Phytoremediation of Lead, Arsenic and Chromium Polluted Soil Using *Opuntia* spp. (Dilang-Baka)

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Abstract
Phytoremediation capabilities of *Opuntia* spp. were tested on heavy metal polluted soil collected in a metallurgical factory in Carmona Cavite. Initial soil testing shows traces of Arsenic: 8.48 mg/Kg, Chromium: 116 mg/Kg, and Lead: 79 mg/Kg. pH and moisture are neutral and dry. *Opuntia* was compared to *A. vera*, within two months. Final results show both can decrease concentrations of Arsenic and Lead but Chromium from *Opuntia* have increased concentration might be due to the reduction of Cr(VI) to Cr(III).

Keywords: Phytoremediation, *Opuntia* spp., *A. vera*, Arsenic, Chromium, Lead

Introduction
Phytoremediation is a technology that uses living plants to clean up soil, air, and water contaminated with hazardous contaminants such as heavy metals (Bruni & Mcleskey, 2013). Since phytoremediation utilizes plants, it is a cheap way of removing contaminants in a certain environment. The plant roots stabilize the soil and prevents movement of pollutants. It is also done on the spot which means less transportation and off-site costs for industries that wishes to remove soil or water contaminants in their area. (Bruni & Mcleskey, 2013). Phytoremediation is an affordable technique that combines several methods to remediate soil contaminated with heavy metals, and other toxic pollutants (Belliturk et al., 2015). Despite their cost-effectiveness and environment friendliness, field applications of these technologies have only been reported in developed countries. In most developing countries, these are yet to become commercially available technologies possibly due to the inadequate awareness of their inherent advantages and principles of operation. With greater awareness by the governments and the public of the implications of contaminated soils on human and animal health, there has been increasing interest amongst the scientific community in the development of technologies to remediate contaminated sites (Wuana & Okieimen, 2011).

The researchers focused on the following heavy metals; arsenic, chromium, and lead, mainly because these are one of the heavy metals that can be seen in contaminated soil (Magahud et al., 2015).
Methodology

The study was conducted from April to May 2021. Proper protocols have been followed to conduct accurate results for the propagation of plants. The soil was collected from metallurgy factories around the industrial zone of Bancal Carmona, Cavite. The soil samples in different sites near the factories were collected in a zig-zag pattern to ensure homogeneity. The plants used for this study were harvested from General Trias Cavite in a residential area. The plants were initially planted directly in the soil and transferred to separate plots two months before the experiment to ensure that care and maintenance for the plants were uniform. Museum of Natural History then authenticated the plants in the University of the Philippines Los Baños Laguna.

Watering the plants was done deeply but infrequently, and the surrounding soil was allowed to dry before watering again to avoid root rotting (Farmer’s Almanac, 2020). Distilled water was also used to avoid contaminants added to the soil, and the soil was cultivated once a week. The plants were kept roofed to avoid exposure to harsh environmental factors such as extreme sunlight and heavy rains or wind.

For the pH and moisture, the researchers used the 3-way meter. The 3-way meter is stuck to the ground about 5 cm in depth, and this is done three times in each pot to get the average pH and moisture.

The collected soil was subjected to initial soil analysis before the propagation of plants to establish a baseline for this study’s variables. Soil analysis in this study included basic pH and moisture measurement and specific heavy metal testing. Mach Union Laboratories did the testing.
Results

1. Other Factors Affecting Phytoremediation capabilities of *Opuntia* spp.

Table 1 Initial and Final pH and Moisture test results of plots for *Opuntia* spp.

<table>
<thead>
<tr>
<th>Plant Code</th>
<th>Initial pH</th>
<th>Initial Moisture</th>
<th>Final pH</th>
<th>Final Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant A (E1)</td>
<td>7.2</td>
<td>5.5</td>
<td>7.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Plant B (E1)</td>
<td>7.9</td>
<td>3.0</td>
<td>7.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Plant C (E1)</td>
<td>8.0</td>
<td>2.5</td>
<td>8.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Plant D (E2)</td>
<td>7.5</td>
<td>4.0</td>
<td>7.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Plant E (E2)</td>
<td>7.9</td>
<td>3.0</td>
<td>7.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Plant F (E2)</td>
<td>7.6</td>
<td>3.0</td>
<td>7.9</td>
<td>3.5</td>
</tr>
<tr>
<td>μ</td>
<td>7.6</td>
<td>3.5</td>
<td>7.6</td>
<td>4.75</td>
</tr>
</tbody>
</table>

Legend: For moisture levels; 1-3 Dry; 4-7 Moist; 8-10 Wet

Table 1 shows the initial pH and moisture test results of *Opuntia* spp. Plots. Each plot’s initial and final pH remained the same; Plant A: 7.2; Plant B: 7.9; Plant C: 8.0; Plant D: 7.5; Plant E: 7.9; and Plant F: 7.6. This gave a mean of 7.6. In terms of moisture, there were significant increases for all plant pots; Plant A: 5.5 to 7.0; Plant B: 3.0 to 4.5; Plant C: 2.5 to 3.5; Plant D: 4.0 to 6.0; Plant E: 8.0 to 4.0; and Plant F: 3.0 to 3.5; with a mean of 3.5 for the initial testing and 4.75 for the final moisture test.

2. Final Heavy Metal Concentration from the Composite soil sample of Positive Control and Experimental Set-up

Table 2 Initial and final heavy metal test results

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Normal Range</th>
<th>Initial Results</th>
<th>Final Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>μ 5 mg/Kg</td>
<td>8.48 mg/Kg</td>
<td>&lt;0.09 mg/Kg</td>
</tr>
<tr>
<td>Chromium</td>
<td>14-70 mg/Kg</td>
<td>116 mg/Kg</td>
<td>58 mg/Kg</td>
</tr>
<tr>
<td>Lead</td>
<td>10 – 50 mg/Kg</td>
<td>79 mg/Kg</td>
<td>64.5 mg/Kg</td>
</tr>
</tbody>
</table>

Table 2 shows the Initial Heavy metal concentration of the soil sample. Arsenic with 8.48 m/Kg; Chromium with 116 mg/Kg; and Lead with 79 mg/Kg. All of the target heavy metals are beyond the normal levels as seen in the normal values indicated in the table. This table also presents the final heavy metal concentrations obtained from the soil samples of both the positive and experimental set up. Both *A. vera* and *Opuntia* spp. had a significant decrease into <0.09 mg/Kg; Chromium decreased for *A. vera* at 58mg/Kg but increased for the *Opuntia* spp plots with 137 mg/Kg; Lastly lead had a significant decrease for both plants; *A. vera* with 64.5 mg/Kg and *Opuntia* spp. with 64.6 mg/Kg.

3. Percent Difference Between Positive and Negative Control

Table 3 Percent difference between the positive control *A. vera* (Sabila) and *Opuntia* spp. (Dilang-baka) treated soil in terms of final heavy metal concentration

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>Concentration With Positive Control <em>Aloe Vera</em></th>
<th>Concentration With Experimental <em>Opuntia</em> spp.</th>
<th>Difference</th>
<th>Average</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>&lt;0.09</td>
<td>&lt;0.09</td>
<td>0</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>Chromium</td>
<td>58</td>
<td>137</td>
<td>79</td>
<td>108</td>
<td>73.15</td>
</tr>
<tr>
<td>Lead</td>
<td>64.5</td>
<td>64.6</td>
<td>0.10</td>
<td>64.55</td>
<td>0.155</td>
</tr>
</tbody>
</table>

Table 3 presents the percent difference between the positive control *A. vera* and the experimental *Opuntia* spp. The first column shows the heavy metals in the soil sample Arsenic, Chromium, and Lead. The second column shows the final concentration of heavy metals in *A. vera*: As <0.09; Cr 58; Pb 64.5; The third column shows the final concentration of heavy metals in *Opuntia* spp.: As <0.09; Cr 137; Pb 64.6; The fourth column indicates the difference between the heavy metal concentration of the positive control *A. vera* and the experimental *Opuntia* spp.
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The results are As 0; Cr 79; Pb 0.10; the following findings can be computed by subtracting the heavy metal concentration value of experimental Opuntia spp. to the value of the positive control A. vera. The fifth column shows the heavy metal average concentration value of the Opuntia spp. with the A. vera, As 0.09; Cr 108; Pb 64.55; it can be done by adding the heavy concentration value of experimental Opuntia spp. to the value of the positive control A. vera, and dividing its sum by 2. The last column shows the percent difference between A. vera and Opuntia spp.

On the other hand, in the final testing results, A. vera, the positive control of the study, decreased all heavy metal concentrations significantly. In a 2018 study conducted by Elhag et al., they evaluated the effectivity of A. vera on heavy metal-contaminated soil. It was shown that succulent species of plants, such as the Aloe, have an ability called phytovolatilization, where the plant takes up water-containing contaminants through their roots, converts them to gaseous form, and then releases them into the atmosphere. Lead might not be within the normal range, but it significantly decreased from the original amount of 79 mg/Kg. In the same study by Elhag et al. (2018), the duration of their experiment was one year, and they proved the efficiency of Aloe as a long-term phytoremediator of Lead.

Opuntia spp., on the other hand, significantly decreased Arsenic levels of the soil at <0.09 mg/Kg and Lead at 64.6 mg/Kg, the same as the positive control. Another genus of cacti, such as the Nopalea, have proven to be effective phytoremediators of chemicals used in textile manufacturing. In the study of Adki et al. (2012), they used cell cultures of Nopalea cohenillifera Salm. Dyck to phytoremediation textile dye. These proponents have successfully used cell cultures of Nopalea to transform various toxic textile dyes into less phytotoxic metabolites. Chromium, on the other hand, increased to 137 mg/Kg from the initial 116 mg/Kg. In congruence to another study by Adki et al. (2013), they used Nopalea cohenillifera as a hyperaccumulator of Chromium (VI) in vitro cultures. It was shown in the study that Nopalea can assimilate enormous amounts of Cr(VI) without significant changes in root growth; in the study it has accumulated 25, 263.369 mg/kg of Cr(VI) without any negative effects to the plant. In another study conducted by Zayed and Terry in 2015, they concluded that some plants accumulate Cr(VI) but release Cr(III) in the soil as an output. Cr(VI), the hexavalent form, is dangerous and carcinogenic, while Cr(III) is tetravalent, considered stable and safe for living organisms. In the heavy metal testing conducted in this study, the chromium that was obtained is not specified; it is the Cr(total) or Total Chromium content of the soil. This might be the possible reason why there is an increased amount of chromium in the soil sample of Opuntia spp.

In Arsenic, there is no difference between A. vera and Opuntia spp. with 0.00 %. This can prove that the two plants used for the study have phytoextracting

Discussion

The soil sample has a mean pH of 7.6, neutral for the initial and final testing. In the study of Lenart and Wolny-Koladka (2012), the soil pH can be alkaline to neutral but still have high concentrations of heavy metals, particularly zinc, cadmium, chromium, lead, nickel, iron, and manganese. The solubility of heavy metals is inversely proportional to pH of the soil; the higher the solubility, the lower the pH, thus resulting in a much higher metal absorption of the plants (Fassler & Robinson, et al., 2010). Moisture levels, on the other hand, varied in the initial and final testing: 3.5 or dry during the initial measurement and 4.75 or moist in the final testing. This change in moisture can be attributed to the plant maintenance technique of the researchers, and it also helped the plant uptake heavy metal contaminants that are present. According to the study by Angle et al. (2013), the higher the moisture content is, the easier it is for plants to do phytoextraction of soil contaminants.

According to ATSDR, 2007, the natural concentration of Arsenic in the soil is usually around 1 to 40 mg/kg with a mean of 5 mg/kg; the results from the collected soil were elevated, meaning there is an increased amount of Arsenic in the soil compared to the normal mean value. This is also high for the soil to be considered healthy and suitable for plant growth, as the ideal level of arsenic for plant flourishing should only be within 0.61 mg/kg to 0.7 mg/kg (Wade, 2019). The initial result for chromium is relatively high above the normal value as per the WHO, 2000, chromium concentrations should range from 14 to about 70 mg/kg, and the results from the collected soil came back at 116 mg/kg. The initial result of lead shows elevated amounts from the normal values at 79 mg/kg. Lead naturally should be 10 – 50 mg/Kg (University of Massachusetts Amherst, 2020).

On the other hand, in the final testing results, A. vera, the positive control of the study, decreased all heavy metal concentrations significantly. In a 2018 study conducted by Elhag et al., they evaluated the effectivity of A. vera on heavy metal-contaminated soil. It was shown that succulent species of plants, such as the Aloe, have an ability called phytovolatilization, where the plant takes up water-containing contaminants through their roots, converts them to gaseous form, and then releases them into the atmosphere. Lead might not be within the normal range, but it significantly decreased from the original amount of 79 mg/Kg. In the same study by Elhag et al. (2018), the duration of their experiment was one year, and they proved the efficiency of Aloe as a long-term phytoremediator of Lead.

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In Arsenic, there is no difference between A. vera and Opuntia spp. with 0.00 %. This can prove that the two plants used for the study have phytoextracting
abilities for phytoremediation soil. As stated in the study of Elhag et al. (2018), succulents have a high capacity to absorb heavy metals.

There is a negligible difference between the A. vera and Opuntia spp for Lead, computed at 0.15%. As explained by Elhag et al. (2018), the longer the duration of planting, the higher the chance of the plant removing contaminants in the soil. Lead for instance can be absorbed at higher rates once the roots are a stable rate of growth.

Lastly, chromium differs by 73.15% between A. vera and Opuntia spp. The computation was done by subtracting the absolute value of the heavy metal concentration of experimental Opuntia spp. to the value of the positive control A. vera and then dividing it by the quotient of the heavy metal concentration value of experimental Opuntia spp. plus the value of the positive control A. vera. The answer is then multiplied by 100. The high percent difference of 73.15% of Chromium between A. vera and Opuntia spp. might be due to the reduction of Cr (VI) to Cr (III), the reduction is possible even at slightly alkalic soil, and it is most common in aerobic soil as stated in the study of Zayed & Terry in 2015. Upon the absorption of Cr(VI), the plant, instead of doing phytovolatilization and releasing the product in the atmosphere, does a different pathway and rereleases the byproduct in the soil as a safe form.

**Conclusion**

Based on the findings of this research, Opuntia spp., the experimental plant, can be used for phytoremediation because it can reduce the amount of certain heavy metals in the soil, making the soil suitable for planting. However, specific recommendations should be made to improve this technique, such as longer duration and addition of trials for the experiment; trying onsite phytoremediation or collecting more soil samples from multiple locations; using extract or cell culture to phytoremediation; conducting specific chromium testing to support the literature cited; and consider the effect of other factors to the phytoremediation capabilities of the Opuntia spp., such as the microorganisms present in the soil and the presence and effect of fertilizers or other chemicals in the sample.

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