B. bassiana and A. indica: Potential Bioremedial Agents For Heavy Metals Contamination

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Abstract
For the present study B. bassiana was isolated and purified from the ground soil. Leaves of A. indica were extracted in aqueous phase. The fungal biomass tolerance to heavy metals was tested upto 100 ppm and found tolerant. Different concentration of heavy metal solutions to be tested were prepared and incubated with both i.e. B. bassiana inoculum and crude extract of A. indica. Biosorption potential of both were estimated (individually as well as by mixing equi volume of both agents to study their cumulative effect) using various parameters. Both agents were showing better biosorption % individually rather than with each other for lead as well as cadmium. Maximum biosorption % of lead was shown by aqueous crude extract of A. indica at 50 ppm, 2500 µl with 95.17 % and of cadmium by B. bassiana at 100 ppm, 2500 µl with 96.7 %. Whereas due to some antagonist effect of inoculum on each other their cumulative biosorption activity showed decreased percentage i.e. 47 % and 36 % in case of lead and cadmium respectively, with comparison to control. Biosorption of lead and cadmium and not their biodegradation was confirmed by further colorimetric analysis of Fungal Biomass.

Keywords- B. bassiana, A. indica, Biosorption, Lead, Cadmium

Introduction
Heavy metal pollution is now a day’s one of the most important environmental concerns. Activities like mining, smelting, agriculture waste disposal, industrial discharge of a variety of metals such as Pb, Cd, Co etc. produce harmful effects on human health [9]. Cadmium occurs in the
environment through process like erosion and abrasion of rocks and soil, volcanic eruptions etc. It is also produced as a byproduct from mining, smelting and refining of sulphide ores of zinc. As it is non degradable therefore leads to kidney issues, lung damage and fragile bones [3]. Lead is a micro element present in trace amounts in all biological materials. The main source are smelting, paints, lead waste, cell batteries etc. In children it leads to central nervous damage, brain and kidney damage [11]. EPA has determined lead as probable human carcinogen. Physical and chemical methods have been proposed for the removal; nevertheless have some disadvantages as cost effectiveness limitation, generation of hazardous byproducts, and inefficiency due to low amounts. Biological methods solve these drawbacks since they are easy to operate, do not produce secondary pollution and shows higher efficiency. Micro organisms and plants are usually used for the removal of heavy metal [2]. Mechanism by which micro organism act on heavy metal includes biosorption, bioleaching etc. Biosorption is a useful alternative to conventional systems for removal of toxic metals because it’s a passive process, property of living as well as inactive- dead- microbial biomass that is cost effective and by which metal recovery is possible [7].

**Material and methods**

**A. Materials-**

1. **Culture of B.bassiana and A.indica leaves**

**B. Method**

1. *B.bassiana* was isolated from ground soil by serial dilution method using potassium phosphate buffer 232 mM as diluent. Serial diluted PDA plates were incubated at 28°C for 46-96 hrs for fungal growth. The pure fungal strain was isolated from the plates by sub culturing it on PDA slants and plates. Confirmation of isolated fungal strain was subjected to morphological studies.

2. *A.indica* leaves were collected from garden of D.A.V College MuzaffarNagar. Disease free healthy leaves were collected, cleaned and air dried. These were grounded, sieved to obtain fine powder and then subjected for extraction of crude extract of Neem.

3. Biosorption of lead and cadmium by these two were estimated according to the method given by Aluyoo, 2009 [1] and Saltzman, 1953 [10]. 2 sets of experiments each containing chemicals with 100 ml PDA and heavy metals i.e. lead and Cadmium at varying concentration (25 ppm, 50 ppm, 75 ppm, 100 ppm) were prepared respectively. Set 3 was prepared containing 100ml PDA only. Set 1, 2 and 3 petriplates were prepared and spreaded with 500ul of isolated fungi culture and incubated at 28°C for 96 hrs.

Experimental sets were established to quantify the biosorption of lead and Cadmium, individually as well as with *B.bassiana* and crude extract of *A.indica*.
together (50:50 volume) to quantify the extent of biosorption done by their cumulative effect. *B. bassiana* was screened for heavy metal tolerance, effect of varying conc. Of heavy metal on fungal growth and maximum effective inoculum amount was quantified. All the experiments were carried out in triplets.

4. The fungal extract was at last screened for their mode of activity as Biosorption or Biodegradation and was confirmed by the method given by Joshi *et al.*, 2011 [5].

- Culture media and Buffer- Potato Dextrose Agar, Potato Dextrose Broth, Phosphate buffer
- Chemicals- The chemicals were obtained from LOBA and FLUKA chemicals.

### III. Observations

#### A. Isolated fungal strain

#### B. Morphological Study

### B. *bassiana*

#### III. Observations-

#### A. Screening for tolerance

#### B. Effect of varying concentration of heavy metals (lead and Cadmium) on Biosorption by *B.bassiana* and crude extract of *A.indica*
C. Inoculum Effect

<table>
<thead>
<tr>
<th>Metal</th>
<th>Conc. (ppm)</th>
<th>Biosorption % (with comparison to control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A.indica</td>
<td>B.bassiana</td>
</tr>
<tr>
<td>Pb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>49.5</td>
<td>4.5</td>
</tr>
<tr>
<td>50</td>
<td>40.5</td>
<td>24.32</td>
</tr>
<tr>
<td>75</td>
<td>18.03</td>
<td>26.98</td>
</tr>
<tr>
<td>100</td>
<td>15.77</td>
<td>28.27</td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>62.43</td>
<td>41.8</td>
</tr>
<tr>
<td>50</td>
<td>62.30</td>
<td>16.93</td>
</tr>
<tr>
<td>75</td>
<td>60.02</td>
<td>21.50</td>
</tr>
<tr>
<td>100</td>
<td>71.14</td>
<td>39.65</td>
</tr>
</tbody>
</table>
D. Maximum Effective Biosorption value for Lead and Cadmium.

E. 1. Estimation of fungal biomass biosorption of lead by *B. bassiana* at different Concentrations (µl)

2. Estimation of fungal biomass for biosorption of Cadmium by *B. bassiana* at different concentrations (µl)
3. Estimation of fungal biomass with crude extract of *A. indica* for biosorption of lead at different concentrations (µl)

![Biosorption % of lead by Fungal Biomass In Association With Neem Extract](image1)

4. Estimation of fungal biomass with crude extract of *A. indica* for biosorption of Cadmium at different concentrations (µl)

![Biosorption % of Cadmium by Fungal Biomass In Association With Neem Extract](image2)

**Result and Discussion**

The mechanism of action for heavy metal biodegradation of *B. bassiana* is Biosorption and Bioaccumulation while Biosorption in the case of *A. indica*. These results were supported by the work of Aluyoo and Audu, 2009 [1] and K.S Suantak, 2010 [6] who reported the same findings. The fungal biomass was found tolerant tolerance upto 100 ppm of both heavy metals present in the sample.

- **With 500 µl of Inoculum and varying concentration of Heavy Metals**-

  Maximum biosorption % of 28.27 % lead by *B. bassiana* was obtained at 100 ppm whereas of cadmium it was 41.8 % at 25 ppm. In case of *A. indica* max. biosorption of
lead was 49.5 % at 25 ppm and of Cadmium was 71.14 % at 100 ppm.

- **With different amounts of Innoculum and varying concentration of Heavy Metals together-**

  With increasing volume of inocula biosorption % increased successively upto an amount and after that it shows a decline. It was 92.5 % and 96.7 % for 2500 µl inocula at 100 ppm in case of lead and cadmium respectively by *B. bassiana*. Whereas in case of *A. indica* was 95.17 % for 2500 µl at 50 ppm for lead and 95.52 % for 2500 µl at 75 ppm for cadmium.

- **Maximum cumulative effect of *B. bassiana* and crude extract of *A. indica* on lead and cadmium-**

  Maximum cumulative effect for biosorption by both innoculants was seen at 25 ppm, 3000 µl for 47 % in case of lead and at 50 ppm, 2500 µl for 36.26 % in case of Cadmium. Thus some antagonist effect of inoculum on each other was seen which resulted in decreased biosorption percentage. Whereas as seen in paper of Hussein et al, 2011 [4] biosorption of lead by *B. bassiana* was seen max. at pH 6 and under different conditions like time (mins). Also in paper of Malik et al, 2012 [8] biosorption by neem leaves appears to be much greater but in only 300 mins of time period.

**Conclusion**

Thus we can conclude by the present study that mechanism of action by fungi is Biosorption as well as Bioaccumulation and not Biodegradation. Biological control by means of biosorption by *B. bassiana* as well as *A. indica* is quite effective measures against heavy metal toxicity of lead and Cadmium but individually. Due to some antagonistic effect on each other they are not able to show such good results. However as a whole it can be concluded that *B. bassiana* is a better bioremedial agent than *A. indica*.

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