



**SDI Review Form 1.6**

Journal Name:	<a href="#">Advances in Research</a>
Manuscript Number:	Ms_AIR_31986
Title of the Manuscript:	Chromium (VI) reducing <i>Brevibacillus brevis</i> OZF6 inoculation enhances pea growth and decreases metal uptake in pea plants
Type of the Article	Original Research Paper

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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**PART 1: Review Comments**

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b><u>Compulsory</u></b> REVISION comments	<p>Introduction:</p> <ul style="list-style-type: none"> <li>- Line 44: <i>Escherichia coli</i></li> <li>- The introduction is a space where we should present an overview of the subject and the problem involved. These two criteria were fulfilled.</li> </ul> <p>However, it lacks an approach of why use <i>Brevibacillus brevis</i> associated with phytoremediation. So, I have some questions that may help:</p> <p>Is there a large contamination of chromium in the crops? If so, what are the sources of these contaminants? (Your work is in the agricultural area, so you must present these biases in the introduction)</p> <p>Is there a relationship between rhizobacteria and a better ability to degrade chromium?</p> <p>Is there any record in the literature of chromium-reducing <i>Brevibacillus</i>?</p> <p>Is the information contained between lines 59 and 67 is necessary? Remember that your focus is on agriculture.</p> <p>Material and methods:</p> <p>I strongly recommend that you describe in one or two lines the origin of this isolate. Is it an isolate belonging to the collection of microorganisms from your laboratory? Is it an isolate from another study? Did you isolate it?</p> <p>How many repetitions you use for 2.1 and 2.2 experiments?</p> <p>Line 77: Grams per litre?</p> <p>Line 81: I suggest that instead of grams you use the number of microorganisms in millilitre per gram (Cells / mL or CFU / mL or Cells / mg or CFU / mg). 1g (fresh weight) does not give us an accurate picture of how many cells were effectively immobilized. The same problem for line 93.</p> <p>Line 100: Was the soil autoclaved?</p> <p>Line 104: What is the concentration of microorganisms that each seed received? (Cells / mL or CFU / mL). To compare effectively, it is necessary to standard the inoculum.</p> <p>Line 136: It cannot be said that it was significantly different in relation to the control without a</p>	<p>At line 44, <i>Escherichia coli</i> is corrected.</p> <p>Source of contamination in the agricultural field in introduction has been added as per suggestion. Relationship between rhizobacteria and chromium reduction has been added.</p> <p>Relationship between bacteria and reduction is also already in the introduction.</p> <p>There is no record of chromium (VI) reducing <i>Brevibacillus</i>. The sentence between line 59 and 67 is important as it shows the mechanism of reduction. Origin of the strain OZF6 has been included in the materials methods.</p> <p>Experiments in section</p>



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	<p>statistical test. I suggest the use of another term.</p> <p>Discussion:  Lines 182 to 184: This information has already been put in the introduction. Review the writing.  Line 187 and 188: The information about the microorganism should be in the materials and methods section.  Line 191 to 196: The results were properly compared with other studies; however, you did not point out a value for Cr (VI) removal (mM). I suggest you to justify that bacteria immobilized by some substance have better results. You need to explain why bacteria immobilized by alginate are better than compared to free cells.  Line 216: What is the hypothesis of the authors to explain that seeds with microorganisms allow the germination of the seeds even in the presence of the metal?</p>	<p>2.1 and 2.2 were repeated three times which has been included in section 2.1 and 2.2. At line 77 the volume has been added. Line 81. Cells were not added as cfu/ml but one gram of the cell mass was added which was over night grown culture so no cfu/ml was calculated. This gram is as per the research conducted by various researchers published in some reputed international journals. L 100. Soil was autoclaved which has been added. Line 104. Each seed received <math>10^8</math> cells which has been added to the paper. L. 136. Significantly has been replaced with "reduced highest concentration of chromium (VI)". Line 182-184. The information has been reviewed. Line 187-188. The information has been added in the materials and methods. Line 191-196. Justification has been added as per</p>
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		<p>suggestion. Line 216. In this study bio-inoculant reduced the toxicity of the metal and thus increased the seed germination in the presence of the metal.</p>
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<b>Minor</b> REVISION comments	<p>Introduction:</p> <ul style="list-style-type: none"> <li>- I suggest the organization of the first paragraph in the following order: General information on chromium, biological importance, sources of contamination and sources of remediation. Here is my suggestion:</li> </ul> <p>Chromium (Cr) occurs either in trivalent or hexavalent forms. Although hexavalent chromium is the most toxic form, trivalent is an essential micronutrient for animals, plants and humans which is involved in glucose metabolism [7], stimulation of enzyme system [8] and stabilization of nucleic acids by increasing the processivity of DNA polymerase [9]. High solubility of Cr (VI) makes it more toxic, ease to pass through biological membranes and can easily damage proteins and nucleic acids particularly DNA, thus inhibits the number of species of the microbes and their growth [4,5,6]. The contamination of Cr (VI) is mainly is due to the use of Cr (VI) in leather, tanning, metallurgy, electroplating, textile, and pigment manufacturing industries [1-3]. Reduction of toxic Cr (VI) to Cr (III) is a useful process for remediation of Cr (VI) affected environments [10] and can be readily used to save our soil and water from the toxic effects of these metals. The reduction of Cr (VI) has been reported in <i>Bacillus</i> [11,12], <i>Pseudomonas</i> [13-14], <i>Escherichia coli</i> [15], <i>Microbacterium</i> [2], <i>Ochrobactrum intermedium</i> [16] and <i>Micrococcus</i> [17].</p> <ul style="list-style-type: none"> <li>-Line 55: It's the first time that you are using this expression, please say that is Plant Growth promoting Rhizobacteria and put PGPR in parentheses.</li> <li>-Line 47 to 50: I suggest that you split the sentence in two new sentences, one for direct method and another for indirect method.</li> <li>- Line 52 to 54: It is not necessary to cite again the role of chromium reductase, just say that the enzyme can work on anaerobic and aerobic environment. That said, phrases from lines 50 to 54 can be converted into one.</li> <li>-Lines 56 to 57: This information is repeated from lines 48 and 49. Chromium in line 57 is missing a "c".</li> <li>-Line 190: Correlation is not the best word to describe it, it is better say according.</li> </ul>	<p>Introduction has been rearranged as per suggestion.</p> <p>Line 55. Plant growth promoting rhizobacteria has been added.</p> <p>Line 47-50. The sentence as been broken down into two sentences as per suggestion.</p> <p>Line 52-54. The sentences has been converted into one.</p> <p>"C " has been added to chromium</p> <p>Correlation has been replaced with agree.</p>
<b>Optional/General</b> comments	<p>The work is very relevant in the face of our current agricultural management practices. The work presents scientific consistency, the structure is well substantiated and all the criteria to evaluate the bioremediation of the chromium VI were fulfilled.</p>	

### Reviewer Details:

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