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Root and shoot length, nodulation, chlorophyll, seed yield and grain protein content of pigeonpea influenced by chromium reducing strains *Rhizobium* strain RP2 and BP7 *Bacillus* strain in chromium related soils in Aligarh

Huma Naz, Asma Naz, Ayesha, Samiya Maqsood and Shabbir Ashraf

Abstract
In this experiment chromium-reducing and plant growth promoting *Rhizobium* strain RP2 was used to assess its bioremediation potential in pot house conditions using pigeonpea as a test legume crop. Among the three concentration of Cr (VI) at 60.52 mg/kg soil had the largest toxic effects decreased root length 55.82%, shoot length 28.27%, nodule numbers 69.73% and nodule dry weight 75.21%, at 80 days, root length 44.44%, shoot length 34.01%, at 110 day as compared to control. In comparison when, *Rhizobium* strain RP2 was also added, it increased the root length by 4.00%, shoot length 3.16 %, nodule numbers 11.69%, nodule dry weight 5.62% at 80 days (root length 6.13%, shoot length 2.38% at 110 days as compared to control. Chromium at concentration 60.52 mg/kg was the most toxic and decreased the chlorophyll and by 63.89% as compared to control. In comparison, the bioinoculant showed a maximum increase in the chlorophyll content of 8.41%, at 60.52 mg Cr /kg soil compared to control. Chromium at 60.52 mg/kg was the most toxic and decreased the seed yield and grain protein by 48.94% and 17.86% as compared to control. In comparison, the bioinoculant showed a maximum increase in the seed yield and protein content of 8.27% and 16.42% at 60.52 mg Cr /kg soil compared to control. *Bacillus* strain BP7 was used to assess its bioremediation potential in pot house conditions using pigeonpea as a test legume crop. Among the three concentration of Cr (VI) at 60.52 mg/kg soil had the highest toxic effects decreased root length 55.82%, shoot length 28.27%, nodule numbers 69.73% and nodule dry weight 75.21%, at 80 days, root length 44.44%, shoot length 34.01%, at 110 day as compared to the control. In comparison when, *Bacillus* strain BP7 was also added, it increased the root length, 7.69%, shoot length 3.80%, nodule numbers 16.31%, nodule dry weight 6.25% at 80 days, root length 8.67%, shoot length 2.52% respectively at 110 days as compared to control. Chromium at 60.52 mg/kg was the most toxic and decreased the chlorophyll by 63.89% as compared to control. In comparison, the bioinoculant showed a maximum increase in the chlorophyll content of 6.30%, at 60.52 mg Cr /kg soil compared to control. Chromium at 60.52 mg/kg was most toxic and decreased the seed yield and grain protein by 48.94% and 17.86% respectively as compared to control. In comparison, the bioinoculant showed a maximum increase in the seed yield and protein content of 7.18% and 13%, respectively at 60.52 mg Cr /kg soil as compared to control.

Keywords: *Rhizobium, Bacillus,* pigeonpea, chromium, toxic

Introduction
Heavy metals excessive levels in agricultural soils cause serious risks not only for normal plant growth and crop yield but also for the human health they are widespread environmental pollutants and among heavy metals, chromium is a highly toxic metal to living organisms with many adverse effects reported in humans, animals, plants, and microorganisms 9C (Cervantes *et al.*, 2001; P. Sangwan *et al.*, 2014) [5, 6]. Plants, including legumes, are able to uptake heavy metals like chromium from soils that result in many adverse effects, such as inhibition of seed germination and seedling development, reduction in root and shoot biomass, quality of flowers, and crop yield (Gill *et al*., 2015; DalCorso *et al*., 2012) [1, 2]. These effects of heavy metals are connected with inhibition of certain metabolic processes, including biosynthesis of chlorophylls and proteins (Sangwan *et al*., 2014; Bibi and Hussain, 2005; Xue, 2014) [6, 3, 4, 9]. As a result, progressive chlorosis, necrosis, and decreased protein content are typical signs of heavy metal toxicity to plants (Cervantes *et al.*, 2001, Panda and Choudhury 2005*;* Baryla *et al.*, 2012) [5, 8].

In the nature, chromium exists in two valence forms: Cr^{3+} and Cr^{6+}, which chromite minerals are mainly composed of (Bagchi *et al*., 2002; Gill *et al*., 2016; Panda and Choudhury 2005; Kumar and Maiti 2013; Zayed and Terry, 2003) [10, 11, 8, 12, 13]. Chromates and dichromates are the most abundant anionic forms of chromium in the environment (Kubrak *et al*., 2010; Lushchak *et al*., 2009) [14, 15]. Toxicity of chromium for plants depends on its valence state with Cr^{6+} being more toxic and mobile than Cr^{3+} (Panda and Choudhury 2005, Shah *et al*., 2010, Valko *et al*., 2005) [8]. The hexavalent chromium is toxic for agricultural plants at concentrations of about 0.5-5.0 mg ml^{-1} in the nutrient solution and 5-100 mg g^{-1} in the soil. Under physiological conditions, concentration of chromium ions in plants is less than 1 μg g^{-1} (Oliveira, 2012; Ali *et al*., 2015) [16, 17].

Material Methods

Bioremediation studies using metal resistant Plant Growth Promoting *Rhizobacterial* isolates

The *Rhizobial* strain of *Rhizobium* RP2 resistant to chromium and phosphate solubilizers *Bacillus* BP7 resistant to chromium isolated in the present investigation were used for bioremediation studies using pigeonpea as a test crop, when grown in the presence and absence of different heavy metals. The strain showing the highest tolerance to specific metal was chosen for plant inoculation in the presence and absence of a particular metal.

Microbial treatments, metal application and legume growth

The experimental soil for bioremediation studies was sandy clay loam. Prior to inoculation of soil with PGPR, the cell suspension of isolate was grown in YEM broth (for *Rhizobia*) and NB broth (for phosphate solubilizers) in flasks shaken at 120 rpm at 28±2⁰C for 5 and 3 days respectively to a cell density of 6×10^8 (*Rhizobia*) of 3×10^7 cell/ml (for phosphate solubilizers). Seeds of pigeonpea (var. Bahar) were surface sterilized (Vincent, 1970) [33] and were coated with metal resistant plant growth promoting *Rhizobium* strain RP2, respectively. Among the phosphate solubilizers, *Bacillus* strain BP7 was used to inoculate pigeonpea seeds. Seeds of each legume were soaked in liquid culture medium for 24 h using 10% gum arabic as adhesive agent to deliver approximately 10^8 cells/seed for *Rhizobia* and 10^7 cells/seed for *Bacillus*. The non-coated sterilized seeds used as control were soaked in sterile water only. The non-inoculated sterilized seeds (10 seeds per pot) were so on June, 10 Pigeonpea, 2007 in clay pots (25 cm high, 22cm internal diameter) using three kg unsterilized soil with control the three treatments. Treatments for each crop and metals were as follows- Pigeonpea inoculated with or without strain RP2, BP7 were grown in soils treated individually with 15.13, 30.26 and 60.52 mg/kg of Cr.

Table 1: Effect of three concentrations of Cr (VI) on growth and nodulation of pigeonpea plants grown in soil inoculated with and without *Rhizobium* sp. RP2

<table>
<tr><th rowspan="4">Treatment</th><th rowspan="4">Dose rate (mg kg-1 soil)</th><th colspan="14">Length (cm plant-1)</th><th>Nodulation (No.plant-1)</th><th>Nodulation</th><th rowspan="4">Chlorophyll content (mg g-1)</th><th rowspan="4">Seed yield (g plant-1)</th><th rowspan="4">Grain protein (mg g-1)</th></tr>
<tr><th colspan="7">Root</th><th colspan="7">Shoot</th><th rowspan="3">80DAS</th><th>Dry mass (mg plant-1)</th></tr>
<tr><th colspan="3">80DAS</th><th colspan="4">110DAS</th><th colspan="3">80DAS</th><th colspan="4">110DAS</th><th>80DAS</th></tr>
<tr></tr>
<tr><td>Uninoculated</td><td>Control</td><td colspan="3">30.15</td><td colspan="4">37.15</td><td colspan="3">76.46</td><td colspan="4">98.94</td><td>22.00</td><td>45.13</td><td>28.38</td><td>46.33</td><td>27.26</td></tr>
<tr><td></td><td>0.5x</td><td colspan="3">26.06</td><td colspan="4">30.31</td><td colspan="3">74.39</td><td colspan="4">88.07</td><td>16.66</td><td>29.21</td><td>18.19</td><td>41.66</td><td>25.56</td></tr>
<tr><td></td><td>1.0x</td><td colspan="3">20.79</td><td colspan="4">25.31</td><td colspan="3">67.83</td><td colspan="4">77.84</td><td>8.33</td><td>19.21</td><td>14.23</td><td>32.33</td><td>24.18</td></tr>
<tr><td></td><td>2.0x</td><td colspan="3">13.32</td><td colspan="4">20.64</td><td colspan="3">54.85</td><td colspan="4">65.30</td><td>6.66</td><td>11.19</td><td>10.25</td><td>23.66</td><td>22.39</td></tr>
<tr><td>Inoculated</td><td>Control</td><td colspan="3">34.21</td><td colspan="4">39.14</td><td colspan="3">81.60</td><td colspan="4">102.50</td><td>25.66</td><td>48.45</td><td>32.35</td><td>52.33</td><td>28.25</td></tr>
<tr><td></td><td>0.5x</td><td colspan="3">36.62</td><td colspan="4">41.20</td><td colspan="3">83.71</td><td colspan="4">103.96</td><td>28.66</td><td>49.36</td><td>34.51</td><td>55.33</td><td>30.58</td></tr>
<tr><td></td><td>1.0x</td><td colspan="3">37.17</td><td colspan="4">42.51</td><td colspan="3">84.48</td><td colspan="4">104.58</td><td>31.33</td><td>49.85</td><td>35.02</td><td>56.33</td><td>29.15</td></tr>
<tr><td></td><td>2.0x</td><td colspan="3">35.58</td><td colspan="4">41.54</td><td colspan="3">84.18</td><td colspan="4">104.94</td><td>28.66</td><td>51.18</td><td>35.07</td><td>56.66</td><td>32.89</td></tr>
<tr><td></td><td></td><td>a</td><td>b</td><td>c</td><td>a×b</td><td>b×a</td><td>c×a</td><td>a×b×c</td><td>a</td><td>.719</td><td>c</td><td>a×b</td><td>b×a</td><td>c×a</td><td>a×b×c</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>SE±m</td><td></td><td>.113</td><td>.160</td><td>.113</td><td>.227</td><td>.160</td><td>.227</td><td>.321</td><td>.164</td><td>2.15</td><td>.164</td><td>.328</td><td>.232</td><td>.328</td><td>.465</td><td>.656</td><td>.937</td><td>.322</td><td>.833</td><td>.745</td></tr>
<tr><td>CD at 5%</td><td></td><td>.315</td><td>.446</td><td>.315</td><td>.630</td><td>.446</td><td>.630</td><td>.892</td><td>.464</td><td>2.96</td><td>.464</td><td>.928</td><td>.656</td><td>.928</td><td>1.313</td><td>1.967</td><td>2.81</td><td>.966</td><td>2.498</td><td>3.28</td></tr>
<tr><td>CD at 1%</td><td></td><td>.443</td><td>.627</td><td>.443</td><td>.887</td><td>.627</td><td>.887</td><td>1.25</td><td>.641</td><td>4.69</td><td>.641</td><td>1.282</td><td>.906</td><td>1.282</td><td>1.813</td><td>2.709</td><td>3.87</td><td>1.33</td><td>3.441</td><td>2.67</td></tr>
<tr><td>CV</td><td></td><td colspan="7">2.19</td><td colspan="7">.952</td><td>5.411</td><td>4.27</td><td>2.147</td><td>3.166</td><td>5.17</td></tr>
<tr><td colspan="20">a= Metal, b= Concentration, c= Days, ab= Metal x Concentration, ba=Concentration x Metal, ca = Days x Metal, abc= Metal x Concentration x Days</td><td></td></tr>
</table>

Values are mean of three replicates where each replicate constituted three plants/ pot In this table, value 0.5x of metal concentration indicate half, 1.0x indicate normal and 2.0x indicate double values of metal, symbol indicate, Cr for chromium

Table 2: Effect of three concentrations of Cr (VI) on growth and nodulation of pigeonpea plants grown in soil inoculated with and without BP7 *Bacillus* sp.

<table>
<tr><th rowspan="3">Treatment</th><th rowspan="3">Dose rate (mg kg⁻¹ soil)</th><th colspan="14">Length (cm plant⁻¹)</th><th>Nodulation (No. Plant⁻¹)</th><th>Nodulation</th><th rowspan="3">Chlorophyll content (mg g⁻¹)</th><th rowspan="3">Seed yield (g plant⁻¹)</th><th rowspan="3">Grain protein (mg g⁻¹)</th></tr>
<tr><th colspan="7">Root</th><th colspan="7">Shoot</th><th rowspan="2">80DAS</th><th>Dry mass (mg plant⁻¹)</th></tr>
<tr><th colspan="3">80DAS</th><th colspan="4">110DAS</th><th colspan="3">80DAS</th><th colspan="4">110DAS</th><th>80DAS</th></tr>
<tr><td>**Uninoculated**</td><td>**Control**</td><td colspan="3">30.15</td><td colspan="4">37.15</td><td colspan="3">76.46</td><td colspan="4">98.94</td><td>22.00</td><td>45.13</td><td>28.38</td><td>46.33</td><td>27.26</td></tr>
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<tr><td></td><td>1.0x</td><td colspan="3">20.79</td><td colspan="4">25.31</td><td colspan="3">67.83</td><td colspan="4">77.84</td><td>8.33</td><td>19.21</td><td>14.23</td><td>32.33</td><td>24.18</td></tr>
<tr><td></td><td>**2.0x**</td><td colspan="3">13.32</td><td colspan="4">20.64</td><td colspan="3">54.85</td><td colspan="4">65.30</td><td>6.66</td><td>11.19</td><td>10.25</td><td>23.66</td><td>22.39</td></tr>
<tr><td>**Inoculated**</td><td>**Control**</td><td colspan="3">34.07</td><td colspan="4">39.31</td><td colspan="3">82.50</td><td colspan="4">103.53</td><td>30.66</td><td>49.37</td><td>34.26</td><td>55.66</td><td>29.60</td></tr>
<tr><td></td><td>0.5x</td><td colspan="3">34.90</td><td colspan="4">40.87</td><td colspan="3">84.70</td><td colspan="4">105.60</td><td>34.33</td><td>52.46</td><td>34.92</td><td>58.00</td><td>31.54</td></tr>
<tr><td></td><td>1.0x</td><td colspan="3">35.57</td><td colspan="4">41.54</td><td colspan="3">84.93</td><td colspan="4">105.78</td><td>34.33</td><td>52.43</td><td>35.99</td><td>58.66</td><td>30.45</td></tr>
<tr><td></td><td>2.0x</td><td colspan="3">36.69</td><td colspan="4">42.72</td><td colspan="3">85.64</td><td colspan="4">106.14</td><td>35.66</td><td>52.37</td><td>36.42</td><td>59.66</td><td>33.45</td></tr>
<tr><td></td><td></td><td>a</td><td>b</td><td>c</td><td>a×b</td><td>b×a</td><td>c×a</td><td>a×b×c</td><td>a</td><td>b</td><td>c</td><td>a×b</td><td>b×a</td><td>c×a</td><td>a×b×c</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>SE±m</td><td></td><td>.134</td><td>.190</td><td>.134</td><td>.269</td><td>.190</td><td>.269</td><td>.381</td><td>.167</td><td>.236</td><td>.167</td><td>.335</td><td>.236</td><td>.335</td><td>.473</td><td>.656</td><td>1.00</td><td>.186</td><td>.799</td><td>.790</td></tr>
<tr><td>CD at 5%</td><td></td><td>.374</td><td>.529</td><td>.374</td><td>.748</td><td>.529</td><td>.748</td><td>1.058</td><td>.464</td><td>.656</td><td>.464</td><td>.928</td><td>.656</td><td>.928</td><td>1.313</td><td>1.96</td><td>3.01</td><td>.559</td><td>2.39</td><td>3.99</td></tr>
<tr><td>CD at 1%</td><td></td><td>.532</td><td>.754</td><td>4.88</td><td>.895</td><td>.754</td><td>.895</td><td>1.095</td><td>.652</td><td>.923</td><td>.652</td><td>1.305</td><td>.923</td><td>1.305</td><td>1.846</td><td>2.70</td><td>4.16</td><td>.770</td><td>3.30</td><td>2.89</td></tr>
<tr><td>CV</td><td></td><td colspan="7">2.076</td><td colspan="7">.963</td><td>4.81</td><td>4.48</td><td>1.21</td><td>2.94</td><td>5.65</td></tr>
<tr><td colspan="20">a= Metal, b= Concentration, c= Days, ab= Metal x Concentration, ba=Concentration x Metal, ca = Days x Metal, abc= Metal x Concentration x Days</td><td></td></tr>
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Values are mean of three replicates where each replicate constituted three plants/ pot In this table, value 0.5x of metal concentration indicate half, 1.0x indicate normal and 2.0x indicate double values of metal, symbol indicate Cr for chromium

Results

Growth of pigeonpea influenced by chromium reducing *Rhizobium* RP2 in chromium related soils

Pigeonpea plant growth and nodulation

In this experiment chromium-reducing and plant growth promoting *Rhizobium* strain RP2 was used to assess its bioremediation potential in pot house conditions using pigeonpea as a test legume crop. The pigeonpea plants grew poorly when the soil was amended with Cr. Generally, the growth and nodulation decreased progressively with increasing concentration of Cr (VI). Among the three concentration of Cr (VI) at 60.52 mg/kg soil had the largest toxic effects and significantly ($P \leq 0.05$ and 0.01) decreased root length 55.82%, shoot length 28.27%, nodule numbers 69.73% and nodule dry weight 75.21%, at 80 days, root length 44.44%, shoot length 34.01%, at 110 day as compared to control. In comparison when, *Rhizobium* strain RP2 was also added, it increased the root length by 4.00%, shoot length 3.16 %, nodule numbers 11.69%, nodule dry weight 5.62% at 80 days (Table 1); root length 6.13%, shoot length 2.38% respectively at 110 days as compared to control. The inoculant strain protected the plants from Cr toxicity possibly through the soluble chromate reductase or by providing plants with the sufficient amounts of growth-promoting substances. The inoculant strain reduced the Cr uptake by plant organs. In conclusion, the strain RP2 showed a potential for Cr (VI) reduction, produced plant growth-promoting substances under Cr stress and enhanced the growth and yield of pigeonpea, both in Cr stress and Cr free conditions. Due to the multifarious activity, the strain RP2 could therefore, be utilized for growth promotion as well as for the bioremediation of Cr polluted soil.

Chlorophyll Content

Chlorophyll content of plant decreased consistently with increasing concentrations of chromium (Table 1) without the inoculation of strain RP2. Chromium at concentration 60.52 mg/kg was the most toxic and decreased the chlorophyll and by 63.89% as compared to control. In comparison, the bioinoculant showed a maximum increase in the chlorophyll content of 8.41%, at 60.52 mg Cr /kg soil compared to control (Table 1).

Seed yield and grain protein

Seed yield and grain protein, decreased consistently with increasing concentrations of chromium (Table 1) without the inoculation of strain RP2. Chromium at 60.52 mg/kg was the most toxic and decreased the seed yield and grain protein by 48.94% and 17.86% as compared to control. In comparison, the bioinoculant showed a maximum increase in the seed yield and protein content of 8.27% and 16.42%, respectively, at 60.52 mg Cr /kg soil compared to control (Table 1).

Chromium tolerant *Bacillus* BP7 affecting pigeonpea in chromium treated soils

Pigeonpea plant growth and nodulation

In this experiment chromium-reducing and plant growth promoting *Bacillus* strain BP7 was used to assess its bioremediation potential in pot house conditions using pigeonpea as a test legume crop. The pigeonpea plants grew poorly when the soil was amended with Cr. Generally, the growth and nodulation decreased progressively with increasing concentration of Cr (VI). Among the three concentration of Cr (VI), Cr (VI) at 60.52 mg/kg soil had the highest toxic effects and significantly ($P \leq 0.05$ and 0.01) decreased root length 55.82%, shoot length 28.27%, nodule numbers 69.73% and nodule dry weight 75.21%, at 80 days, root length 44.44%, shoot length 34.01%, at 110 day as compared to the control. In comparison when, *Bacillus* strain BP7 was also added, it increased the root length, 7.69%, shoot length 3.80%, nodule numbers 16.31%, nodule dry weight 6.25% at 80 days respectively (Table 41), root length 8.67%, shoot length 2.52% respectively at 110 days as compared to control. The inoculant strain protected the plants from Cr toxicity possibly through the soluble chromate reductase or by providing plants with the sufficient amounts of growth-promoting substances. The inoculant strain reduced the Cr uptake by plant organs. In conclusion, the strain BP7 showed a potential for Cr (VI) reduction, produced plant growth-promoting substances under Cr stress and enhanced the growth and yield of pigeonpea, both in Cr stress and Cr free conditions. Due to the multifarious activity, the strain BC5 could therefore, be utilized for growth promotion as well as for the bioremediation of Cr polluted soil.

Chlorophyll Content

Chlorophyll content of plant decreased consistently with increasing concentrations of chromium (Table 2) without the inoculation of strain BP7. Chromium at 60.52 mg/kg was the most toxic and decreased the chlorophyll by 63.89% as compared to control. In comparison, the bioinoculant showed a maximum increase in the chlorophyll content of 6.30%, at 60.52 mg Cr /kg soil compared to control (Table 2).

Seed yield and grain protein

Seed yield and grain protein, decreased consistently with increasing concentrations of chromium (Table 2) without the inoculation of strain BP7. Chromium at 60.52 mg/kg was most toxic and decreased the seed yield and grain protein by 48.94% and 17.86% respectively as compared to control. In comparison, the bioinoculant showed a maximum increase in the seed yield and protein content of 7.18% and 13%, respectively at 60.52 mg Cr /kg soil as compared to control (Table 2).

Discussion

Bioremediation Studies

Bioremediation involves the use of living dead organism to degrade or transform the toxic heavy metals into less toxic forms. It involves the use of naturally occurring microflora and plants to reduce, eliminate, contain transform metals to being products, present in soils, sediments, water or air. Plant microbe interactions have been proposed to increase the bioremediation capacity of plants (Zhuang *et al*.,2007; Khan *et al*., 2008) [31, 32] and legumes in association with different species of rhizobia and other PGPR strains are receiving greater attention for their application and use in the remediation of metal contaminated soils (Ike *et al*., 2007) [30]. Bioremediation depends on the presence of the sufficient number of variable organism and the proper conditions suitable for their growth. In this context, metal tolerant strains of *Rhizobium* RP2 and phosphate solubilising bacteria *Bacillus* BP7 possessing the ability, of phytohormone production, to tolerate high concentration of metal ions and to form function symbiosis (only N_2 fixers) with their respective legume host plants were used to evaluate their bioremediation potential using their specific legume host grown in soils treated with Cr (for pigeon pea). In this experiment pigeon pea grow poorly when soil was treated with different concentrations of heavy metals single or combination for pigeon pea for reasons explained earlier.

Generally, though, with increase in metal concentration, there was decrease in the measured parameters even in the inoculated legumes, but there parameters of inoculated legumes even at the highest dose of each metal, compared to the un-inoculated plants grown with the same highest rates of each individual metal. The highly metal tolerant and symbiotically effective strains of, phosphate solubilizer (BP7) and *Rhizobium* (RP2) substantially increased the measured parameters of their respective host plants, compared to un-inoculated but metal treated control plants. Similarly, the improved symbiotic relationship expressed in term of nodulation on the respective legume host in metal amended soil suggested the establishment of rhizobial species and its ability to form functional nodules on legumes even in the presence of heavy metal. However, the development of nodules on the root system at un-inoculated or inoculated with metal tolerant *Rhizobium* (RP2), *Bacillus* (BP7) legumes suggested that the nodules might have been produced by some indigenous rhizobial population, because soil used in this study was non-sterilized.

PGPR including N_2 fixers can affect plant development either indirectly by circumventing the toxic effect of metals or directly by synthesizing the plant growth regulating substances. Moreover, the phytohormone is reported to reduce the effect of high concentration of certain metals (e.g. Cd) on the growth of non-inoculated soybean plants (Ghorbanli *et al*., 1999) [29]. Inoculation of metal tolerant and phytohormone producing strains augmented the growth of the legumes when the bacterial strains ((RP2 and BP7) were applied as seed inoculants in metal amended soil. The study consolidated the fact the selected strains possesses metal reducing/tolerant ability which in turn might have provided the protection to legume plants against the inhibitory effects of each metal (Faisal & Hasnain, 2006). Furthermore, the siderophore and IAA producing ability of these strains might also have enhanced root growth & uptake of soil minerals by the host plant (Zaidi *et al*., 2006) [28]. The IAA produced by the rhizoidal strains promotes root growth directly by stimulating plant cell elongation or cell division (Minamisauia & Fukai, 1991) [27]. The result thus suggests that the application of these strains as a seed bio-inoculants increased the metal tolerance through their PGP activities promoted overall growth of legume plants. Similar evidence of increase in plant growth in metal amended soil has been reported (Faisal & Hasnain, 2005; Pajuelo *et al*., 2007) [25, 26]. Burd *et al*., (2000) [24], observed an increase in the growth of tomato, canola and mustard, when these plants were grown in presence of *Kluyvera ascorbata* in Ni, Pb and Zn amended soil. However, reports on the effect of metals on *Rhizobium*-legume symbiosis are conflicting. For example, Chaudri *et al*., (2000) [23], observed a significant reduction in nodulation, when field grown pea was grown in soil amended with Zn and Cu. While Ibekwe *et al*. (1995) [22] reported a considerable increase in nodulation of alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) when grown in metal amended soil.

Moreover, the chlorophyll content in fresh leaves of pigeonpea measured, leghaemoglobin in fresh nodules of pigeonpea; nitrogen content in roots and shoots of each legume and nitrogen content in roots and shoots of each legume and seed yield and grain protein decreased consistently with increase in the concentration of chromium (pigeonpea) in the absence of bio inoculants. In comparison, the plants grown in the presence of bioinoculants increased the measured parameters under the influence of metals. For example, the chlorophyll content of pigeonpea plants inoculated with RP2 and grown in amended with 60.52 mg Cr/kg soil, increased the chlorophyll content significantly by 8.41%, above the un-inoculated but amended with the same dose of chromium. Similar findings have been reported by (Wani *et al*., 2007b) [21].

Seed yield and grain protein increase of pigeonpea by 7.18, 19.51%) grown in soil amended solely with the same dose of chromium. Burd *et al*. (2002) observed an increase in the protein content of tomato, canole and Indian mustard when plants were grown in the presence of *Kluyvera ascorbata*

SOD165 in the presence of high concentration of nitrogen, lead and zinc.

The accumulation of Cr uninoculated and an inoculated for pigeonpea (at 60, 80 and 110 DAS).

For pigeonpea at 60, 80 and 110 in plants organs (roots, and shoots) differed among treatments. The uptake of each metal by the roots, shoots and grains of two legumes used in this study increased with increase in the concentration of tested metal.

This study demonstrated that the inoculation of *Rhizobium* (RP2) specific to pigeonpea and *Bacillus* BP7, BP5 used as seed inoculants showed dual effects on pigeonpea plants grown in metal treated soil. Such bacterial strains endowed with multiple properties of growth promotion and ability to reduce the toxicity of tested metals, therefore, can be used as bioinoculant for chickpea grow in waste water irrigated soils with metals, as used in this study. Furthermore, the remediation of heavy metal contaminated sites using PGPR including symbiotic nitrogen fixers is an exciting area of research, because these can easily and experimentally be mass produced for the inoculant of legume crops compared to other means of remediation.

Growth of Pigeonpea

Chromium at elevated levels inhibits the growth of plants (Zayad & Terry 2003) [13] but, when strain was also added, it increased the measured parameters. The inoculant strain protected the plants from Cr toxicity possibly through the soluble chromate reductase or by providing plants with the sufficient amounts of growth-promoting substances. The inoculant strain reduced the Cr uptake by plant organs. Similarly, accumulation of Cd and Cr in greengram has been reported (Wani *et al.*; 2007c, d) [18, 19]. In conclusion, the strain RP2 showed a potential for Cr reduction, produced plant growth-promoting substances under Cr stress and enhanced the growth and yield of pigeonpea, both in Cr stress and Cr free conditions. Due to the multifarious activity, the strain RP2 could therefore, be utilized for growth promotion as well as for the bioremediation of Cr polluted soil.

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