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RESEARCH ARTICLE

# REMOVAL OF HEAVY METALS USING BACTERIA ISOLATED FROM LIGNITE MINING ENVIRONMENT

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## ABSTRACT

In industrialized areas, high concentrations of heavy metals have been often found in effluent, soils and wastes, establishing a serious ecological risk. Microorganisms are the first biota that undergoes direct and indirect impacts of heavy metals. The present study was conducted to isolate the heavy metal resistant bacteria from metal rich soil from lignite mining site to assess its capacity to remove heavy metals. Five different bacterial strains were isolated and designated as HMB1, HMB2, HMB3, HMB4 and HMB5 and they were tested against different concentrations of heavy metals *viz.,* Hg, Cr and Ni. Three different methods such as living cells, dead cells and immobilization techniques were used for assessment of capacity of all the five strains to remove metals from the solution containing 100 mg/L. The results of the present study indicated that the maximum heavy metal removal was found to be high in immobilization technique followed by dead cells and living cells. Among the five strains, the HMB2 was high efficient than the others strains in all the methods. Based on the morphological and biochemical characterization the strains were identified. The bacterial strain HMB1 was belonged to *Bacillus* sp., the strain HMB2 was belonged to *Bacillus subtilis*, the strains HMB3 and HMB4 were belonged to *Pseudomonas* sp. and the strain HMB5 was belonged to *Serratia* sp. This indicated that the potential use of these bacterial isolates for removal of heavy metals from wastewater and industrial effluents containing higher concentration of heavy metals.



## INTRODUCTION

Agricultural soils in many parts of the world are from slightly to moderately contaminated by heavy metal toxicity such as Cd, Cu, Zn, Ni, Co, Hg, Cr, Pb and As. This could be due to long term use of phosphatic fertilizers, sewage sludge application, dust from smelters, industrial waste and bad watering practices in agricultural lands (Bell *et al*., 2001; Schwartz *et al*., 2001; Passariello *et al*., 2002). Three kinds of heavy metals are of concern including toxic heavy metals such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc., Precious metals such as Pd, Pt, Ag, Au, Ru etc and Radionuclides such as U, Th, Ra, Am, etc (Wang and Chen, 2006). Small amounts of heavy metals can be necessary for health, but too much may cause acute or chronic toxicity (poisoning). Many of the heavy metals released in the mining and burning of coal are environmentally and biologically toxic elements, such as lead, mercury, nickel, tin, cadmium, antimony, chromium and arsenic, as well as radio isotopes of thorium and strontium (Jeff Goodell, 2006).

Heavy metal pollution by industrial activities and technological development is posing significant threats to the environment and public health because of its toxicity, non-biodegradability and bio-accumulation (Bahadir *et al*., 2007; Perez-Maren *et al*., 2008; Reddad *et al*., 2003). Application of biological processes for decontaminating the contaminated/polluted sites is a challenging task because heavy metals cannot be degraded and hence persist in the soil (Kidd *et al*., 2009; Lebeau *et al*., 2008; Rajkumar *et al*., 2010; Ma *et al*., 2011a). Conventional techniques commonly applied to remove heavy metals from waste water and contaminated soil includes chemical (precipitation, neutralization) or physical (ion exchange, membrane separation, electro dialysis and activated carbon adsorption) methods (Atkinson, 1998). Moreover, these processes may be non–viable at low concentrations. Further, these processes are expensive and not ecofriendly (Gadd and Griffith, 1978; Volesky, 1987). Bioremediation is a technique that uses living organisms in order to degrade or transform contaminants into their less toxic forms (Vidali, 2001).Microorganisms exposed to the higher concentration of toxic heavy metals may develop resistance against the elevated levels of these metals (Habi and Daba, 2009). The present study deals with the isolation and characterization of heavy metal-tolerant bacterial strains isolated from soil of lignite mining site of Neyveli, TamilNadu and the ability of the isolated native microbial strains towards removal of Hg, Cr and Ni using living, dead and immobilized bacterial cells were evaluated and compared.

## MATERIALS AND METHODS

### *Isolation of heavy metal tolerant bacterial strains*

Basal media Nutrient Agar (NA) incorporated with 50 μg/ml salts of heavy metals (Hg, Cr and Ni) were prepared separately and used for selective isolation of heavy metal resistant bacteria. The soil sample collected from lignite mine was serially diluted and

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directly transferred on Nutrient medium from $10^{-6}$ dilution and incubated at 37˚C for 24 hrs. After the incubation period the plates were observed for growth on the media (Virender Singh *et al.*, 2010). The isolated and distinct colonies on these selective media were sub-cultured repeatedly on the same media for purification. The isolated bacterial cultures were directly streaked on different concentration of heavy metals (20, 40, 60, 80, 100,120 mg/L of $HgCl_2$; 50, 100, 150, 200, 250,300 mg/L of $K_2Cr_2O_7$ and 50, 100, 150, 200, 250,300 mg/L of $NiCl_2$) incorporated Nutrient Agar medium and incubated at 37˚C for 48 hrs for the study of maximum heavy metal tolerance level of the isolates.

***Bioremediation of heavy metals by the bacterial isolates***

The stock solutions of the heavy metals were prepared by mixing 1g of respective heavy metal *viz.* $HgCl_2$, $K_2Cr_2O_7$ and $NiCl_2$ in one litre of deionized water (Semra IIhan *et al*., 2004).

***Heavy metal adsorption by living microbial cells (Bioaccumulation) (Vargas et al., 2009)***

About 1% living microbial biomass (Bacterial isolates) were suspended individually in a solution (100 ml) supplemented with heavy metals and incubated for 48 hrs. After incubation, cells were harvested by centrifugation. The supernatants of the samples were analysed and the quantity of each metal removed was measured using AAS and expressed as mg/lit.

***Heavy metal adsorption by dead microbial cells (Biosorption) (Vargas et al., 2009)***

Biomass (bacterial isolates) from the isolates grown in respective broth were harvested by centrifugation and washed with distilled water three times. The pellet was dried and milled. Aliquots of dried microbial cells (200 mg/L) were prepared in distilled water and homogenized in a mixer to destroy aggregated cells. About 1ml of cell suspensions were added to the metal solution (100 ml) prepared and incubated. After incubation, the suspensions were centrifuged and filtered for biomass removal. Heavy metal concentration in the supernatant was measured as previously described.

***Heavy metal adsorption by immobilized microbial cells (Johncy Rani et al., 2010b)***

The microbial cells (bacterial isolates) were immobilized as beads according to the procedure of Leung *et al*. (2000). The beads (1g) containing $>10^5$ cfu/ml biomass were added to the conical flask containing 100 ml of metal solution and incubated for 48 hrs. After which the samples were withdrawn for heavy metal analysis using AAS. Among three methods, immobilization showed best results.

***Identification of the isolates***

The strains isolated and used in this present study was identified by morphological and biochemical characterization as per the method suggested by Gerhardt *et al*. (1994).

## RESULTS AND DISCUSSION

***Isolation of heavy metal tolerant bacterial isolates from lignite mine soil***

Five strains were isolated and designated as (Heavy Metal Bacteria) HMB1, HMB2, HMB3, HMB4 and HMB5. The growth response of these strains against different concentrations of heavy metals (Hg, Cr and Ni) was tested and the results are presented in Table - 1. The strains HMB1, HMB2, HMB3 and HMB4 were able to grow at 100 mg/L of Mercury. Whereas, the strain HMB5 was able to grow upto 60 mg/L. Likewise, the strains HMB2, HMB3 and HMB4 were able to grow in chromium upto 250 mg/L. Whereas, the strain HMB1 was able to grow upto 200 mg/L and HMB5 was upto 100 mg/L of chromium. In nickel, the strains HMB1, HMB2, HMB3 and HMB4 were able to grow upto 250 mg/L, but HMB5 was upto 150 mg/L of nickel only.

***Heavy metal removal by the bacterial isolates***

Bioremediation (Bioaccumulation, Biosorption and Immobilization) of heavy metals was studied by using live cultures, dead cells and immobilized cells. The results revealed that all the types of cells were found to remove heavy metals. The results of the three methods are presented in Table - 2.

***Heavy metal adsorption by living bacterial cells (Bioaccumulation)***

The bioaccumulation (using living bacterial culture) studies revealed higher amount of heavy metal adsorption was by the strain HMB2 and the values are 43.1 mg/L for Hg, 55.8 mg/L for Cr and 56.2 mg/L for Ni, followed by HMB3, HMB4 and HMB1. Whereas, the strain HMB5 showed the lowest activity of heavy metal adsorption (38.2 mg/L for Hg, 49.0 mg/L for Cr and 52.4 mg/L for Ni ). Pan *et al.* (2009) observed similar results using *Pseudomonas* sp. and *Bacillus* sp. as bioremediation agent, as well as Ting and Choong (2009) in their comparison between the ability of a *Trichoderma* isolate to bioaccumulate and bioabsorb. The three strains of *Pseudomonas* isolated from heavy metal contaminated soil accumulated 29, 25 and 26 mg $g^{-1}$ dry weight of cells, respectively at the zinc concentration of 1.6 mM (Munees Ahemad and Abdul Malik, 2011). Ahmad *et al.* (2005) reported that Gram negative bacteria showed higher bioaccumulation capacity to heavy metals than the Gram positive counter parts due to their higher level of intrinsic metal resistance. This difference was based on the chemical composition of their cell wall. Noghabi *et al*. (2007) reported that the high capability of heavy metals bioaccumulation by Gram negative bacteria.

***Heavy metal adsorption by dead bacterial cells (Biosorption)***

In biosorption studies (using dead bacterial cells) the isolate HMB2 showed the maximum heavy metal adsorption (56.3 mg/L for Hg, 66.1 mg/L for Cr and 67.1 mg/L for Ni) followed by HMB3, HMB4 and HMB1. The isolate HMB5 showed the minimum adsorption of the heavy metals (50.3 mg/L for Hg, 59.2 mg/L for Cr and 60.2 mg/L for Ni). As observed in the present study, Hussein *et al*. (2004) reported that the maximum adsorption of heavy metals reached upto 88% by *Pseudomonas* sp. Several of the reports revealed that *Pseudomonas* sp. was a suitable biosorbent to remove heavy metals like Cu, Cd and Pb from aqueous solution (Zaied *et al*., 2008). In the present study, dead cells were found efficient than living cells whereas, several authors have described this higher efficiency was by living microbial cells. Zucconi *et al.* (2003) found that living cells of *Azospirillum* sp., showed a higher capacity than dead cells. Al-Garni *et al*. (2009) reported a decrease between 15.2 mg/L, 44.6 mg/L for living and dead cells of *Bacillus* sp., 18.9 mg/L, 59.8 mg/L for living and dead cells of *Azotobacter* sp. This difference in living and dead cells might be probably as a consequence of the method used to prepare the dead biomass, which affects the efficiency of the heavy metal biosorbing capacity of the organisms (Bishnoi and Garima, 2005).

**Table 1 Maximum** heavy metal tolerance level of the bacterial isolates

| Isolates | Heavy metals (mg/L) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $HgCl_2$ | | | | | | $K_2Cr_2O_7$ | | | | | | $NiCl_2$ | | | | | |
| | 20 | 40 | 60 | 80 | 100 | 120 | 50 | 100 | 150 | 200 | 250 | 300 | 50 | 100 | 150 | 200 | 250 | 300 |
| HMB1 | + | + | + | + | + | - | + | + | + | + | - | - | + | + | + | + | + | - |
| HMB2 | + | + | + | + | + | - | + | + | + | + | + | - | + | + | + | + | + | - |
| HMB3 | + | + | + | + | + | - | + | + | + | + | + | - | + | + | + | + | + | - |
| HMB4 | + | + | + | + | + | - | + | + | + | + | + | - | + | + | + | + | + | - |
| HMB5 | + | + | + | - | - | - | + | + | - | - | - | - | + | + | + | - | - | - |

**Table 2** Heavy metal removal by bacterial isolates in different methods

| S.No | Isolates | Heavy metals (Hg, Cr, Ni) initial concentration: 100 mg/L | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Living cells | | | Dead cells | | | Immobilized cells | | |
| | | Hg adsorbed (mg/L) | Cr adsorbed (mg/L) | Ni adsorbed (mg/L) | Hg adsorbed (mg/L) | Cr adsorbed (mg/L) | Ni adsorbed (mg/L) | Hg adsorbed (mg/L) | Cr adsorbed (mg/L) | Ni adsorbed (mg/L) |
| 1. | HMB1 | 41.2 | 52.2 | 53.0 | 52.5 | 62.1 | 62.5 | 68.2 | 72.1 | 72.8 |
| 2. | HMB2 | 43.1 | 55.8 | 56.2 | 56.3 | 66.1 | 67.1 | 74.5 | 78.2 | 79.0 |
| 3. | HMB3 | 42.8 | 55.2 | 56.0 | 56.0 | 65.4 | 66.2 | 74.1 | 77.6 | 78.2 |
| 4. | HMB4 | 42.6 | 54.8 | 55.6 | 55.8 | 64.9 | 65.7 | 73.6 | 76.8 | 77.0 |
| 5. | HMB5 | 38.2 | 49.0 | 52.4 | 50.3 | 59.2 | 60.2 | 63.0 | 67.0 | 69.1 |

### *Heavy metal adsorption by Immobilized bacterial cells*

In immobilization studies, the strain HMB2 showed maximum heavy metal adsorption (74.5 mg/L for Hg, 78.2 mg/L for Cr and 79.0 mg/L for Ni) followed by HMB3, HMB4 and HMB1,whereas HMB5 showed least in heavy metal adsorption (63.0 mg/L for Hg, 67.0 mg/L for Cr and 69.1 mg/L for Ni). It was reported that the immobilized bacterial cells have greater adsorption capacity than that of dead or living cells because the bacterial cells consists of small particles with low density, poor mechanical strength and little rigidity in their cell surface (Leusch *et al*., 2005). These results are also supported by other authors (Costa and Leite, 2000; Sudha and Abraham, 2003; Wei Bin *et al*., 2006; Vijayaraghavan and Yeoung Sang, 2007). The immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems (Holan and Volesky, 1998). Also, immobilized beads are hard enough to withstand the application, pressures, water retention capacity, porous, transparent to metal ion sorbate species and have high and fast sorption uptake even after repeated regeneration cycles. In addition because of immobilization, the biosorbents will have better shelf life and offer easy and convenient usage compared to free biomass, which is easily biodegradable (Volesky and May Phillips, 2000).

### *Identification of the bacterial isolates*

The strains used in the present study were identified based on morphological and biochemical characteristics. According to the morphological and biochemical characteristics, the strain HMB1 was belong to *Bacillus* genera, HMB2 was *Bacillus subtilis*, two strains were belongs to *Pseudomonas* (HMB3 & HMB4), whereas the strain HMB5 was belongs to *Serratia* sp.

## CONCLUSION

Heavy metal tolerant bacteria isolated from lignite mining environment have proven to be efficient as detoxification agents in multi-polluted heavy metals aqueous solutions, especially *Bacillus subtilis* (HMB2) and strains of *Pseudomonas* (HMB3, HMB4). In all the cases, immobilized cells showed higher activity than living and nonliving cells. Although, further studies are needed, these results are very promising as a starting point for a potential application of these microorganisms in bioremediation of industrial effluent, sewage sludge and industrial wastes.

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