yield was observed at harvest, respectively, when soil was treated with 12 mg Cd/kg soil and 68 mgCr/kg soil compared to 6 mg Cd/kg and 34 mg Cr/kg soil. The average maximum reduction in seed yield among combination treatments was 20% when 24 and 1338 mg/kg of cadmium-copper was applied together, relative to the control. The average maximum
increase of 7% in seed yield was observed for cadmium-chromium (6 and 34 mg/kg soil) which decreased consistently with increase in metal concentrations of each mixture treatment, compared to control. Cadmium and chromium in general, progressively increased the grain protein (GP) with increasing concentrations of tested metals (Table 40). The average maximum GP was observed with 24 mg Cd/kg (232 mg/g) and 136 mg Cr/kg (230 mg/g). In comparison, copper used either alone or as mixture decreased the GP consistently with increasing levels, relative to control. Cadmium (24 mg/kg) with copper (1338 mg/g) declined the GP by 7% compared to control. The mixtures of metals in general, had the greatest toxic effect on GP compared to single metal application.

4.12.4.5 Glutathione reductase activity
The glutathione reductase, an antioxidant enzyme, synthesized within roots and nodules under metal stress, increased considerably with increase in the concentration of cadmium, chromium and copper (Table 41). In this experiment, the maximum increase in GR activity was observed for cadmium at 24 mg/kg which increased the GR activity of roots by 260 and 306% 90 and 120 days after sowing respectively, compared to those observed for control at 90 (20 n mol/mg protein) and 120 DAS (16 n mol/mg protein), respectively. In comparison, the same concentration of cadmium increased the GR activity in nodules by 319 and 307% at 90 and 120 DAS, respectively, compared to control. Comparison between the three dose rates of cadmium demonstrated a profound increase of 100 and 103% (at 90 DAS) and 124 and 104% (at 120 DAS) in GR activity of roots and nodules, respectively, at 24 mg Cd/kg soil, compared to those observed at 6 mg/kg soil. Similarly, copper at 1338 mg/kg soil increased the GR activity of roots and nodules by 17 and 22% (at 90 DAS) and 26 and 19% (at 120 DAS) compared to 334.5 mg/kg of copper applied to soils. In combination treatments, the maximum increase in GR activity in roots was assayed when cadmium was applied with copper (24 and 1338 mg/kg) which increased the GR activity by 280 and 319% after 90 and 120 DAS, respectively, compared to control. Similarly, the GR activity in nodules increased by 338 (at 90 DAS) and 329% (at 120 DAS) at 24 mg Cd/kg and 1338 mg Cu/kg soil compared to control plants. Generally, the GR activity was recorded more in roots compared to that of nodules. The combined application of metals showed the greatest GR activity in both roots and nodules, compared to single metal application, which decreased consistently with increase in plant age.
4.12.4.6 Phytoaccumulation of heavy metals

The accumulation of cadmium, chromium and copper in roots and shoots (at 90 and 120 DAS) and grains at 120 DAS differed among treatments. The concentration of metals in plant organs were affected invariably by the dose rates of each metal applied. A higher amount of cadmium (Fig. 84), chromium (Fig. 85) and copper (Fig. 86) in roots, shoots and grains, were observed when these metals were used individually compared with dual metal application. The pea plants showed a maximum accumulation of cadmium in roots (1.5 and 2.1 μg/g) and shoots (0.62 and 1.1 μg/g) at 90 and 120 DAS respectively, and grains (0.32 μg/g) at harvest with 24 mg/kg soil (Fig. 84). In comparison, the higher concentration of chromium in roots at 90 and 120 DAS was 24.5 and 28.4 μg/g and in shoots was 15.5 and 17.9 μg/g, respectively while in grains, it was 2.7 μg/g (Fig. 85). The application of 1338 mg/kg soil of copper showed the higher accumulation of copper in roots at 90 and 120 DAS (14.4 and 17.7 μg/g) and shoots (8.5 and 11.7 μg/g) and at harvest in grains as 3.7 μg/g (Fig. 86). The concentration of cadmium, chromium and copper in plant organs were however, reduced marginally when 24 mg/kg of cadmium was applied with 136 mg/kg of chromium (Fig. 87 and 88) or 1338 mg/kg of copper (Fig. 89 and 90) and when 136 mg/kg of chromium was used with 1338 mg/kg of copper (Fig. 91 and 92). Generally, the phytoaccumulation of heavy metals was higher in roots compared to the shoots or grains at all levels of metals. Moreover, the simultaneous application of metals reduced the uptake of these metals by plant organs.

4.13 Bioremediation studies

4.13.1 Growth of chickpea influenced by chromium reducing Mesorhizobium RC3 in chromium treated soils

4.13.1.1 Plant growth and nodulation

In this experiment, chromium-reducing and plant growth promoting Mesorhizobium strain RC3 was used to assess its bioremediation potential in pot house conditions using chickpea as a test legume crop. The chickpea plants grew poorly, when the soil was amended only with chromium (Plate 6). Generally, the growth and nodulation decreased progressively with increasing concentration of Cr (VI). Among the three concentration of Cr (VI), Cr (VI) at 136 mg/kg soil had the largest toxic effects and significantly (P ≤ 0.05) decreased root length, shoot length, dry root weight, dry shoot weight, nodule numbers, nodule dry weight and total dry weight by 28, 24, 20, 21, 16, 3 and 18% at 90 DAS, and root length, shoot length, dry root
weight, dry shoot weight and total dry weight by 25, 18, 38, 12 and 17% at 135 DAS, respectively, relative to the control. In comparison, when *Mesorhizobium* sp. RC3 was also used with Cr (VI), it increased the measured parameters (Table 42). The bio-inoculant when added with 136 mg Cr/kg increased the root length, shoot length, dry shoot weight, nodule numbers, nodule dry weight and total dry weight by 11, 8, 48, 44, 47 and 33% at 90 DAS, respectively, while these parameters increased marginally at 135 DAS, compared to control (Table 42). While comparing the effects of different concentrations of chromium on inoculated plants, 136 mg Cr/kg soil showed a maximum increase of 72, 56, 43, 92, 86, 55 and 71% at 90 DAS, in root length, shoot length, dry root weight, dry shoot weight, nodule numbers, dry nodule mass and total dry mass, respectively, and 67, 39, 85, 22 and 31% at 135 DAS in root length, shoot length, dry root weight, dry shoot weight and total dry mass, respectively, compared to non-inoculated plants but having the same concentration of chromium. The two way ANOVA revealed that the individual effects of inoculation and chromium was significant (P ≤ 0.05) for the measured parameters except the individual effects of chromium on dry root weight at 90 and 135 DAS, dry shoot weight at 135 DAS, dry nodule weight at 90 and 120 DAS and total dry mass at 90 and 135 DAS. However, the interactive effect of inoculation and Cr (inoculation x Cr) was non significant for dry root weight at 90 and 135 DAS, dry shoot weight at 135 DAS, dry nodule weight at 90 DAS and total dry matter production at 135 DAS.

### 4.13.1.2 Chlorophyll and leghaemoglobin content

Chlorophyll and leghaemoglobin content measured at 90 DAS, decreased consistently with increasing concentrations of chromium (Table 43) without the inoculation of strain RC3. Chromium at 136 mg/kg was the most toxic and decreased the chlorophyll and leghaemoglobin content by 11 and 46%, respectively, compared to uninoculated control. In comparison, the bioinoculant showed a maximum increase in the chlorophyll and leghaemoglobin content of 13 and 27%, respectively, at 68 mg Cr/kg soil compared to inoculated but without chromium which was 18 and 6% higher than the values obtained at 136 mg Cr/kg treated soils. While comparing the effects of different concentrations of chromium on inoculated plants, a maximum increase of 27 and 129% in chlorophyll and leghaemoglobin content, respectively, was observed at 136 mg Cr/kg soil compared to non-inoculated but treated with the same concentration of chromium. Two factor ANOVA revealed that the individual effects of inoculation and chromium and their interaction were significant (P ≤ 0.05)
for the measured parameters, except the individual effect of chromium and interaction on chlorophyll content.

4.13.1.3 Nitrogen content, seed yield and grain protein
Nitrogen content in roots and shoots at 90 and 135 DAS and seed yield and grain protein at harvest (135 DAS) of chickpea plant decreased consistently with increase in the concentration of chromium (Table 43). Chromium at 136 mg/kg soil, decreased root N content by 19 and 27%, shoot N by 13 and 9%, at 90 and 135 DAS, respectively, and seed yield and grain protein by 15 and 4%, respectively, compared to the control. In comparison, the bio-inoculant increased the root N by 6% each at 90 and 135 DAS, shoot N by 8 and 12% at 90 and 135 DAS, respectively, and seed yield and grain protein by 9 and 4%, respectively, at 68 mg Cr/kg compared to inoculated but untreated control. While comparing the effects of different concentrations of chromium on inoculated plants, a maximum increase of 46 and 45% in root N at 90 and 135 DAS, 40 and 26% in shoot N at 90 and 135 DAS, 27% in seed yield and 8% in grain protein was observed at 136 mg Cr/kg soil compared to non-inoculated but having the same concentration of chromium. Two factor ANOVA revealed that the individual effects of inoculation and chromium and their interaction (inoculation x chromium) were significant (P < 0.05) for the measured parameters.

4.13.1.4 Chromium uptake
Accumulation of Cr in the roots and shoots at 90 DAS and 135 DAS and grains at 135 DAS, increased with increase in the concentration of Cr (VI) in soil. The average maximum accumulation of 42 µg/g and 29 µg Cr/g in roots and shoots (Fig 93) after 90 DAS and 64, 36 and 17 µg/g was observed in roots, shoots and grains (Fig. 94) after 135 DAS at 136 mg Cr/kg soil, when chickpea plants were grown in the absence of bio-inoculant. In contrast, a maximum accumulation of 36 and 19 µg Cr/g in roots and shoots (Fig 93) after 90 DAS and 58, 19 and 12 µg Cr/g was observed in roots, shoots and grains (Fig. 94) after 135 DAS at 136 mg Cr/kg soil respectively, when plants were grown in the presence of bio-inoculant.

4.13.2 Chromium tolerant Bacillus PSB10 affecting chickpea in chromium treated soils
4.13.2.1 Plant growth and nodulation
In this study, the chickpea were severely affected by the chromium toxicity in the absence of bio-inoculant but when chickpea seeds were inoculated with plant growth promoting rhizobacterium, Bacillus sp. PSB10 and grown in sandy clay loam soils amended with different
concentrations of Cr (VI) applied separately, increased the measured parameters (Table 44). The *Bacillus* PSB10 when used with 68 mg/kg Cr (VI) increased the root length, shoot length, dry root weight, dry shoot weight, nodule numbers, nodule dry weight and total dry weight by 17, 13, 21, 17, 19, 40 and 18%, at 90 DAS, respectively, compared to control and root length, shoot length, dry root weight, dry shoot weight and total dry weight by 11, 12, 19, 25 and 24%, respectively, after 135 DAS, compared to inoculated control. While comparing the effects of *Bacillus* PSB10 on chickpea applied with different concentrations of chromium, a maximum increase of 72, 60, 115, 56, 62, 15 and 39% at 90 DAS, in root length, shoot length, dry root weight, dry shoot weight, nodule numbers, dry nodule mass and total dry mass, respectively, and 58, 35, 115, 59 and 71% at 135 DAS, in root length, shoot length, dry root weight, dry shoot weight and total dry mass respectively, was observed at 136 mg Cr/kg soil compared to non-inoculated but having same concentration of chromium. The two way ANOVA revealed that the individual effects of inoculation and chromium was significant (P ≤ 0.05) for the measured parameters except the individual effects of inoculation on nodule numbers at 90 DAS, individual effect of chromium on dry root weight at 90 and 135 DAS, dry shoot weight at 135 DAS and dry nodule weight at 90 DAS. The interactive effect of inoculation and chromium was non significant for dry root weight at 90, dry shoot weight at 90 and 135 DAS, dry nodule weight at 90 DAS and total dry matter production both at 90 and 135 DAS.

4.13.2.2 Chlorophyll and leghaemoglobin content

Chlorophyll and leghaemoglobin content at 90 DAS decreased consistently with increase in the concentration of chromium (Table 45) without the inoculation of *Bacillus* PSB10 strain. Chromium at 136 mg/kg decreased the chlorophyll and leghaemoglobin content by 11 and 46%, respectively, compared to un-inoculated (0.84 mg/g and 0.13 m mol/gfm for chlorophyll and leghaemoglobin, respectively) control. In comparison, the bio-inoculant increased the chlorophyll content by 7% while it enhanced the leghaemoglobin content by 25%, at 68 mg Cr/kg soil compared to inoculated but without chromium. While comparing the effects of different concentrations of chromium on inoculated plants, a maximum increase of 23 and 143% in chlorophyll and leghaemoglobin was recorded at 136 mg Cr/kg soil compared to non-inoculated but having the same concentration of chromium. Two factor ANOVA revealed that the individual effects of inoculation and chromium and their interaction (inoculation x Cr)
were significant (P < 0.05) for the measured parameters, except the individual effect of Cr on chlorophyll content.

**4.13.2.3 Nitrogen content, seed yield and grain protein**

Nitrogen content determined at 90 and 135 DAS and seed yield and grain protein assayed at 135 DAS of chickpea declined progressively with increasing rates of chromium (Table 45). The N content in roots at 90 (13 mg/g) and 135 DAS (11 mg/g), and in shoots at 90 (20 mg/g) and 135 DAS (19.7 mg/g) decreased significantly following 136 mg Cr/kg soil application, compared to control. Similarly, the seed yield (4.4 g/plant) and grain protein (220 mg/g) declined by 15 and 4%, respectively, compared to inoculated control (5.2 g/plant and 230 mg/plant for seed yield and grain protein, respectively). In comparison, the chromium reducing *Bacillus* strain PSB10 increased the root N by 4 and 11%, shoot N by 3% each at 90 and 135 DAS respectively, seed yield and grain protein by 4 and 1% respectively, at 136 mg/kg soil, compared to inoculated but metal free control. While comparing the effects of different concentrations of Cr on inoculated plants, a maximum increase of 100 and 127%, in root N and 70 and 52% in shoot N at 90 and 135 DAS, 32% in seed yield and 10% in grain protein was recorded at 136 mg Cr/kg soil, compared to non-inoculated but amended with the same concentration of Cr. Two factor ANOVA revealed that the individual effects of inoculation and Cr and their interaction (inoculation x Cr) were significant for the measured parameters.

**4.13.2.4 Chromium uptake**

Accumulation of chromium in the roots and shoots at 90 and 135 DAS and grains at 135 DAS increased with increasing dose of Cr (VI), added to soil. A maximum uptake of 42 μg/g and 29 μg Cr/g was observed in roots and shoots (Fig 95) at 90 DAS while at 135 DAS, the uptake was 64, 36 and 17 μg/g by roots, shoots and grains (Fig. 96) at 136 mg Cr/kg soil, when chickpea plants were raised in the absence of bio-inoculant. In comparison, a maximum accumulation of 30 and 17 μg Cr/g in roots and shoots (Fig 95) at 90 DAS and 50, 22 and 11 μg Cr/g was observed in inoculated roots, shoots and grains (Fig. 121) after 135 DAS at 136 mg Cr/kg soil, respectively.

**4.13.3 Effect of metal tolerant *Bradyrhizobium* RM8 and different concentrations of nickel and zinc on greengram plants**

The production of phytohormones by the metal tolerant bradyrhizobial strain RM8 in the presence and absence of both nickel and zinc prompted to assess the effect of this bio-inoculant
on the performance of greengram, grown in nickel and zinc amended soil. The bacteria inoculated and non-inoculated greengram plants grown in sandy clay loam soil subjected to three levels each of nickel (Table 46) and zinc (Table 47) responded differently in terms of plant growth. The non-inoculated plants exposed to different concentrations of nickel demonstrated a significant (P ≤ 0.05) inhibition in plant growth and nodulation. Generally, the dry matter accumulation and nodulation 50 and 80 days after sowing decreased consistently with increase in the concentration of nickel. In the absence of bio-inoculant, nickel at 580 mg/kg of soil significantly (P ≤ 0.05) decreased the root length, shoot length, root dry weight, shoot dry weight, nodule numbers, nodule dry mass and total dry mass by 8, 33, 21, 23, 25, 13 and 23%, respectively, at 50 DAS and 21, 24, 30, 23, 40, 18 and 24% respectively at 80 DAS, relative to the control. In general, with the increase in concentration of nickel, a progressive decrease in the measured parameters was observed. In contrast, the inoculated plants exposed to different concentration of nickel increased the measured parameters. The bio-inoculant strain RM 8 significantly (P ≤ 0.05) increased the root length, shoot length, dry root weight, dry shoot weight, nodule numbers, nodule dry mass and total dry mass by 42, 15, 20, 16, 54, 56 and 18%, respectively, at 50 DAS and 17, 20, 13, 16, 22, 33 and 21%, respectively, at 80 DAS, when plants were grown in soil amended with 290 mg Ni/kg, compared to inoculated but without metal treated soil. While comparing the effects of different concentrations of nickel on inoculated plants, a significant increase of 80, 67, 41, 44, 82, 75 and 45%, at 50 DAS, and 75, 60, 29, 52, 83, 60 and 52%, at 80 DAS, in root length, shoot length, dry root weight, dry shoot weight, nodule numbers, dry nodule mass and total dry mass, respectively, was observed at 290 mg Ni/kg soil compared to non-inoculated plants. The bio-inoculant further increased the root length, shoot length, dry root weight, dry shoot weight, nodule numbers, dry nodule mass and total dry mass significantly (P ≤ 0.05) by 29, 80, 20, 25, 13 and 24% at 50 DAS and 40, 54, 21, 26, 89, 11 and 25%, at 80 DAS, respectively, even at 580 mg Ni/kg soil, compared to non-inoculated but amended with the same dose of nickel. The two way ANOVA revealed that the individual effects of inoculation and nickel and their interaction (inoculation x nickel) was significant (P ≤ 0.05) for the measured parameters.

The inoculated and non-inoculated plants exposed to three levels of zinc showed a variable plant growth (Table 47). Zinc at a concentration of 9780 mg/kg soil showed greatest phytotoxic effects on greengram plants and decreased root length, shoot length, dry root
weight, dry shoot weight, nodule numbers, dry nodule mass and total dry mass significantly (P ≤ 0.05) by 24, 40, 26, 26, 33 25 and 26%, at 50 DAS, and 32, 35, 30, 25, 40, 27 and 26%, at 80 DAS, respectively, relative to the control. In contrast, plants inoculated with strain RM 8 significantly (P ≤ 0.05) increased root length, shoot length, dry root weight, dry shoot weight, nodule numbers, dry nodule mass and total dry mass significantly by 92, 11, 29, 29, 38, 17 and 28% at 50 DAS and 54, 73, 19, 29, 78, 13 and 28% at 80 DAS, respectively, compared to un-inoculated but amended with the same rate of zinc application. However, a maximum increase of 107, 83, 33, 28, 50, 71 and 28%, at 50 DAS, and 100, 77, 16, 25, 73, 67 and 26%, at 80 DAS, in root length, shoot length, dry root weight, dry shoot weight, nodule numbers, dry nodule mass and total dry mass, respectively, was observed when inoculated plants were exposed to 4890 mg Zn/kg soil, compared to plants grown in the absence of bio-inoculant, but amended with the same concentration of metal. Generally, the bio-inoculant, significantly (P ≤ 0.05) increased the measured parameters when greengram plants were grown in soils exposed to all the three concentration of zinc separately. The two way ANOVA revealed that the individual effects of inoculation and zinc and their interaction (inoculation x zinc) was significant (P ≤ 0.05) for all the measured parameters except the interactive effect of inoculation vs zinc on dry root weight at 50 and 80 DAS and inoculation, zinc and their interaction for nodule numbers at 50 DAS.

Chlorophyll content and leghaemoglobin at 50 DAS, nitrogen content at 50 and 80 DAS and seed yield and seed protein at harvest (80 DAS) decreased consistently with increase in the concentration of nickel (Table 48) and zinc (Table 49) without the inoculation of RM8 strain. At a concentration of 290 mg Ni/kg soil, the percent decrease was 9, 29, 14 and 13 for chlorophyll, leghaemoglobin, root N and shoot N at 50 DAS, and 11, 13, 5 and 6 for root N, shoot N, seed yield and seed protein at 80 DAS, respectively; for 580 mg Ni/kg soil, 13, 43, 35 and 25 for chlorophyll, leghaemoglobin, root N and shoot N at 50 DAS and 16, 21, 20 and 11 for root N, shoot N, seed yield and seed protein at 80 DAS, respectively, compared to the control. In comparison, the inoculated strain RM8 significantly (P ≤ 0.05) increased the chlorophyll, leghaemoglobin, root N and shoot N by 14, 120, 41 and 37%, at 50 DAS and root N, shoot N, seed yield and grain protein by 38, 37, 34 and 13%, respectively, at 80 DAS, at 290 mg Ni/kg soil, compared to un-inoculated but containing 290 mg Ni/kg soil. The measured parameters were also increased at 580 mg Ni/kg soil inoculated with Bradyrhizobium strain.
RMS compared to non-inoculated but nickel amended soil. While comparing the effects of 580 mg Ni/kg soil and inoculation effects, the strain RMS significantly (P < 0.05) increased the chlorophyll and leghaemoglobin content of fresh nodules by 19 and 175%, respectively, at the same rate of nickel concentration. However, the N contents, seed yield and seed protein did not differ significantly (P ≤ 0.05) among inoculated and non-inoculated plants at 580 mg Ni/kg soil. The two factor ANOVA demonstrated that the individual effects of inoculation and nickel and their interaction (inoculation x nickel) were significant (P ≤ 0.05) for the measured parameters except the individual effect of nickel on chlorophyll and leghaemoglobin and the interaction only for chlorophyll contents.

Similarly, the highest effect of zinc was found at 9780 mg/kg soil which decreased the chlorophyll content, leghaemoglobin, root N and shoot N by 5, 29, 35 and 36%, at 50 DAS, and root N, shoot N, seed yield and seed protein by 26, 29, 25 and 13%, at 80 DAS, respectively, compared to the control (Table 49). In comparison, the inoculated strain RMS increased the chlorophyll content, leghaemoglobin, root N and shoot N by 11, 120, 18 and 29 at 50 DAS and root N and shoot N, seed yield and grain protein at 80 DAS by 29, 38, 33 and 9%, respectively, compared to the plants grown in soil exposed to 9780 mg Zn/kg soil. However, zinc at 4890 mg/kg soil inoculated with strain RMS exhibited a greatest stimulatory effect and significantly (P ≤ 0.05) increased the chlorophyll content and leghaemoglobin by 9 and 100%, respectively, at 50 DAS, root N by 47 (50 DAS) and 15 (80 DAS) and shoot N by 42 (50 DAS) and 70% (80 DAS) and seed yield and grain protein by 36% and 13%, respectively, compared to plants grown in soil amended solely with 4890 mg Zn/kg soil. Chlorophyll, leghaemoglobin, seed yield, seed protein and N content were also increased at 580 mg Ni/kg and 9780 mg Zn/kg soil inoculated with *Bradyrhizobium* RM8 compared to uninoculated but nickel and zinc amended soil. The two way ANOVA showed that the individual effects of inoculation and zinc and their interaction (inoculation x zinc) were significant (P ≤ 0.05) for all the measured parameters except the individual effects of zinc and interaction on chlorophyll content.

The accumulation of nickel and zinc in plant tissues differed among treatments. The uptake of nickel and zinc by the roots and shoots at 50 and 80 DAS and grains at harvest of greengram plants increased with increase in the concentration of tested metals. A significantly higher concentration of nickel and zinc in roots and shoots at 50 DAS and roots, shoots and
grains at 80 DAS was observed when greengram plants were grown in the absence of bio-
inoculant but amended with 580 mg Ni/kg soil. The average maximum accumulation of 153.7
and 92.2 μg Ni/g was observed in roots and shoots (Fig. 97) at 50 DAS and 181.4, 138.4 and 32
μg/g was detected in roots, shoots and grains (Fig. 98) at 80 DAS, at 580 mg Ni/kg soil, when
greengram plants were grown in the absence of bio-inoculant. In contrast, a maximum
accumulation of 130.3 and 74.6 μg/g in roots and shoots (Fig 97) at 50 DAS and 139.9, 121.1
and 25 μg/g in roots, shoots and grains (Fig. 98) was observed at 80 DAS, at 580 mg Ni/kg soil,
when plants were grown in the presence of bio-inoculant. The inoculated strain reduced the
concentration of nickel in roots and shoots by 15 and 19% at 50 DAS and roots, shoots and
grains by 23, 13 and 22% at 80 DAS, respectively, when plants were grown in soil amended
with 580 mg Ni/kg soil. Similarly, at a concentration of 9780 mg Zn/kg soil, the maximum
concentration of zinc was found as 496 and 394 μg Zn/g in roots and shoots (Fig. 99) 50 days
after sowing and 555, 448 and 123 μg Zn/g in roots, shoots and grains (Fig 100) 80 days after
sowing, respectively, when greengram plants were grown in the absence of bio-inoculant. On
the contrary, the average maximum accumulation of zinc was determined as 449.8 and 436.6
μg Zn/g in roots and shoots (Fig. 99) 50 days after sowing and 468, 350.1 and 105 μg Zn/g in
roots, shoots and grains (Fig. 100) 80 days after sowing, respectively, at 9780 mg Zn/kg soil
inoculated with strain RM8. Further, the data revealed a lower concentration of nickel and zinc
in tissues and grains of bio-inoculant treatment, compared to the un-inoculated greengram
plants. While for zinc (9780 mg/kg) treated soil, the bio-inoculant decreased the uptake of zinc
in roots and shoots by 9 and 11 % at 50 DAS and in roots, shoots and grains by 9, 11 and 15%
at 80 DAS, respectively, compared to plants grown in the absence of bio-inoculant.

4.13.4 Impact of metal tolerant Rhizobium RL9 on lentil grown in metal amended soil

4.13.4.1 Plant growth and nodulation

The nickel, lead and zinc tolerant Rhizobium strain RL9 was used to assess its impact on lentil
plants sown in soils treated separately with three concentrations of nickel, lead and zinc to
which strain RL9 showed tolerance under in vitro conditions. Lentil plants grown in soil
amended with different rates of nickel showed a variable growth and nodulation (Table 50).
Generally, length and weights of plant organs (roots and shoots) and nodulation at 90 and 120
DAS, decreased progressively with increase in rates of nickel. Nickel at 580 mg/kg soil had the
greatest phytotoxic effect and significantly (P ≤ 0.05) decreased the length of roots and shoots

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by 43 and 39%, at 90 DAS, and 41 and 33%, at 120 DAS, dry weight of roots and shoots by 27 
and 19% at 90 DAS, and 28 and 19% at 120 DAS, nodule numbers and nodule dry weights by 
58 and 54% at 90 DAS, and 47 and 50%, at 120 DAS, respectively. Similarly, the total dry 
weights of lentil plants decreased by 23% and 24% at 90 and 120 DAS, respectively, compared 
to control. In contrast, plants inoculated with strain RL9 increased the measured parameters, 
even in the presence of nickel (Plate 7A). Rhizobial strain when used with 290 mg Ni/kg had 
the highest stimulatory effect and increased the root length, shoots length, dry root weight, dry 
shoots weight, nodule numbers and nodule dry weight by 73, 71, 205, 145, 50 and 157% at 90 
DAS and 79, 33, 172, 140, 82 and 109% at 120 DAS, respectively, compared to un-inoculated 
but 290 mg Ni/kg amended soil. Likewise, the dry matter accumulation in whole lentil plants 
increased by 160 and 147%, at 90 and 120 DAS, respectively, when 290 mg Ni/kg soil was also 
applied with inoculant strain, compared to un-inoculated but amended with 290 mg/kg nickel 
(Table 50). Furthermore, the growth and nodulation also increased even at 580 mg Ni/kg soil in 
the presence of bio-inoculant. The two way ANOVA revealed that the individual effects of 
inoculation and nickel and their interaction (inoculation x Ni) was significant (P ≤ 0.05) for the 
measured parameters at 90 and 120 DAS.

The toxicity of lead to lentil plants increased with increase in the concentration, both in 
presence and absence of bio-inoculant (Table 51). Lead at 390 mg/kg soil had the greatest 
toxicity to lentil plants and significantly (P ≤ 0.05) decreased the length of roots and shoots by 
33 and 39% at 90 DAS and 33 and 29% at 120 DAS, dry weight of roots and shoots by 25 and 
17% at 90 DAS and 23 and 15% at 120 DAS and total dry biomass by 22 and 19% at 90 and 
120 DAS, respectively, compared to control. Symbiotic parameters assessed in this study were 
also adversely affected following lead application to soils. Generally, the lead toxicity to 
symbiotic properties increased with increasing dose of lead. A maximum decline in symbiotic 
properties (nodule numbers and nodule dry mass) was observed at 390 mg/kg soil that 
significantly reduced the nodule numbers by 42 and 33%, at 90 and 120 DAS, compared to 
control (12 and 15 nodules per plant at 90 and 120 DAS, respectively). The decrease in nodule 
umber accompanying a significant decrease in the nodule mass both at 90 and 120 DAS and 
was metal concentration dependent. In contrast, plants inoculated with RL9 increased the 
measured parameters, even in the presence of lead (Plate 7B). Rhizobial strain with 195 mg 
Pb/kg had the highest growth promoting effect and increased the root length, shoots length, dry
root weight, dry shoots weight, nodule numbers, nodule dry weight and total dry weight by 67, 87, 255, 145, 100, 138 and 172% at 90 DAS and 71, 52, 192, 146, 83, 100 and 159% at 120 DAS, respectively, compared to un-inoculated but 195 mg Pb/kg amended soil. Furthermore, growth and nodulation also increased even at 390 mg Pb/kg soil in the presence of bio-inoculant. The bio-inoculant considerably increased the measured parameters even at the highest rate of Pb, compared to sole application of Pb (Table 51). The two way ANOVA revealed that the individual effects of inoculation and Pb and their interaction (inoculation x Pb) was significant (P ≤ 0.05) for the measured parameters both at 90 and 120 DAS.

Similarly, the toxicity of zinc to lentil increased with increase in the concentration, both in the presence and absence of bio-inoculant (Table 52). Zinc at 9780 mg/kg soil had the greatest toxicity to lentil and significantly (P ≤ 0.05) decreased the length of roots and shoots by 24 (16 cm) and 33% (12 cm) at 90 DAS and 26 (20 cm) and 21% (19 cm) at 120 DAS, dry weight of roots and shoots by 18 (36 mg/plant) and 15% (105 mg/plant) at 90 DAS and 19 (105 mg/plant) and 11% (120 mg/plant) at 120 DAS, respectively, relative to control. Nodule numbers and nodule dry weights were decreased by 25 and 31% at 90 DAS and 27 and 25% at 120 DAS, total dry biomass declined by 17% at 90 DAS and 14% at 120 DAS respectively, compared to control. On the contrary, the lentil plants inoculated with RL9 increased the measured parameters, in the presence of zinc (Table 52). Rhizobial strain with 4890 mg Zn/kg had the highest stimulatory effect and increased root length (33 cm), shoots length (31 cm), dry root weight (17 mg/plant), dry shoots weight (310 mg/plant), nodule numbers (15 per plant), nodule dry mass (19 mg/plant) and total dry mass (500 mg/plant) by 74, 82, 350, 177, 50, 90 and 213% at 90 DAS respectively, compared to un-inoculated but 4890 mg Zn/kg amended soil. Similarly, the measured parameters increased following application of inoculated strain with 4890 mg Zn kg⁻¹ soil. Furthermore, growth and nodulation also increased even at 9780 mg Zn/kg soil in the presence of bio-inoculant. Generally, the measured parameters increased with plant age in both inoculated and un-inoculated plants. The two way ANOVA showed that the individual effects of inoculation and zinc and their interaction (inoculation x zinc) was significant (P ≤ 0.05) for the measured parameters at 90 and 120 DAS.

4.13.4.2 Chlorophyll and leghaemoglobin content
Chlorophyll and leghaemoglobin content at 90 DAS decreased consistently with increase in the concentration of nickel (Table 53), lead (Table 54) and zinc (Table 55) without the inoculation
of strain RL9. Among the three concentrations, nickel at 580 mg kg\(^{-1}\) had the greatest phytotoxic effect on the photosynthetic pigments of lentil plants and decreased the chlorophyll and leghaemoglobin by 39 and 44% compared to un-inoculated control. The bio-inoculant, on the other hand, when used together with 290 mg Ni/kg, increased the chlorophyll in fresh foliage and leghaemoglobin content in fresh nodules by 173 and 133%, compared to inoculated but 290 mg Ni/kg amended soil. Furthermore, chlorophyll and leghaemoglobin content also increased even further at 580 mg Ni/kg soil in the presence of bio-inoculant. Two factor ANOVA revealed that the individual effects of inoculation and nickel and their interaction (inoculation x nickel) were significant \((P \leq 0.05)\) for the measured parameters. Similarly, the toxicity to lentil plants increased with increasing dose of lead (Table 54) and zinc (Table 55), both in the presence and absence of bio-inoculant. Lead at 390 and zinc at 9780 mg/kg soil, decreased the chlorophyll content by 7 and 25% respectively, while the leghaemoglobin content in fresh nodules were reduced by 44% at each rate, compared to control at 90 DAS. In contrast, plants inoculated with RL9 at 195 mg Pb/kg soil increased the chlorophyll and leghaemoglobin by 221 and 100%, compared to inoculated but 195 mg Pb/kg amended soil (Table 54). Similarly, plants inoculated with RL9 at 4890 mg Zn/kg soil increased the chlorophyll and leghaemoglobin by 192 and 86%, compared to inoculated but 4890 mg Zn/kg amended soil (Table 55). Furthermore, chlorophyll and leghaemoglobin content increased even at 390 and 9780 mg/kg of lead and zinc, in the presence of bio-inoculant. Similarly, the toxicity of zinc to lentil increased with increase in the concentration, both in presence and absence of bio-inoculant (Table 55). Two factor ANOVA revealed that the individual effects of inoculation and Pb and Zn and their interaction \([(\text{inoculation x Pb}) \text{ and } (\text{inoculation x Zn})]\) were significant \((P \leq 0.05)\) for chlorophyll and leghaemoglobin content.

**4.13.4.3 Nitrogen content, seed yield and grain protein**

Nitrogen content, seed yield and grain protein decreased progressively with increase in the concentration of nickel in the absence of bio-inoculant (Table 53). Nickel at 580 mg/kg decreased the N content in roots and shoots by 10 and 4% at 90 DAS and 11 and 6% at 120 DAS, seed yield by 22% and grain protein by 8%, compared to control. In contrast, the bio-inoculant increased the N content, seed yield and grain protein even in the presence of different concentration of nickel, the maximum being 14 and 7% at 90 DAS and 19 and 8% in root N and shoot N respectively, 97% in seed yield and 15% in grain protein at 290 mg/kg
compared to non-inoculated but 290 mg Ni/kg amended soil. The bio-inoculant also increased the N content, seed yield and grain protein even at the highest dose of nickel, compared to un-inoculated but amended with same dose rates of nickel. The two way ANOVA showed that the individual effects of inoculation and nickel and their interaction (inoculation x nickel) were significant (P ≤ 0.05) for all the measured parameters except the individual effects of nickel on root N at 90 DAS and shoot N at 120 DAS and the interaction on root N content at 90 and 120 DAS and shoot N content at 120 DAS only.

Like the effect of nickel on lentil plants, lead also reduced the measured parameters considerably both in the presence and absence of bio-inoculant (Table 54). Lead at 390 mg/kg soil showed the greatest toxicity to lentil plants and decreased the N content in roots and shoots by 9 and 5% at 90 DAS, and 10 and 18% at 120 DAS, respectively, compared to control. The seed yield and grain protein were also decreased by 18 and 6%, at 390 mg Pb/kg soil, compared to control. In contrast, inoculated plants grown in 195 mg Pb/kg amended soil increased the N content in roots and shoots by 11 and 7 at 90 DAS and 18 and 9% at 120 DAS respectively, seed yield by 188% and grain protein by 11%, compared to inoculated and 195 mg Pb/kg amended soil (Table 54). Furthermore, chlorophyll and leghaemoglobin increased even at 390 mg Pb/kg soil in the presence of bio-inoculant. The two way ANOVA showed that the individual effects of inoculation and lead and their interaction (inoculation x Pb) were significant (P ≤ 0.05) for all the measured parameters except the effect of interaction on root N and shoot N content at 90 DAS.

Similarly, the toxicity of zinc to lentil increased with increase in the concentration, both in the presence and absence of bio-inoculant (Table 55). Zinc at 9780 mg/kg soil decreased the N content in roots and shoots by 7 and 5% at 90 DAS and 8 and 14% at 120 DAS respectively, seed yield by 14% and grain protein by 4% compared to control. In contrast, plants inoculated with Rhizobium strain RL9 at 4890 mg Zn/kg soil increased the N content in roots and shoots by 12 and 6% at 90 DAS and 17 and 10% at 120 DAS, seed yield by 210% and grain protein by 13%, compared to inoculated but 4890 mg Zn/kg amended soil (Table 55). Furthermore, N content, seed yield and grain protein were not adversely affected even at the highest tested rates of zinc, when strain RL9 was also used. The two way ANOVA showed that the individual effects of inoculation and zinc and their interaction (inoculation x zinc) were significant (P ≤ 0.05) for all the measured parameters except the individual effects of zinc on
shoot N content at 120 DAS and the effect of interaction on shoot N content at 90 and 120 DAS.

4.13.4.4 Glutathione reductase activity
Antioxidant enzyme, the glutathione reductase (GR) activity of roots and nodules, assayed at 90 and 120 DAS, increased considerably with increasing concentration of nickel (Fig 101 and 102), Pb (Fig 103 and 104) and Zn (Fig. 105 and 106), both in un-inoculated and inoculated plants. In un-inoculated plants, 580 mg Ni/kg, 390 mg Pb/kg and 9780 mg Zn/kg increased the GR activity of roots by 250, 200 and 117%, at 90 DAS while at 120 DAS, they enhanced it by 290, 230 and 130%, respectively, compared to control. While nickel, lead and zinc at the same dose rate increased the GR activity of nodules by 210, 140 and 100%, at 90 DAS and 211, 133 and 89% at 120 DAS, respectively, compared to control. In comparison, 580 mg Ni/kg, 390 mg Pb/kg and 9780 mg Zn/kg, when applied with inoculant strain, increased the GR activity of roots by 43, 22 and 38% at 90 DAS and 44, 21 and 43% at 120 DAS, respectively, and GR activity of nodules by 48, 4 and 15% at 90 DAS and 54, 5 and 18% at 120 DAS, respectively, compared to un-inoculated but amended with the same concentration of nickel and zinc.

4.13.4.5 Metal uptake
The uptake of nickel, lead and zinc by plant organs (roots and shoots) at 90 and 120 DAS and grains at harvest (120 DAS) was maximum at 580 mg/kg of nickel (Fig. 107 and 108), 390 mg/kg of lead (Fig. 109 and 110) and 9780 mg/kg of zinc (Fig. 111 and 112) both in the presence and absence of bio-inoculant. Generally, nickel, lead and zinc concentration in plant organs were less in the presence and absence of bio-inoculant at 145, 97.5 and 2445 mg/kg of Ni, Pb and Zn respectively, compared to those observed at the highest dose of each metal. Moreover, the concentrations of nickel, lead and zinc were recorded less in the presence of bio-inoculant compared to the un-inoculated plants. Generally, roots accumulated more concentrations of Ni, Pb and Zn compared to those observed for shoots or grains, under both inoculated and metal stressed condition.

4.13.5 Performance of pea in the presence of nickel and zinc tolerant Rhizobium strain RP5 in metal amended soil
4.13.5.1 Plant growth and symbiosis
In this study, nickel and zinc tolerant rhizobial strain RP5 was used to inoculate pea, which was then grown in the soil treated with different concentrations of nickel and zinc. The Rhizobium
inoculated and non-inoculated pea plants grown in sandy clay loam soil treated with three concentrations, each of nickel (Table 56) and zinc (Table 57) showed variable plant growth. In this experiment, plants grew poorly when soil was treated with different concentration of nickel and zinc. Generally, the length of plant organs, dry matter accumulation in plants and nodulation 90 and 120 days after sowing decreased progressively with increasing concentration of both nickel and zinc. In the absence of bio-inoculant, nickel at 580 mg/kg soil, had the largest phytotoxic effect and decreased the root length, shoot length, root dry weight, shoot dry weight, total dry matter, nodule numbers and nodule dry mass by 25, 18, 30, 10, 14, 13 and 23% at 90 DAS, and 23, 18, 32, 10, 13, 14 and 18 at 120 DAS, respectively, compared to control. On the contrary, when strain RP5 was also used with 580 mg Ni/kg soil, it increased the root length, shoot length, root dry weight, shoot dry weight, total dry matter, nodule numbers and nodule dry mass by 47, 32, 57, 12, 17, 25 and 22% at 90 DAS and 41, 31, 64, 10, 16, 25 and 12% at 120 DAS, respectively, compared to un-inoculated but treated with same dose of nickel (Plate 8B). While comparing the effects of different concentration of nickel on inoculated plants, a substantial increase of 53, 32, 53, 14, 23, 32 and 19% at 90 DAS and 40, 25, 68, 11, 23, 28 and 18% at 120 DAS in root length, shoot length, dry root weight, dry shoot weight, nodule numbers, nodule dry mass and total dry matter, respectively, was observed at 290 mg Ni/kg soil, compared to non-inoculated but amended with the same rate of nickel. The two way ANOVA revealed that the individual effects of inoculation and nickel was significant (P ≤ 0.05) for the measured parameters except the individual effects of inoculant on dry shoot weight at 90 DAS, nickel on dry shoot weight at 90 and 120 DAS, dry nodule weight at 90 DAS and total dry weight at 90 and 120 DAS. However, the interactive effect of inoculant and nickel was non-significant for dry shoot weight at 90 and 120 DAS, dry nodule weight at 90 DAS and total dry matter at both 90 and 120 DAS.

Similarly, the length of plant organs, dry matter production and symbiotic properties (e.g. nodule numbers and nodule mass) of pea plants declined with increasing concentrations of zinc (Table 57). In contrast, when strain RP5 was also added with 4890 mg Zn/kg soil, increased the root length, shoot length, dry root weight, dry shoot weight, nodule numbers, nodule dry mass and total dry matter by 50, 31, 41, 11, 23, 28 and 16% at 90 DAS and 45, 24, 50, 10, 21, 22 and 15% at 120 DAS respectively, compared to plants grown in the absence of bio-inoculant but treated with the same dose of zinc (Plate 8A). Moreover, like sole application
of zinc, the measured parameters also decreased with increasing concentrations of zinc, applied along with RP5, compared to the plants grown in soil treated solely with zinc. The two way ANOVA revealed that the individual effects of inoculation and zinc and their interaction (inoculation x zinc) was significant (P ≤ 0.05) for all the measured parameters except the individual effects of inoculation on shoot dry weight at 90 DAS, zinc on root and shoot dry weight at both 90 and 120 DAS, nodule numbers at 90 DAS and total dry weight at 120 DAS and the effect of interaction on root and shoot dry weight at 90 DAS and 120 DAS, dry nodule mass at 90 DAS and total dry mass at 120 DAS, respectively.

4.13.5.2 Photosynthetic pigments, leghaemoglobin, N content and seed attributes

Nickel and zinc when applied alone, decreased chlorophyll, leghaemoglobin (LH), N content, seed yield and grain protein (GP) consistently with increase in the concentration of Ni (Table 58) and Zn (Table 59). For un-inoculated plants, nickel at 290 mg kg⁻¹ decreased the chlorophyll content, LH, root N and shoot N by 9, 27, 9, and 8% at 90 DAS and root N, shoot N, seed yield and GP by 11, 9, 6 and 2% at 120 DAS, respectively, while 580 mg Ni/kg soil decreased the chlorophyll content, LH, root N and shoot N by 12, 45, 15 and 16% respectively, at 90 DAS and root N, shoot N, seed yield and GP by 18, 16, 10 and 3%, respectively, at 120 DAS, compared to the control. In comparison, the inoculant strain when applied with 290 mg Ni/kg soil, increased the chlorophyll content, LH, root N and shoot N by 19, 112, 26 and 47%, respectively, at 90 DAS and root N, shoot N, seed yield and GP by 40, 55, 26 and 8%, respectively, at 120 DAS, compared to pea plants grown in soil amended solely with the same dose of nickel. The measured parameters also increased even further at 580 mg Ni/kg soil inoculated with strain RP5, compared to non-inoculated but nickel treated soil. Furthermore, the measured parameters differed significantly (P ≤ 0.05) among inoculated and non-inoculated plants at 580 mg Ni/kg soil. Two factor ANOVA revealed that the individual effects of inoculation and Ni and their interaction (inoculation x Ni) were significant (P ≤ 0.05) for the measured parameters, except the individual effect of metal and interaction on chlorophyll content.

Similarly, the highest effect of zinc was recorded at 9780 mg/kg soil, which decreased the chlorophyll content, LH, root N and shoot N by 10, 36, 12 and 11%, respectively at 90 DAS and root N, shoot N, seed yield and GP, by 4, 9, 7 and 2%, respectively, at 120 DAS, compared to the control (Table 59). In comparison, the inoculated strain increased the
chlorophyll content, LH, root N and shoots N by 15, 85, 20 and 36%, at 90 DAS and root N, shoot N, seed yield and GP by 15, 38, 24 and 6%, respectively, at 120 DAS, compared to the plants grown in soil treated only with 9780 mg Zn/kg soil. However, zinc at 4890 mg/kg soil inoculated with strain RP5 showed a highest stimulatory effect and increased the chlorophyll content, LH, root N and shoots N by 16, 89, 22 and 39%, respectively at 90 DAS and root N, shoots N, seed yield and GP by 25, 45, 26 and 7%, at 120 DAS, respectively, compared to the plants grown in soil treated solely with 4890 mg Zn/kg soil. Like nickel application, more N was found in shoots when pea plant was grown in soil amended with zinc and with or without bio-inoculant. The two way ANOVA showed that the individual effects of inoculation and zinc and their interaction (inoculation x zinc) were significant (P ≤ 0.05) for all the measured parameters except the individual effects of zinc on chlorophyll and grain protein and the effect of interaction on chlorophyll and root N content at 120 DAS.

4.13.5.3 Glutathione reductase activity

The antioxidant enzyme production by pea, grown in metal stressed soil, was also determined in roots and nodules of both inoculated and un-inoculated plants. The GR activity in plant organs differed considerably and were influenced by nickel (Fig. 113 and 114) and zinc (Fig. 115 and 116) concentrations added to soils. Generally, a concentration dependent increase in GR activity of roots and nodules was observed for nickel and zinc in inoculated and non-inoculated plants. A maximum GR activity in plant organs for un-inoculated pea was observed at 580 mg Ni/kg which showed an increase of 243 (roots) and 208 % (nodules) at 90 DAS and 291 (roots) and 210% (nodules), respectively, at 120 DAS, over control. In comparison, the GR activity of roots and nodules of inoculated plants measured at 90 DAS was 250 and 281% respectively while at 120 DAS it was 275 and 279%, respectively, at 580 mg Ni/kg soil, compared to inoculated but metal free control. While comparing the effects of different concentration of nickel on GR activity, nickel at 580 mg/kg showed an increase of 46 and 65% at 90 DAS and 40 and 71% at 120 DAS in inoculated roots and nodules respectively, compared to un-inoculated but amended with the same dose of nickel. A trend similar to the effect of nickel on GR activity was observed for zinc, both in roots and nodules of inoculated and un-inoculated pea plants. In un-inoculated plants, 9780 mg Zn/kg increased the GR activity of roots by 114 and 117% at 90 and 120 DAS, respectively, compared to control. While zinc at the same dose rate increased the GR activity of nodules by 117% and 130%, 90
and 120 days after sowing, respectively, compared to control. In comparison, 9780 mg Zn/kg, when applied with inoculant strain, increased the GR activity of roots by 47 and 54% at 90 and 120 DAS, respectively, and GR activity of nodules by 54 and 52% at 90 and 120 DAS respectively, compared to un-inoculated but amended with the same concentration of zinc. Roots in general, showed more GR activity for both inoculated and un-inoculated plants grown in nickel and zinc stressed soil.

4.13.5.4 Nickel and zinc uptake

The uptake of nickel and zinc by roots and shoots at 90 and 120 DAS and grains at 120 DAS increased with increasing concentrations of nickel and zinc both for un-inoculated and inoculated plants. The average maximum accumulation of nickel and zinc in roots and shoots was 140 and 92 μg/g (Fig. 117), respectively, at 90 DAS and 165, 100 and 25 μg/g (Fig. 118) at 120 DAS, respectively, for plants grown in the absence of Rhizobium strain RP5 at 580 mg Ni/kg soil. In contrast, the maximum accumulation of nickel in roots and shoots of inoculated plant was 95 and 60 μg/g, respectively, at 90 DAS and 115, 75 and 18 μg/g, respectively, at 120 DAS, at the same rate of nickel applied to soil. The inoculated strain decreased the concentration of nickel in roots and shoots by 32 and 35 %, at 90 DAS and in roots, shoots and grains by 30, 25 and 28%, at 120 DAS, respectively, when plants were grown in soil treated with 580 mg Ni/kg soil, compared to non-inoculated plants. Similarly, for zinc (9780 mg/kg) treated soil, the bio-inoculant strain declined the uptake of zinc by 7 and 9% in roots and shoots at 90 DAS (Fig. 119) and roots, shoots and grains by 10, 9 and 25% at 120 DAS (Fig. 120), respectively, compared to plants grown in the absence of strain RP5. Moreover, roots in general, showed more uptake of nickel and zinc compared to shoots or grains of both inoculated and un-inoculated plants.