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Research Article

# *IN VITRO* ASSAY OF BIOMOLECULES, SYNTHESIS OF STRESS REDUCING PROLINE AND PERFORMANCE OF METAL TOLERANT *BRADYRHIZOBIUM* INOCULATED GREENGRAM (*Vigna radiata* L. Wilczek) UNDER METAL STRESS

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**Abstract-** Heavy metals are serious environmental pollutants and deleteriously affect the sustainability of microbes, plants and humans. Considering the toxicity of heavy metals, the present study was designed to isolate metal tolerant plant growth promoting rhizobacteria and to assess their plant growth promoting activities in the presence and absence of heavy metals. The best performing metal tolerant *Bradyrhizobium* strain C4 was selected to evaluate its impact on biological and chemical properties of greengram grown under metal stress. *Bradyrhizobium* sp. (vigna) strain C4 showed maximum tolerance to copper (1600 µg/ml), cadmium (200 µg/ml) and chromium (400 µg/ml) and produced siderophore, ammonia, cyanogenic compounds and synthesized indole-3-acetic acid under metal stress. *Bradyrhizobium* strain C4 enhanced the overall growth of greengram plants grown in soils stressed with/without varying concentrations of copper, cadmium and chromium. Proline concentration in greengram plants increased with increasing concentration of metals but declined significantly in *Bradyrhizobium* sp. (vigna) inoculated plants compared to control plants. The intrinsic abilities of growth promotion, enhanced performance of metal tolerant *Bradyrhizobium* sp. (vigna) inoculated plants and reduction in proline level of the inoculated plants grown under metal stress are indicative that *Bradyrhizobium* sp. (vigna) could be used for developing rhizobial inoculant for optimizing the production of greengram in soils polluted even with copper, cadmium and chromium.

**Keywords**- Bioremediation, *Bradyrhizobium*, Greengram, Heavy Metals, Proline.

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## Introduction

Heavy metals discharged from industrial operations such as smelting, mining, metal forging, manufacturing of alkaline storage batteries and combustion of fossil fuel are considered a major threat to the environmental sustainability [1]. Moreover, the agricultural activities like application of agrochemicals and use of sewage in agricultural fields also adds a considerable amount of metals to the soils [2]. The discharged heavy metals persist in the environment because they cannot be degraded biologically [3]. And hence, following uptake, metals severely affect the composition and functions of microbiota [4] and plants [5] and via food chains, the animals [6] and humans [7]. Phytotoxically, heavy metal inhibits antioxidative enzymes, impair ionic transport and redox potential of the cell and damage DNA [8,9]. Also, heavy metals adversely affect respiration process, protein synthesis and carbohydrate metabolism of plants [10]. To overcome metal stress, plants have evolved certain mechanisms. For instance, they store toxic metals in roots (bioaccumulation) and hence, prevent its translocation to other organs [11]. Also, plants secrete complex compounds that reduce metal availability in soil, exclude metal through selective uptake, immobilize and accumulate metal within vacuoles, increase production of metal-binding compounds and metal-tolerant enzymes. In this regard, amino acid for example, proline, an antioxidant and a free radical scavenger accumulates when plants are exposed to excessive stress. And hence, the accumulation of proline is considered one of the most important physiological strategies employed by higher plants to cope with toxicity under various stresses like heavy metals, salinity and drought [12]. Proline secreted by plants protects the cell membrane and enzymes [13] and also provide energy for growth and survival under stressed conditions [14]. Additionally, there are reports which suggest that proline can influence- (a) mitochondrial functions (b) cell proliferation or cell death and (iii) specific gene expression leading to protection of plants from abnormal environmental conditions. Plant growth promoting rhizobacteria (PGPR) among heterogeneously distributed soil microbiota obviate metal toxicity involving mechanisms such as biosorption, immobilization through the excretion of organic acids or bioleaching, bio-mineralization, intracellular accumulation and enzyme catalyzed transformation [4]. Consequently, after application, they improve the health of the plants in polluted soils [15] by supplying important plant nutrients like N (nitrogen fixers) and P (phosphate solubilizers), production of phytohormones and by disease suppression [16]. Considering such physiologically important traits, the use of agronomically inexpensive and vital metal tolerant PGPR has become one of the most preferred choices due in part to its easy to operate option and safety in bioremediation strategies. The present study was therefore, aimed at identifying metal tolerant PGPR, evaluating their plant growth promoting activities under metal stress and assessing the impact of metal tolerant strain on biological and chemical properties of greengram, grown under metal stressed soils.

## Materials and Methods

### Isolation of bacterial cultures and sensitivity/resistance to metals

Soil samples were collected from the rhizospheres of garlic *(Allium sativum)* and cabbage *(Brassica oleracea)* grown in fields receiving sewage. Soil samples were serially diluted in sterile normal saline solution and spread evenly onto nutrient

agar (NA), King's B (KB), yeast extract mannitol (YEM) agar and Jensen's medium (JM) for quantifying total bacterial populations, *Pseudomonas, Rhizobium* and *Azotobacter* populations, respectively. Plates were incubated at 28±2°C for two days (NA), two to three days (KB), six to seven days (YEMA) and four to five days (JM) and populations were counted. Each bacterial isolate appearing on respective plates was streaked three times on the same plates in order to obtain a pure bacterial culture. Bradyrhizobial strain used in this study as bacterial inoculant was recovered from nodules of greengram plants grown in the fields of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, ($27^0 29^1$ latitude and $72^0$ $29^1$ longitude) using Yeast Extract Mannitol (YEM) medium. For this, healthy and undamaged nodules were surface sterilized with sodium hypochlorite (2.5 % for 2 min.). The surface sterilized nodules were rinsed in 95 % ethanol (v/v) and washed with several changes of distilled water (DW). The nodules were crushed gently in Normal Saline Solutions (NSS). Nodule suspensions were serially diluted in NSS and 100 µl of each diluent was spread plated on YEM agar medium (Hi Media Laboratories Pvt. Ltd. Mumbai, India) containing 2.5% Congo red indicator dye. The inoculated plates were incubated at 28±2 °C for 5 days. A- single colony was picked and streaked four times on the same medium to ascertain the purity of the culture and the selected strain was maintained on YEM (g/l: mannitol 10; $K_2HPO_4$ 0.5; $MgSO_4$. $7H_2O$ 0.2; NaCl 0.1; yeast extract 1; $CaCO_3$ 1 and pH was adjusted to 7) agar medium at 4 °C until use. *Bradyrhizobium* strain C4 was subjected to plant infection test (nodulation test) using greengram as a host plant in order to validate its host specificity. Minimum inhibitory concentration (MIC) of metals was determined by plate dilution method [17] using nutrient agar plates. A varying concentrations ranging from 25 µg/ml to 1600 µg/ml of salts of Cu [($CuSO_4$. $5H_2O$; purity 98.5%; mol. weight (g/mol) 249.68; CAS No. 7758-99-8; Qualigens], Cd [($CdCl_2.2H_2O$; purity 98%; mol. weight (g/mol) 201.33; CAS No. 34330-64-8; Qualigens] and Cr ( $K_2Cr_2O_7$; purity 99.5%; mol. weight (g/mol) 294.19; CAS No. 7778-50-9; Qualigens] was added to the autoclaved nutrient agar medium. Each bacterial isolate was spot inoculated onto the plates and incubated at 28±2°C for 24–48 h. The concentration of metals inhibiting the growth was defined as MIC while the concentration supporting the growth was defined as maximum tolerance level (MTL).

***In vitro* bioassay of plant growth promoting activities**

**Indole acetic acid, siderophores, ammonia and cyanide production**

Indole acetic acid (IAA) was quantitatively assayed by the method of Gordon and Weber [18], later modified by Brick *et al*. [19]. Bacterial cultures ($10^8$ cells $ml^{-1}$) were inoculated into Luria Bertani (LB) broth (g $l^{-1}$: tryptone 10; yeast extract 5; NaCl 10 and pH 7.5; supplemented with different concentrations of tryptophan (0, 100, 200, 400 and 500 µg/ml) and were incubated for 48 h at 28±2 °C with shaking at 125g for 72 h. Fully grown cultures were centrifuged at 9000g for 30 min. Two drops of orthophosphoric acid and foul milliliter of Salkowski reagent (50 ml, 35% of perchloric acid, 1ml 0.5M $FeCl_3$ solution) was added to two milliliter supernatant and incubated at room temperature for 20-30 min. Development of pink color indicated IAA production. Optical density was taken at 530 nm using Spectronic 20 $D^+$. The IAA was determined using a calibration curve of pure IAA as a standard. Siderophores was evaluated by $FeCl_3$ method. Autoclaved nutrient broth was inoculated with cultures and incubated at 28±2°C for four days. After incubation, cultures were centrifuged at 5000 r/min. for 15 min. One milliliter of 2% ferric chloride was added to one milliliter of supernatant and change in color from orange to reddish brown was observed. The production of ammonia and cyanide were determined by the method of Dye [20] and Bakker and Schipper [21], respectively. The plant growth promoting substances were also determined under metal stressed environment. For this, 25 to 400 µg/ml of Cu, Cd and Cr were added to each respective medium and the growth regulators were assessed by the method similar to those adopted for metal free environment.

**Bacterial preparation, seed treatments and planting**

*Bradyrhizobium* sp. (vigna) identified by plant infection test was selected on the basis of its metal tolerant abilities and plant growth promoting activities to evaluate its effect on greengram (var. K-851) grown in small thermocol cups (6.8 cm x 8 cm), each consisting of 150 g of sterilized (autoclaved) soil (sandy clay loam; organic C 0.4%; Kjeldahl N 0.75 g/kg; Olsen P 16 mg/kg; cation exchange capacity 11.7 cmol $kg^{-1}$, anion exchange capacity; 5.1 cmol/ kg, salt conductivity 59.1 $\mu Sm^{-1;}$ WHC 0.44 ml/g ; pH7.2). The experiment was conducted using sterile soils to avoid competition/interaction from indigenous microflora and hence, to assess the real effects of test organism. The soil was treated with three concentrations (normal, double and three times more of normal rates) of Cu, Cd and Cr. The normal dose of Cu (527 mg/kg soil), Cd (11 mg/kg soil) and Cr (68 mg/kg soil) was comparable to those found in sewage soil used for greengram production. Each metal was used alone and in combination both in the presence and absence of *Bradyrhizobium* sp. (vigna). Heavy metals were added to soils two days before sowing. Healthy seeds of greengram were surface sterilized (70 % ethanol, 3 min; 3 % sodium hypochlorite, 3 min), rinsed 6 times with sterile water and dried. Surface sterilized seeds were inoculated by soaking seeds for 2 h using 10 % gum arabic as sticker to deliver approximately $10^8$ cells per seed of *Bradyrhizobium* strain C4. A total of six healthy seeds of greengram var. K-851 were sown in each pot. Untreated and uninoculated seeds and untreated but *Bradyrhizobium* inoculated seeds served as control. The experiment consisted of 38 treatments and three replications with pots distributed in a completely randomized design. The treatments were- (1) T1= Cu 527 mg/kg (2) T2= Cu 1054 mg/kg (3) T3= Cu 1581 mg/kg (4) T4= Cu 527mg/kg+ *Bradyrhizobium* sp. (vigna) (5) T5= Cu 1054 mg/kg+ *Bradyrhizobium* sp. (vigna) (6) T6= Cu 1581mg/kg+ *Bradyrhizobium* sp. (vigna) (7) T7= Cd 11 mg/kg (8) T8= Cd 22 mg/kg (9) T9= Cd 33 mg/kg (10) T10= Cd 11 mg/kg+ *Bradyrhizobium* sp. (vigna) (11) T11= Cd 22 mg/kg+ *Bradyrhizobium* sp. (vigna) (12) T12= Cd 33 mg/kg+ *Bradyrhizobium* sp. (vigna) (13) T13= Cr 68 mg/kg (14) T14= Cr 137 mg/kg (15) T15= Cr 205 mg/kg (16) T16= Cr 68 mg/kg+ *Bradyrhizobium* sp. (vigna) (17) T17= Cr 137 mg/kg+ *Bradyrhizobium* sp. (vigna) (18) T18= Cr 205 mg/kg+ *Bradyrhizobium* sp. (vigna) (19) T19= Cu 527 mg/kg+ Cd 11 mg/kg (20) T20= Cu 1054 mg/kg+ Cd 22 mg/kg (21) T21= Cu 1581 mg/kg+ Cd 33 mg/kg (22) T22= Cu 527 mg/kg+ Cd 11 mg/kg+ *Bradyrhizobium* sp. (vigna) (23) T23= Cu 1054 mg/kg+ Cd 22 mg/kg+ *Bradyrhizobium* sp. (vigna) (24) T24= Cu 1581 mg/kg+ Cd 33 mg/kg+ *Bradyrhizobium* sp. (vigna) (25) T25= Cd 11 mg/kg+ Cr 68 mg/kg (26) T26= Cd 22 mg/kg+ Cr 137 mg/kg (27) T27= Cd 33 mg/kg+ Cr 205 mg/kg (28) T28= Cd 11 mg/kg+ Cr 68 mg/kg+ *Bradyrhizobium* sp. (vigna) (29) T29= Cd 22 mg/kg+ Cr 137 mg/kg+ *Bradyrhizobium* sp. (vigna) (30) T30= Cd 33 mg/kg+ Cr 205 mg/kg+ *Bradyrhizobium* sp. (vigna) (31) T31= Cu 527 mg/kg+ Cr 68 mg/kg (32) T32= Cu 1054 mg/kg+ Cr 137 mg/kg (33) T33= Cu 1581 mg/kg+ Cr 205 mg/kg (34) T34= Cu 527 mg/kg+ Cr 68 mg/kg+ *Bradyrhizobium* sp. (vigna) (35) T35= Cu 1054 mg/kg+ Cr 137 mg/kg+ *Bradyrhizobium* sp. (vigna) (36) T36= Cu 1581 mg/kg+ Cr 205 mg/kg+ *Bradyrhizobium* sp. (vigna) (37) T37= Control (without metal and without *Bradyrhizobium* sp.) (38) T38= Control (without metal but with *Bradyrhizobium* sp.). Seven days after germination, pots were thinned to three plants per pot. Experimental pots were watered regularly with tap water and were maintained in open field conditions throughout the experiment. The growth of plants such as length of roots, shoots and whole plant length was recorded at 20 and 40 days after sowing (DAS) only due to small experimental pot size. Since the experimental pots were small in size, the yield parameters were not assessed due to fear of lodging of greengram plants and poor availability of plant nutrients available in pots. Fresh and dry matter accumulation was also recorded. The data were subjected to two-way ANOVA and significant partial difference (LSD) was calculated at 5% probability level.

**Estimation of proline and chlorophyll**

Plants uprooted at 20 DAS were used to assay proline content [22]. For this, 0.5g of plant tissue was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. The homogenate was filtered through Whatman No. 2 filter paper and two ml of this filtrate was taken in a test tube and two ml of glacial acetic acid and two ml of acid ninhydrin were added to it. The tubes were heated in boiling water bath for 1h. The reaction was terminated by placing the tubes in ice bath. A- 4 ml of toluene was added to the reaction mixture and stirred well for 20 to 30 sec. Colored

complex was extracted in toluene and toluene layer was separated. The red color intensity was measured at 520 nm. A series of standard with pure proline was run in a similar way by dissolving proline in 3% sulfosalicylic acid and a standard curve was prepared. Amount of proline was determined from the standard curve and proline content on fresh weight basis (μ moles per gram of tissue) was expressed as:

$$\text{Proline content} = \frac{\mu g\ proline/\ ml \times ml\ of\ toluene}{115.5} \times \frac{5}{g\ of\ sample};$$

Where 115.5 is the molecular weight of proline. Total chlorophyll content in fresh foliage was estimated at 40 DAS by the method of Arnon [23].

## Results and Discussion

The free living PGPR strains were isolated from garlic and cabbage rhizospheres while the symbiotic *Bradyrhizobum* was recovered from nodules of greengram plants. Both free living and symbiotic PGPR were primarily identified by Gram's reaction and morphological and biochemical tests. Bacterial strains even-though showed a variable Gram's reaction but all cultures were rod shaped. Of these, isolates G2, G5, C1, C2, C3, C4 and C5 were Gram negative whereas G1, G3 and G4 were Gram positive. Comparing the morphological and biochemical properties observed in this study and those given in Bergey's Manual of Determinative Bacteriology [24], the bacterial cultures were presumptively identified to genus level as *Bacillus* (G1, G3 and G4), *Pseudomonas* (G2, G5, C1 and C2), *Rhizobium* and *Bradyrhizobium* (C3 and C4, respectively) and *Azotobacter* (C5). Furthermore, Cu, Cd and Cr ranging between 25 to1600 μg/ml were used to identify metal tolerant PGPR strains. The toxicity of heavy metals to *Bradyrhizobium* strain varied considerably. However, the toxicity of heavy metals decreased in the following order: Cd>Cr >Cu and was concentration and metal species dependent. *Bradyrhizobium* strain C4 was highly tolerant toward Cu and could grow even at 1600 μg/ml which was followed by Cr (400 μg/ml). In contrast, strain C4 was found highly sensitive to Cd (200 μg/ml) [Table-1]. Similar variation in metal tolerance among other rhizobia for example *R. leguminosarum* biovar viciae, when grown with different concentrations of Zn, Pb, Co, Cd, Ni, and Cr is reported [25]. The variation in the ability of bacteria to tolerate different levels of metals could be due to the differences in the types of media used and growth conditions employed. In a study, Wani *et al*. [26] recorded similar higher level of Ni and Zn tolerance among *R. leguminosarum* strain RP5.. After assessing the metal tolerance ability, the bacterial cultures were tested further to evaluate their ability to produce plant growth regulators such as IAA, HCN, ammonia and siderophores. All bacterial strains used in this study produced a considerable amount of IAA, which increased progressively with increasing concentrations of tryptophan. Among PGPR, *Bradyrhizobium* strain C4 produced maximum (86 μg/ml) amounts of IAA when it was grown in LB medium treated with 500 μg/ml tryptophan [Table-1]. The phytohormone, IAA is reported to control cell division, root initiation, phototropism and apical dominance in plants [4] and PGPR capable of secreting IAA has been found to facilitate plant growth [27]. Among all isolates, strains of *Pseudomonas* and *Bradyrhizobium* were positive for HCN and ammonia production. Furthermore, 57% of the total PGPR could synthesize siderophore after three days of incubation. In other investigation, soil bacteria, in general, have been reported to induce the synthesis of siderophores in heavy metal contaminated soils because of Fe deficiency [28]. The maximum heavy metal tolerance and secretion of active biomolecules under metal free environment prompted to assess the plant growth promoting potential of *Bradyrhizobium* strain C4 in medium treated with varying levels of heavy metals. During this experiment, it was found that the amount of IAA decreased regularly with increasing concentrations of Cu, Cd and Cr, added to the medium. For example, strain C4 produced 58 μg/ml of IAA when 25 μg/ml of Cu was added to the medium which however, decreased further to 36 μg/ml at 400 μg Cu/ml. Similarly, in the presence of 25 μg/ml of Cd, 56 μg/ml of IAA was produced by *Bradyrhizobium* strain C4 which decreased consistently to 34 μg/ml, when strain C4 was grown in the presence of 400 μg/ml Cd [Table-2].

**Table-1** *Minimum inhibitory concentration (MIC) of heavy metals determined by spot inoculation method and quantitative assay of indole acetic acid synthesized by rhizobacterial strains at different concentrations of tryptophan*

| Bacterial isolates | MIC (μg/ml) | | | Indole acetic acid (μg/ml) | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cu | Cd | Cr | 0T* | 100T* | 200T* | 400T* | 500T* |
| G1 | 800 | 200 | 800 | 10 | 26 | 38 | 42 | 53 |
| G2 | 1600 | 200 | 800 | 24 | 43 | 52 | 67 | 79 |
| G3 | 1600 | 200 | 800 | 30 | 44 | 65 | 77 | 82 |
| G4 | 800 | 25 | 800 | 22 | 50 | 68 | 84 | 75 |
| G5 | 400 | 50 | 800 | 18 | 32 | 53 | 76 | 69 |
| C1 | 800 | 50 | 200 | 33 | 51 | 66 | 70 | 80 |
| C2 | 800 | 25 | 400 | 26 | 40 | 55 | 68 | 73 |
| C3 | 400 | 100 | 400 | 35 | 46 | 64 | 72 | 80 |
| C4 | 1600 | 200 | 400 | 39 | 50 | 66 | 78 | 86 |
| C5 | 800 | 100 | 400 | 28 | 37 | 54 | 74 | 85 |

T* indicates concentration of tryptophan in μg/ml; Values are mean of three independent replicates.

**Table-2** *Effect of varying concentrations of heavy metals on active biomolecules synthesized by Bradyrhizobium strain C4 under in vitro conditions*

| Metals used | Dose rate (μg/ml) | Plant Growth Promoting activities | | | |
|---|---|---|---|---|---|
| | | $NH_3$ production | Siderophore production | IAA* (μg/ml) | HCN production |
| Control | 0 | +++ | + | 66 | ++ |
| Cu | 25 | ++ | + | 58 | – |
| | 50 | ++ | + | 54 | – |
| | 100 | ++ | + | 46 | – |
| | 200 | ++ | + | 36 | – |
| | 400 | ++ | + | 40 | – |
| Cd | 25 | + | + | 56 | – |
| | 50 | + | + | 52 | – |
| | 100 | + | + | 48 | – |
| | 200 | – | + | 40 | – |
| | 400 | – | + | 34 | – |
| Cr | 25 | + | + | 60 | – |
| | 50 | + | + | 55 | – |
| | 100 | + | + | 52 | – |
| | 200 | – | + | 43 | – |
| | 400 | – | + | 40 | – |

+ indicates positive activity; – indicates no activity; *IAA were determined at 200 μg/ml of tryptophan added to Luria Bertani broth medium; Values are mean of three independent replicates

In addition, *Bradyrhizobium* strain C4 produced ammonia at all concentrations of Cu ranging from 25–400 µg/ml, added to peptone water. The intensity of ammonia production however, declined in the presence of Cd and ammonia was not detected when 200 and 400 µg Cd/ml was added to the medium. Similar results were obtained for Cr also. Moreover, strain C4 showed positive reaction for siderophore irrespective of the metal species. Strain C4 however, did not produce HCN when grown in the presence of heavy metals. The ability of nodule bacterium to tolerate high level of metals and to secrete important plant growth promoting active biomolecules even in the presence of heavy metals as observed in this study in addition to their intrinsic property of $N_2$ fixation makes them one of the most suitable and valuable choices for legume production in metal contaminated soils.

Considering these properties, *Bradyrhizobium* strain C4 was used to assess its plant growth promoting abilities against greengram, grown in pots filled with sandy clay loam soils treated with varying levels of Cu, Cd and Cr. Plants grown in soil treated differently with varying concentration of heavy metals had stunted growth relative to untreated soils. Generally, the toxicity of metals to biological properties of uninoculated and inoculated plants enhanced with progressively increasing concentration of each metal. Among the three metals used, all the three concentrations of Cr (VI) in particular were highly toxic to greengram. Moreover, Cr (VI) in general, had a visible phytotoxic effect on leaves which was evident by- (i) yellowing of leaves (ii) small leaf size (iii) curling of leaves and (iv) poor shoot length. On the contrary, a significant difference in the length of roots and shoots was observed among *Bradyrhizobium* inoculated and uninoculated plants grown in soils amended with metals. The shoot length of *Bradyrhizobium* inoculated plants uprooted at 20 DAS was increased by 13% (8.5 cm) compared to the shoot length of uninoculated plants grown in the presence of 1054 mg Cu/kg [Table-3]. On the other hand, the root length of inoculated plants grown in soils treated with 11 mg Cd/kg, uprooted at 20 DAS, increased from 3.2 cm to 3.7 cm [Table-4]. Also, the shoot lengths of inoculated plants grown with 68 mg Cr/kg and uprooted at 20 DAS was better than uninoculated plants. The root length of plants however, decreased by 63% (1.5 cm) at 68 mgCr/kg compared with uninoculated control (4 cm) [Table-5]. In contrast, *Bradyrhizobium* in the presence of 68 mg Cr/kg, increased the root length (1.6 cm) and shoot length (2.6 cm) by 25% and 23%, respectively, relative to the uninoculated plants uprooted at 40 DAS. In general, Cu had least toxic effects and a marginal 5% (3.8 cm) decrease in root length was observed when soil was treated with 1054 mg Cu/kg over uninoculated control at 40 DAS. Like Cr and Cd, *Bradyrhizobium* in the presence of Cu, increased the root length by 8 (5 cm) and 16% (4.5 cm) at 1x and 2x concentrations of Cu, respectively at 40 DAS. In the presence of 68 mg Cr/kg, the dry matter accumulation in plants increased from 0.09 g (20 DAS) to 0.12 g (40 DAS). A similar increase in dry matter accumulation was recorded at 40 DAS when plants were grown in the presence of 11 mg Cd/kg. *Bradyrhizobium* inoculation however, further improved the dry biomass of greengram plants at 40 DAS compared to those observed at 20 DAS. For instance, at 40 DAS, there was 0.18 g biomass accumulated within *Bradyrhizobium* inoculated plants grown at 11 mg Cd/kg.

**Table-3** *Effect of copper on biological properties of greengram plants*

| Treatment | Length of plant organs (cm) | | | | Total plant length (cm) | | Dry weight (g/plant) | | Proline content (µg/g fresh weight) |
|---|---|---|---|---|---|---|---|---|---|
| | Root | | Shoot | | | | | | |
| | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS |
| Control | 3.5±0.5 | 4.0±0.3 | 9.7±0.6 | 10.1±0.8 | 13.2±0.8 | 14.1±1.0 | 0.18±0.06 | 0.19±0.03 | 1.92±0.11 |
| Cu (527) | 4.3±0.3 | 4.6±0.2 | 7.6±0.9 | 7.7±0.8 | 11.8±0.8 | 11.7±0.6 | 0.12±0.06 | 0.12±0.02 | 2.46±0.59 |
| Cu (1054) | 3.7±0.4 | 3.8±0.3 | 7.4±0.1 | 7.2±0.6 | 11.0±0.5 | 11.1±0.6 | 0.09±0.02 | 0.10±0.03 | 2.27±0.33 |
| Cu (1581) | 3.8±0.3 | 4.2±0.3 | 7.1±0.6 | 6.8±0.3 | 11.0±0.6 | 11.0±0.5 | 0.07±0.02 | 0.09±0.03 | 5.56±0.57 |
| Control+R | 4.0±0.2 | 4.4±0.4 | 10.0±0.3 | 10.8±0.4 | 14.0±0.8 | 15.2±0.3 | 0.19±0.03 | 0.20±0.03 | 2.48±0.11 |
| Cu (527)+R | 4.0±0.4 | 5.0±0.2 | 8.4±0.9 | 7.5±0.7 | 12.3±0.8 | 12.5±0.7 | 0.13±0.02 | 0.14±0.03 | 1.71±0.20 |
| Cu (1054)+R | 3.6±0.3 | 4.5±0.5 | 8.5±0.6 | 8.1±0.5 | 12.1±0.1 | 12.6±0.8 | 0.12±0.06 | 0.14±0.02 | 1.88±0.13 |
| Cu (1581)+R | 3.2±0.3 | 4.1±0.5 | 8.2±0.7 | 7.8±0.6 | 11.4±1.6 | 11.9±0.8 | 0.11±0.03 | 0.13±0.04 | 4.13±0.12 |
| LSD | 0.588 | 0.530 | 1.652 | 1.893 | 1.649 | 1.746 | 0.013 | 0.004 | 0.566 |

Values in parenthesis indicate the metal dose rate (mg/kg soil); Each value represents mean ± standard deviation of three replicates where each replicate constituted three plants/pot; 'R' indicates *Bradyrhizobium* strain C4

**Table-4** *Effect of cadmium on biological properties of greengram plants*

| Treatment | Length of plant organs (cm) | | | | Total plant length (cm) | | Dry weight (g/plant) | | Proline content (µg/g fresh weight) |
|---|---|---|---|---|---|---|---|---|---|
| | Root | | Shoot | | | | | | |
| | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS |
| Control | 3.5±0.5 | 4.0±0.3 | 9.7±0.6 | 10.1±0.8 | 13.2±0.8 | 14.1±1.0 | 0.18±0.06 | 0.19±0.03 | 1.92±0.11 |
| Cd (11) | 3.2±0.2 | 3.3±0.3 | 8.3±0.5 | 9.2±0.8 | 11.5±0.5 | 12.5±0.5 | 0.16±0.06 | 0.17±0.03 | 3.12±1.02 |
| Cd (22) | 2.8±0.7 | 3.0±0.7 | 7.7±1.0 | 8.2±0.6 | 10.5±0.7 | 11.2±0.6 | 0.17±0.02 | 0.18±0.03 | 4.52±1.01 |
| Cd (33) | 2.4±0.4 | 2.7±0.3 | 6.5±0.1 | 6.8±0.8 | 8.9±0.4 | 9.5±0.5 | 0.14±0.02 | 0.15±0.03 | 9.37±0.84 |
| Control +R | 4.0±0.2 | 4.4±0.4 | 10.0±0.3 | 10.8±0.4 | 14.0±0.8 | 15.2±0.3 | 0.19±0.03 | 0.20±0.03 | 2.48±0.11 |
| Cd (11)+R | 3.7±0.3 | 4.3±0.3 | 8.3±0.8 | 9.2±0.3 | 12.0±1.0 | 13.5±0.5 | 0.16±0.06 | 0.18±0.02 | 2.68±0.73 |
| Cd (22)+R | 3.1±0.6 | 3.3±0.5 | 7.7±1.0 | 7.9±0.9 | 10.8±0.8 | 11.2±0.6 | 0.09±0.06 | 0.13±0.03 | 4.48±0.58 |
| Cd (33)+R | 3.3±0.4 | 3.6±0.1 | 6.7±0.4 | 8.0±0.5 | 10.0±0.5 | 11.6±0.3 | 0.15±0.06 | 0.15±0.02 | 6.40±1.0 |
| LSD | 0.726 | 0.582 | 1.221 | 1.576 | 1.741 | 1.522 | 0.006 | 0.015 | 0.711 |

Values in parenthesis indicate the metal dose rate (mg/kg soil); Each value represents mean ± standard deviation of three replicates where each replicate constituted three plants/pot; 'R' indicates *Bradyrhizobium* strain C4.

Among metals, Cu in general showed little inhibitory effect on the measured parameters. A trend similar to those observed for single metal application was also recorded for composite metals. The root length of plants grown in the presence of mixture of Cu (527 mg/kg) and Cd (11 mg/kg) increased from 2.3 cm to 3.2 cm at 20 DAS and 40 DAS, respectively. However, the composite application of Cu (527 mg/kg) and Cd (11 mg/kg) slightly increased the shoot length both at 20 and 40 DAS [Table-6]. Interestingly, the measured parameters were increased further in the presence of *Bradyrhizobium* which could possibly be due to reduction in the stress levels in inoculated plants. On the other hand, when grown with 11 mg Cd/kg + 68 mg Cr/kg, the plants exhibited a significant stunted growth. As a result, the root length of 20 days old plants was recorded as 1.1 cm which increased to 1.6 cm at 40 DAS. These values were remarkably low compared to the root length of control plants (4 cm) at 40 DAS [Table-7]. Also, the root length of plants increased from 1.1 to 1.4 cm at 20 and 40 DAS, when grown in the presence of 527 mg Cu/kg+ 68 mg Cr/kg. No significant difference was however, observed in shoot length. Interestingly, *Bradyrhizobium* application reduced the toxicity of combined metals and enhanced the root length of inoculated plants at 40 DAS [Table-8]. However, the yield parameters were not measured due to small size of experimental pots. Moreover, the pod and grain formation requires considerably higher amounts of plant nutrients, but due to small pot size, the nutrient pool was

**Table-5** *Effect of chromium on biological properties of greengram plants*

| Treatment | Length of plant organs (cm) | | | | Total plant length (cm) | | Dry weight (g/plant) | | Proline content (µg/g fresh weight) |
|---|---|---|---|---|---|---|---|---|---|
| | Root | | Shoot | | | | | | |
| | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS |
| Control | 3.5±0.5 | 4.0±0.3 | 9.7±0.6 | 10.1±0.8 | 13.2±0.8 | 14.1±1.0 | 0.18±0.06 | 0.19±0.03 | 1.92±0.11 |
| Cr (68) | 1.4±0.4 | 1.5±0.5 | 3.0±0.1 | 3.0±0.5 | 4.4±0.3 | 4.5±0.3 | 0.09±0.04 | 0.12±0.02 | 6.49 ± 1.12 |
| Cr (137) | 1.2±0.3 | 1.6±0.2 | 2.4±0.4 | 2.1±0.6 | 3.6±0.4 | 3.7±0.5 | 0.08±0.05 | 0.09±0.03 | 11.32 ± 0.75 |
| Cr (205) | 0.9±0.1 | 1.2±0.3 | 2.2±0.4 | 2.0±0.4 | 3.1±0.6 | 3.2±0.8 | 0.01±0.02 | 0.02±0.02 | 18.38 ± 0.76 |
| Control+R | 4.0±0.2 | 4.4±0.4 | 10.0±0.3 | 10.8±0.4 | 14.0±0.8 | 15.2±0.3 | 0.19±0.03 | 0.20±0.03 | 2.48±0.11 |
| Cr (68)+R | 0.9±0.2 | 1.0±0.2 | 3.5±0.2 | 3.5±0.4 | 4.4±0.4 | 4.6±0.5 | 0.19±0.05 | 0.20±0.06 | 6.33 ± 1.10 |
| Cr (137)+R | 1.4±0.4 | 1.5±0.0 | 2.8±0.3 | 2.8±0.6 | 4.2±0.4 | 4.3±0.3 | 0.04±0.03 | 0.05±0.03 | 9.49 ± 0.72 |
| Cr (205)+R | 1.3±0.2 | 1.6±0.5 | 2.6±0.3 | 2.6±0.8 | 3.9±0.3 | 4.2±0.3 | 0.02±0.02 | 0.02±0.02 | 13.94 ± 0.55 |
| LSD | 0.432 | 0.523 | 0.633 | 0.877 | 0.751 | 0.975 | 0.007 | 0.004 | 0.783 |

Values in parenthesis indicate the metal dose rate (mg/kg soil); Each value represents mean ± standard deviation of three replicates where each replicate constituted three plants/pot; 'R' indicates *Bradyrhizobium* strain C4

**Table-6** *Effect of mixture of copper and cadmium on biological properties of greengram plants*

| Treatment | Length of plant organs (cm) | | | | Total plant length (cm) | | Dry weight (g/plant) | | Proline content (µg/g fresh weight) |
|---|---|---|---|---|---|---|---|---|---|
| | Root | | Shoot | | | | | | |
| | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS |
| Control | 3.5±0.5 | 4.0±0.3 | 9.7±0.6 | 10.1±0.8 | 13.2±0.8 | 14.1±1.0 | 0.18±0.06 | 0.19±0.03 | 1.92±0.11 |
| Cu (527)+Cd (11) | 2.3±0.3 | 3.2±0.3 | 9.7±0.8 | 9.8±0.8 | 12.8±0.8 | 13.0±0.5 | 0.16±0.02 | 0.16±0.02 | 3.67 +0.61 |
| Cu (1054)+Cd (22) | 1.8±0.2 | 2.2±0.3 | 9.9±0.5 | 10.4±0.4 | 12.3±0.3 | 12.6±0.2 | 0.18±0.02 | 0.19±0.03 | 3.98+0.45 |
| Cu (1581)+Cd (33) | 1.2±0.2 | 1.7±0.3 | 9.3±0.6 | 9.3±0.4 | 10.5±0.9 | 11.0±0.5 | 0.09±0.07 | 0.09±0.03 | 3.79+0.15 |
| Control+R | 4.0±0.2 | 4.4±0.4 | 10.0±0.3 | 10.8±0.4 | 14.0±0.8 | 15.2±0.3 | 0.19±0.03 | 0.20±0.03 | 2.48±0.11 |
| Cu (527)+Cd (11)+R | 3.2±0.3 | 3.6±0.1 | 10.6±0.2 | 9.9±0.4 | 12.9±0.4 | 13.4±0.4 | 0.18±0.01 | 0.18±0.03 | 3.31+0.43 |
| Cu (1054)+Cd (22)+R | 2.3±0.2 | 2.7±0.3 | 10.3±0.4 | 10.3±0.8 | 12.2±0.3 | 13.0±0.6 | 0.16±0.04 | 0.16±0.05 | 3.26+0.42 |
| Cu (1581)+Cd (33)+R | 1.3±0.3 | 2.0±0.5 | 9.9±0.6 | 10.0±0.7 | 11.2±0.3 | 12.0±0.3 | 0.09±0.04 | 0.09±0.05 | 5.47+0.79 |
| LSD | 0.499 | 0.474 | 0.843 | 1.083 | 0.891 | 0.943 | 0.011 | 0.005 | 0.789 |

Values in parenthesis indicate the metal dose rate (mg/kg soil); Each value represents mean ± standard deviation of three replicates where each replicate constituted three plants/pot; 'R' indicates *Bradyrhizobium* strain C4.

**Table-7** *Effect of mixture of cadmium and chromium on biological properties of greengram plants*

| Treatment | Length of plant organs (cm) | | | | Total plant length (cm) | | Dry weight (g/plant) | | Proline content (µg/g fresh weight) |
|---|---|---|---|---|---|---|---|---|---|
| | Root | | Shoot | | | | | | |
| | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS |
| Control | 3.5±0.5 | 4.0±0.3 | 9.7±0.6 | 10.1±0.8 | 13.2±0.8 | 14.1±1.0 | 0.18±0.06 | 0.19±0.03 | 1.92±0.11 |
| Cd (11)+Cr (68) | 1.1±0.2 | 1.6±0.1 | 3.1±0.3 | 2.8±0.3 | 4.3±0.3 | 4.3±0.3 | 0.10±0.01 | 0.10±0.03 | 5.62±0.74 |
| Cd (22)+Cr (137) | 1.3±0.3 | 1.6±0.2 | 2.4±0.6 | 2.4±0.7 | 3.8±0.7 | 4.0±0.6 | 0.08±0.05 | 0.08±0.03 | 9.69±0.36 |
| Cd (33)+Cr (205) | 0.8±0.3 | 0.9±0.1 | 2.6±0.4 | 2.4±0.4 | 3.4±0.4 | 3.3±0.3 | 0.03±0.03 | 0.04±0.03 | 12.84±0.43 |
| Control+R | 4.0±0.2 | 4.4±0.4 | 10.0±0.3 | 10.8±0.4 | 14.0±0.8 | 15.2±0.3 | 0.19±0.03 | 0.20±0.03 | 2.48±0.11 |
| Cd (11)+Cr (68)+R | 1.2±0.3 | 2.0±0.3 | 3.2±0.5 | 2.4±0.5 | 4.3±0.5 | 4.4±0.4 | 0.11±0.01 | 0.11±0.08 | 4.30±0.53 |
| Cd (22)+Cr (137)+R | 1.4±0.2 | 1.9±0.1 | 3.0±0.6 | 2.3±0.4 | 4.3±0.4 | 4.2±0.4 | 0.09±0.03 | 0.09±0.03 | 7.43±0.62 |
| Cd (33)+Cr (205)+R | 0.8±0.4 | 1.1±0.4 | 3.2±0.3 | 3.0±0.5 | 4.0±0.2 | 4.1±0.1 | 0.04±0.03 | 0.04±0.03 | 8.64±0.37 |
| LSD | 0.512 | 0.454 | 0.834 | 0.889 | 0.823 | 0.806 | 0.011 | 0.006 | 0.612 |

Values in parenthesis indicate the metal dose rate (mg/kg soil); Each value represents mean ± standard deviation of three replicates where each replicate constituted three plants/pot; 'R' indicates *Bradyrhizobium* strain C4.

**Table-8** *Effect of mixture of copper and chromium on biological properties of greengram plants*

| Treatment | Length of plant organs (cm) | | | | Total plant length (cm) | | Dry weight (g/plant) | | Proline content (µg/g fresh weight) |
|---|---|---|---|---|---|---|---|---|---|
| | Root | | Shoot | | | | | | |
| | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS | 40 DAS | 20 DAS |
| Control | 3.5±0.5 | 4.0±0.3 | 9.7±0.6 | 10.1±0.8 | 13.2±0.8 | 14.1±1.0 | 0.18±0.06 | 0.19±0.03 | 1.92±0.11 |
| Cu (527)+Cr (68) | 1.1±0.1 | 1.4±0.2 | 3.6±0.3 | 3.5±0.5 | 4.6±0.4 | 4.9±0.4 | 0.02±0.04 | 0.02±0.01 | 5.49±0.70 |
| Cu (1054)+Cr (137) | 0.8±0.4 | 1.2±0.4 | 3.4±0.4 | 3.2±0.2 | 4.2±0.2 | 4.3±0.3 | 0.02±0.04 | 0.02±0.02 | 9.58±0.22 |
| Cu (1581)+Cr (205) | 1.1±0.1 | 1.3±0.3 | 2.2±0.4 | 2.2±0.6 | 3.3±0.4 | 3.5±0.5 | 0.01±0.03 | 0.02±0.02 | 11.87±0.33 |
| Control+R | 4.0±0.2 | 4.4±0.4 | 10.0±0.3 | 10.8±0.4 | 14.0±0.8 | 15.2±0.3 | 0.19±0.03 | 0.20±0.03 | 2.48±0.11 |
| Cu (527)+Cr (68)+R | 1.1±0.1 | 1.9±0.1 | 3.2±0.3 | 3.2±0.3 | 4.7±0.2 | 5.1±0.4 | 0.09±0.12 | 0.10±0.03 | 4.58±0.62 |
| Cu (1054)+Cr (137)+R | 1.3±0.3 | 1.5±0.4 | 3.1±0.6 | 3.1±0.4 | 4.4±0.4 | 4.6±0.2 | 0.02±0.02 | 0.03±0.02 | 6.65±0.63 |
| Cu (1581)+Cr (205)+R | 1.5±0.3 | 1.8±0.2 | 2.6±0.5 | 2.2±0.4 | 4.1±0.3 | 4.0±0.5 | 0.02±0.04 | 0.02±0.02 | 8.09±0.70 |
| LSD | 0.518 | 0.535 | 0.732 | 0.886 | 0.669 | 0.891 | 0.005 | 0.003 | 0.825 |

Values in parenthesis indicate the metal dose rate (mg/kg soil); Each value represents mean ± standard deviation of three replicates where each replicate constituted three plants/pot; 'R' indicates *Bradyrhizobium* strain C4.

possibly reduced and hence experiment was intentionally terminated before yield stage.

Proline is reported to accumulate in plants under stress conditions and protects cell membrane and enzymes from the damaging effects of various stressor molecules. Proline also provides protection against oxidative stress by maintaining redox homeostasis [29]. Considering the importance of proline in stress alleviation, the proline content was detected in inoculated and uninoculated greengram plants grown under metal stress. In general, the metal tolerant *Bradyrhizobium* used in this study reduced the level of proline in whole greengram plants grown in soils treated differently with varying rates and species of metals. Furthermore, the highest level of proline was accumulated in Cr treated greengram plants. While comparing the impact of three concentrations of Cr on proline synthesis in both inoculated and uninoculated greengram plants, 205 mgCr/kg maximally produced the proline in uninoculated plants compared to inoculated plants. A- 18.4 µg/g of

proline was recorded in whole plants grown in soils treated with 205 mgCr/kg, as compared to 1.92 µg/g in control. When metals were used in combination, the highest levels of proline (5.62 µg/g) was recorded in plants grown in soils treated jointly with Cd (11mg/kg) and Cr (68 mg/kg). Among all metal applications, the combination of Cu and Cd produced the lowest amount of proline (3.67 µg/g) compared to other single or dual metals application. Following *Bradyrhizobium* inoculation, the metal stress was reduced and hence, the secretion of proline decreased from 18.4 µg/g to 13.9 µg/g in *Bradyrhizobium* inoculated plants grown with 205mg Cr/kg. Similarly, uninoculated plants grown in soils treated with 33mg Cd/kg produced a considerable amount (9.37µg/g) of proline which decreased by 32% in the presence of *Bradyrhizobium*. Copper at 1581 mg/ kg among all metals however, produced lowest (5.56 µg/g) proline [Table-3-8]. Proline accumulation in inoculated and uninoculated plants was directly proportional to the level of heavy metal stress. Interestingly, the level of proline reduced significantly in *Bradyrhizobium* inoculated greengram plants which could possibly be due to reduction in metal stress mediated by *Bradyrhizobium* strain. In a similar study conducted on *Arachis hypogaea*, the level of proline decreased significantly following PGPR inoculation, when the plants were grown in presence of salt stress [30]. The possible reason for this was the lesser degree of stress faced by PGPR inoculated plants. In yet other experiment, proline content reduced substantially in sugarcane plants inoculated with *Bacillus* sp. growing in soils treated with varying doses of NaCl [31]. This was also attributed to the bioremediation potential of *Bacillus* sp. which resulted in alleviation of stress caused by NaCl. The mean value of proline recorded at 1x, 2x and 3x concentrations of metals was: 3.43 µg/g (Cu), 5.67 µg/g (Cd) and 12.06 µg/g (Cr) as compared to 1.92 µg/g for control. While calculating the impact of the three metals on proline level, the order of metal effects on proline synthesis was: Cr> Cd> Cu. Chlorophyll content in fresh foliage of plants assayed at 40 DAS was variable [Fig-1 and 2]. A consistent decrease in chlorophyll content was however, observed as the dose of metals increased. For instance, 21 mg/g of chlorophyll in fresh weight of leaves recorded at 11mg Cd/kg was decreased to 18 and 15 mg chl/g fresh weight at 22 and 33 mg/kg of Cd added to soil, respectively. Likewise, the plants grown in soils treated with varying doses of Cu showed similar inhibitory effects. The chlorophyll content was maximum (21.5 mg chl/g fresh foliage) at 527 mg Cu/kg, while it was lowest (19 mg/g fresh weight) at 1581mg Cu/kg soil. While comparing the impact of metal toxicity on cholorophyll synthesis of greengram plants, chromium in general was found highly destructive and only 15 mg chl/g fresh weight was detected at 68 mg Cr/kg. The uninoculated and inoculated plants could produce 29 and 31 mg chl/g fresh weight, respectively. Interestingly, the chlorophyll formation in *Bradyrhizobium* sp. (vigna) inoculated greengram plants continued even in the presence of heavy metals.

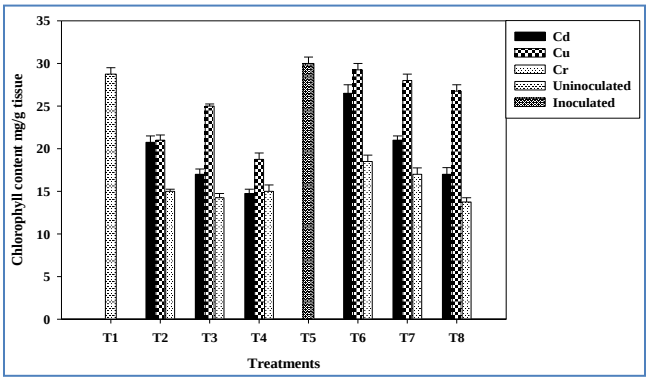


**Fig-1 Chlorophyll content in fresh foliage of greengram plants grown in the presence of copper, cadmium and chromium (mg/kg).:** T1 Uninoculated control and T5 Inoculated control; Single dose of Cu (527), Cd (11) and Cr (68) (Uninoculated,T2 & Inoculated, T6), Double dose of Cu (1054), Cd (22) and Cr (137) (Uninoculated, T3 & Inoculated, T7), Triple dose of Cu (1581), Cd (33) and Cr (205) (Uninoculated, T4 & Inoculated, T8)

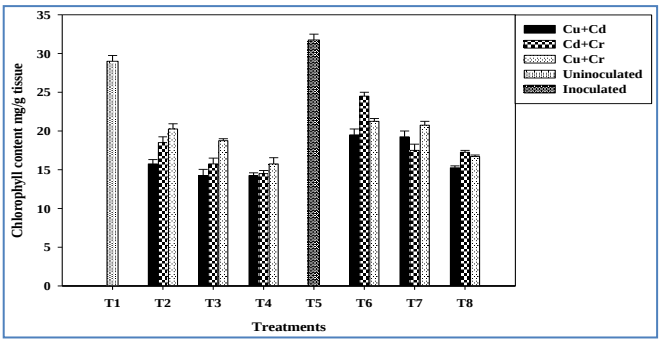


**Fig-2 Chlorophyll content in fresh foliage of greengram plants grown in the presence of combinations of copper, cadmium and chromium (mg/kg):** T1. Uninoculated control; T5 Inoculated control; Single dose of Cu (527)+Cd (11), Cd (11)+Cr (68), Cu (527)+Cr (68) (Uninoculated, T2 & Inoculated, T6), Double dose of Cu (1054)+Cd (22), Cd (22)+Cr (137), Cu (1054)+Cr (137) (Uninoculated, T3) & Inoculated, T7), Triple dose of Cu (1581)+Cd (33), Cd (33)+Cr (205), Cu (1581)+Cr (205) (Uninoculated, T4 & Inoculated, T8)

## Conclusion

The findings of present study demonstrated variation in heavy metal toxicity among the strains of PGPR recovered from vegetables (garlic and cabbage) rhizospheres and pulse nodules. The continued secretion of plant growth promoting active biomolecules even under metal stress by metal tolerant *Bradyrhizobium* sp. (vigna) and reduction in proline formation in inoculated plants grown under metal stress conditions might have accounted for enhancement in the growth of *Bradyrhizobium* sp. (vigna) inoculated greengram plants grown in soils treated with varying rates of copper, cadmium and chromium. This study clearly suggests that the metal tolerant *Bradyrhizobium* could be used to develop rhizobial inoculant for enhancing the performance of greengram in metal polluted soils.

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**Author contributions:** MSK and AZ have conceived and designed the research plan. AR performed the experiments and drafted manuscript. AZ, AR and SS also contributed in data analysis, discussion and overall development of manuscript. All authors have read and approved the final version of manuscript.

**Abbreviations:** MIC- Minimum inhibitory concentration; IAA- Indole-3-acetic acid; HCN- Hydrogen cyanide; DAS- Days after sowing

**Conflict of interest:** The authors declare that there are no conflicts of interest

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