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# Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: A biosorption approach

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**Microorganisms play a vital role in heavy metal contaminated soil and wastewater by the mechanisms of biosorption. In this study, heavy metal resistant bacteria were isolated from an electroplating industrial effluent samples that uses copper, cadmium and lead for plating. These isolates were characterized to evaluate their applicability for heavy metal removal from industrial wastewaters. The physico-chemical parameters of the samples were initially analyzed. The optimum conditions of pH, biomass concentration and heavy metal concentration were determined for the microbial growth on biosorbents and correlated with heavy metal removal. The observed optimum conditions were applied for the biosorption process carried out in immobilized and dead bacterial isolates. The biosorption of immobilized cells of *Bacillus* sp. was 69.34% of Cu, *Pseudomonas* sp. was 90.41% of Cd and *Micrococcus* sp. was 84.27% of Pb, whereas the dead cells of *Bacillus* sp. was 44.73% of Cu, *Pseudomonas* sp. was 86.66% of Cd and *Micrococcus* sp. was 79.22%. Experimental results reveal that all the immobilized isolates have potential application for the removal of Cu, Cd and Pb from industrial wastewater than the dead bacterial cells.**

**Key words:** Biosorption, bacteria, heavy metal, dead bacterial cells, immobilization.

## INTRODUCTION

The current pattern of industrial activity allows the natural flow of materials and introduces novel toxic chemicals into the environment (Faisal and Hasnanin, 2004). Heavy metals include cadmium, lead, chromium, copper and nickel, which contaminate the soils, ground water, sediments and surface waters are extremely toxic to biological and ecological systems. The heavy metals are released due to the discharge of effluent into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc., and the concentration levels of these heavy metals varies widely in the environment. Heavy metals pose a critical concern to human health and environmental issues due to their high occurrence as a contaminant, low solubility in biota and the classification of several heavy metals as carcinogenic and mutagenic (Alloway, 1995; Diels et al., 2002).

Moreover, the metals cannot be degraded to harmless products and hence persist in the environment indefinitely. As a result, several methods have been devised for the treatment and removal of heavy metals in contaminated sites. Conventional physico-chemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation and sorption (Kadirvelu et al., 2001; Kadirvelu et al., 2002) for heavy metal removal are being economically expensive and have disadvantages like incomplete metal removal, higher reagent, energy requirements and generation of toxic sludge. Biological approach has the great potential that contributes for the achievement of this goal and is economical. Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant (Prasenjit and Sumathi, 2005).

The response of microorganisms towards toxic heavy metals is of importance in view of the interest in the reclamation of polluted sites (Shankar et al., 2007). Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Shumate and Strandberg, 1985; Anders et al., 1992; Fourest and Roux,

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**Table 1.** Characteristics of electroplating industrial effluent used for the isolation of heavy metal-resistant bacteria.

| Parameter | Value |
|---|---|
| pH | 7.97 |
| Electrical conductivity (ms) | 240 |
| Total dissolved solids (mg/L) | 151.2[a] (±0.86)[b] |
| Organic carbon (%) | 3.5[a] (±0.59)[b] |
| Organic matter (%) | 6.13[a] (±0.74)[b] |
| **Heavy metals (mg/L)** | |
| Copper | 2.8860[a] (±0.75)[b] |
| Cadmium | 1.9820[a] (±1.40)[b] |
| Lead | 1.2720[a] (±0.37)[b] |

[a] Value represents mean of triplicates.
[b] Values in parentheses represent standard deviation.

1992; Hussein et al., 2001; Hussein et al., 2003). Feasibility studies for large-scale applications demonstrated that, biosorptive processes are more applicable than the bioaccumulative processes, because living systems (active uptake) often require the addition of nutrients and hence increase biological oxygen demand or chemical oxygen demand in the effluent (Hussein et al., 2004). Biosorption is proven to be quite effective for the removal of metal ions from contaminated solution in a low cost and environment friendly manner (Volesky, 1990).

In this present study, the ability of isolated native microbial strains towards biosorption of Cu, Cd and Pb using immobilized and dead bacterial cells were evaluated and compared. Effect of temperature, pH, biomass and tolerance to the heavy metals by the microbial isolates were carried out.

## MATERIALS AND METHODS

### Sampling

Effluent sample were collected from an electroplating industry at Tiruchchirappalli district, Tamil Nadu, India that uses copper, cadmium and lead for plating. The collected sample was transferred to a sterile plastic container and taken immediately to the laboratory and maintained at 4°C for further studies. The effluent characteristics are listed in Table 1 (APHA, 1998; Saxena, 1998). Heavy metal concentrations were analyzed using 400/HGA 900/AS 800–Perkin Elmer Atomic Absorption Spectrophotometer (AAS).

### Isolation and identification of heavy metal-resistant bacteria from the effluents

Cu, Cd and Pb-resistant bacterial isolates were isolated from the effluent using nutrient agar (NA) medium and were prepared using peptic digest of animal tissue (5 g/L), beef extract (3 g/L), NaCl (5 g/L) and agar 15 g/L. The isolated metal-resistant bacteria were amended with 100 mg/L of Cu, Cd and Pb individually. Pour plate was performed in NA medium and was incubated at 37°C for 24 h. Morphological, physiological and biochemical characteristics of the isolates were determined by adopting standard methods (data not shown) (Cuppucino and Sherman, 1983).

### Determination of heavy metal-resistant bacterial isolates by plate diffusion method

Heavy metal resistant bacteria were determined by plate diffusion method (Hassen et al., 1998). Heavy metal salt solutions were prepared in different concentrations, say 10, 20, 40, 60, 80 and 100 mg/L. Each plate was spread with overnight cultures of appropriate organisms. To each of the plate 100 µl of appropriate metal salt solutions were added in each wells of 10 mm in diameter and 4 mm in depth. NA plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured. A zone size less than 1 mm scored as resistance strain.

### Antibiotic resistance test

The disc diffusion method was used to determine antibiotic sensitivity of the isolates. Kanamycin, streptomycin, ampicillin, tetracycline and chloramphenicol were the five antibiotics used and placed at equidistance. Zone of inhibition were measured and were classified as resistance or sensitive isolates (Baurer et al., 1966).

### Optimization for heavy metal removal

Temperature, pH, biomass, heavy metal concentrations are the factors which affects the biosorption process. Particularly, pH (Gourdon et al., 1990), biomass concentration (Gong et al., 2005) and heavy metal concentration (Kiran et al., 2005) on biosorption experiments were investigated by optimization process. The bacterial isolates were inoculated into a series of test tubes containing 5 ml of nutrient broth. The pH was varied from 5 to 9 (5, 6, 7, 8 and 9) by adjusting the medium amended with 25 mg/L of Cu, Cd and Pb. The biomass concentration was varied from 1 to 5% (1, 2, 3, 4 and 5) in the medium containing 25 mg/L of Cu, Cd and Pb. The heavy metal concentration was varied from 20 mg/L to 100 mg/L (20, 40, 60, 80 and 100 mg/L).

### Heavy metal adsorption by dead bacterial cells

The dead bacterial cells (200 mg/L) were suspended in distilled water and homogenized in a mixer to destroy aggregated cells. The cell suspensions were added into the effluent sample. The wet cells were suspended in 100 ml of 0.5% (w/v) NaCl solution at room temperature in order to obtain a suspension with equivalent to the dead bacterial cell concentration of 200 mg/L. NaCl was included to prevent cell damage due to osmotic pressure. The adsorption test was conducted in an incubator shaker (100 rpm) at 30°C. The samples were taken after 24 h incubation (Gourdon et al., 1990). The supernatants of the samples were analyzed and the percentage of each metal removal was measured using AAS.

### Heavy metal adsorption by the immobilized bacterial cells

The immobilized bacterial cells were prepared as beads according to the procedure of Leung et al. (2000) and were maintained in the conical flask containing 50 ml of samples for incubation, after which the samples were withdrawn for heavy metal analysis by using

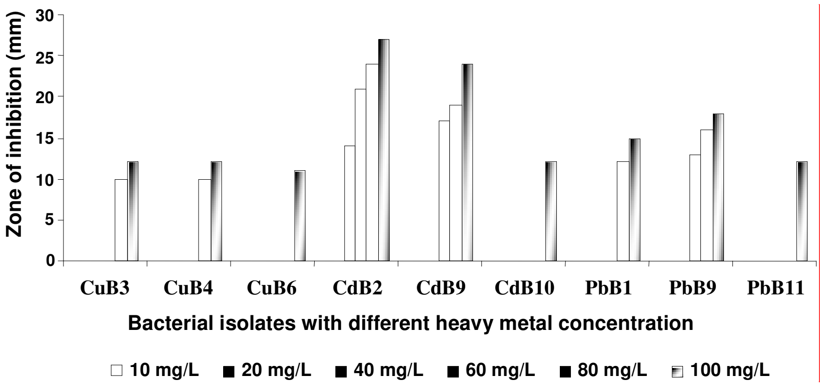


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**Figure 1.** Growth of bacteria in different concentrations of heavy metals by plate diffusion method

**Table 2.** Antibiotic susceptibility of heavy metal resistant bacteria.

| Antibiotics | Disc potency (mcg) | Antibiotic susceptibility pattern | | |
|---|---|---|---|---|
| | | ***Bacillus* sp.** | ***Pseudomonas* sp.** | ***Micrococcus* sp.** |
| Ampicillin | 10 | R* | R | R |
| Chloramphenicol | 10 | R | R | R |
| Kanamycin | 5 | R | R | R |
| Streptomycin | 10 | S* | S | S |
| Tetracycline | 10 | R | R | R |

*R- Resistant strain, S- Sensitive strain.

AAS.

### Statistical analyses

Data were analyzed using SPSS version 11.5. Treatment means were compared using the paired 'T' Test after it was determined that there was a significant treatment effect.

## RESULTS AND DISCUSSION

### Heavy metal resistance efficiency

In plate diffusion method, results of zone formation indicate the ability of the isolates as heavy metal-resistant or sensitive (Duxbury, 1981). Heavy metal-resistant isolates show no inhibition of growth for higher concentration of heavy metals, whereas heavy metal-sensitive isolates show inhibition of growth for higher concentration of heavy metals. Based on this concept, *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. were identified as efficient strains that were resistant to Cu, Cd and Pb respectively. The identified efficient strains were selected for further studies. The results are shown in Figure 1.

In antibiotic resistance test, metal resistance capacities of the microbes are mainly associated with antibiotic resistance. The selected three bacterial isolates were resistant to the antibiotics like tetracycline, kanamycin, chloramphenicol and ampicillin but were sensitive to streptomycin (Table 2). Earlier literatures reveal that there is an interrelationship between the antibiotic and heavy metal resistance capacities of all the microbes (Harnett and Gyles, 1984). Metal tolerance and antibiotic resistance are often closely associated that are found in many clinical isolates (Tmoney et al., 1987).

### Optimization for heavy metal removal

In the pH range studied (5 to 9) all the heavy metal resistant bacterial isolates growth were increased gradually at initial pH 7 and decreased at increased pH (Figure 2). The biosorption of the cell was sensitive to pH (Simie et al., 1998). The cell surface metal binding sites and availability of metal in solution are affected by pH. At low pH, the cell surface sites are closely linked to the $H^+$ ions, thereby making these unavailable for other cations. However, with an increase in pH, there is an increase in ligand with negative charges which results in increased binding of cations (Ahuja et al., 1999).

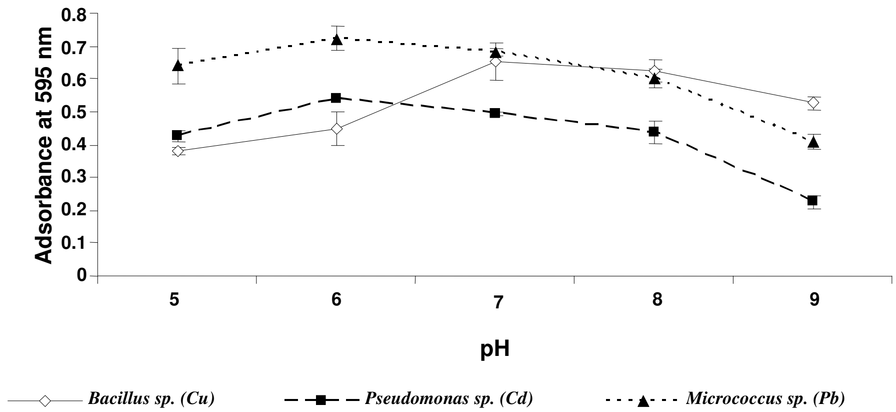


**Figure 2.** Cellular growth of bacterial isolates in response to various pH. Temperature: 37°C, incubation time: 24 h. Each value is the mean of triplicates. Error bars represent standard deviation.

The increase of pH resulted in an increased negative charge on the surface of the cell which favored electrochemical attraction and adsorption of metal (Gourdon et al., 1990). *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. has the ability to adsorb maximum Cu, Cd and Pb at pH 7, 6 and 6 respectively which are similar to the results of Wang and Chen (2006) and Blackwell et al. (1995). In their result, the highest adsorption occurs at pH ranges from 4 - 8. This pH range is widely accepted as being optimal for metal uptake of almost all types of biomass.

The *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. showed the maximal growth of biomass in the presence of Cu, Cd and Pb at the level of 3, 2 and 4% respectively (Figure 3). A similar trend in metal uptake with variations in biosorbents concentration has been reported for Pb adsorption from its synthetic aqueous solutions by *Spirulina maxima* (Gong et al., 2005). From this study, the result indicates that in bacteria when the biomass concentration increases there will be reduction in the growth of organisms and adsorption of heavy metals. Previous study reported that the high biosorbents concentrations are known to cause cell agglomeration and consequent reduction in the inter-cellular distance (Pons and Fuste, 1993). This result indicates a 'screen effect' among the dense layer of cells, leading to 'protection' of the binding sites from metal ions. In other words, the metal uptake is higher when the inter-cellular distance is more (at low biosorbent concentration), as this condition ensures optimal electrostatic interaction between cells with a significant factor of biosorption (Itoh et al., 1975).

The maximum growth of *Bacillus* sp. in the presence of Cu attained at 20 mg/L and it gradually decreased up to 100 mg/L (Figure 4). This result was similar to *Klebsiella aerogenes* in which the adsorption of both metals gradually increased at initial concentration. *Pseudomonas* sp. showed the maximum growth in the presence of Cd at 60 mg/L. This result was similar to maximum adsorption of Cd (II) ion at 60 mg/L on the dead bacterial cells of *Zoogloea ramigera* reported by Norberg (1984). Maximum growth of *Micrococcus* sp. in the presence of Pb was observed at 40 mg/L, which was similar to *Neurospora crassa* that adsorbed Pb from its synthetic aqueous solutions (Kiran et al., 2005). It was explained that at low metal concentration the biosorption of the biosorbents is not fully utilized (Rani and Haripriya, 2003). Several previous studies confirmed Cu, Pb and Zn removal by *Trametes vesicolor* (Gulay et al., 2003), chromium removal by *Bacillus* sp. (Yi-Tin and Chang song, 1995) and *Bacillus firmus* also having less ability to remove Pb, Cu and Zn (Salehizadesh and Shojaosadati, 2003). All these results clearly reveal the existence of infinite heavy metal reduction possibly due to heavy metal toxicity towards the cells.

### Biosorption of heavy metal by dead bacterial cells

The biosorption is basically at lab scale inspite of its development for decades (Wang and Chen, 2006). In the present study, the dead bacterial cells of *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. were used as the biosorbents for the adsorption of Cu, Cd and Pb respectively. *Bacillus* sp. showed 44.73% adsorption of Cu (Figure 5). It was proved that *Bacillus* sp. can grow in significant levels of heavy metal media and have high capacity for heavy metal adsorption (Kim, 2005). *Bacillus*

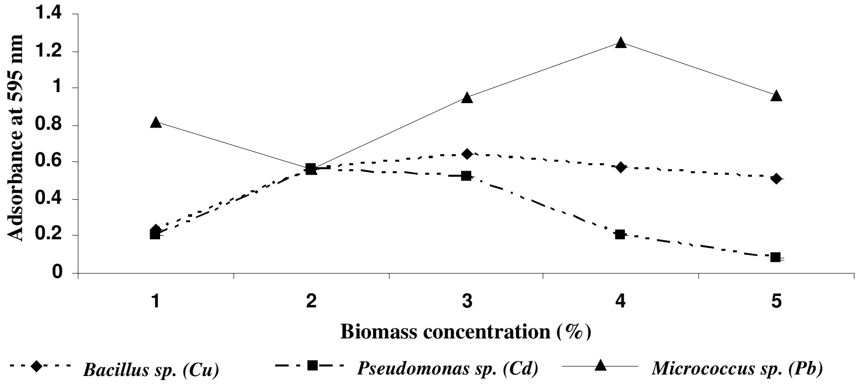


**Figure 3.** Cellular growth of bacterial isolates in response to various biomass concentrations. Temperature: 37°C, incubation time: 24h. Each value is the mean of triplicates. Error bars represent standard deviation.

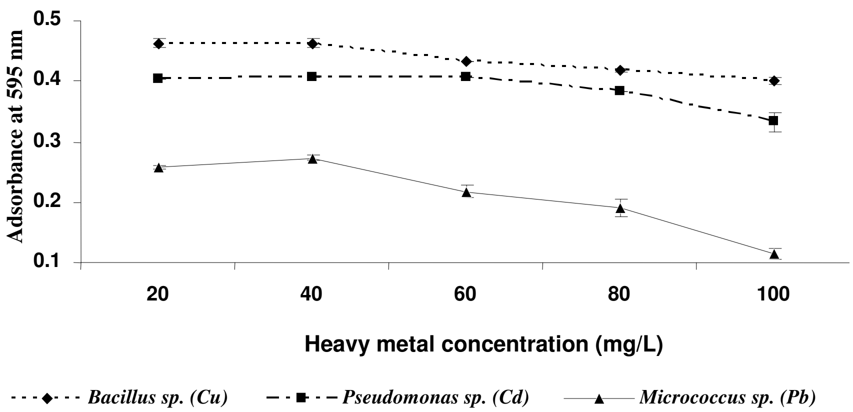


**Figure 4.** Cellular growth of bacterial isolates in response to various heavy metal concentrations. Temperature: 37°C, incubation time: 24h. Each value is the mean of triplicates. Error bars represent standard deviation.

sp. has the ability to adsorb the Cu at a maximum level of 400 mg/L and removed 65% of Cu during the active growth cycle. *Pseudomonas* sp. was considered to be the most effective biosorbent because of its high adsorption capacity when compared to *Bacillus* sp. and *Micrococcus* sp. *Pseudomonas* sp. adsorbed 86.66% of Cd. Previous studies reported that the maximum adsorption of heavy metals reached up to 88% by *Pseudomonas* sp. (Hussein et al., 2004). Most of the reviews reveal that *Pseudomonas* sp. is a suitable biosorbent to remove heavy metals like Cu, Cd and Pb from aqueous solution. *Micrococcus* sp. has the ability to adsorb 79.22% of Pb (Zaied et al., 2008).

**Biosorption of heavy metal by immobilized bacterial cells**

The biosorption of immobilized bacterial cells like *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. adsorbed Cu (69.34%), Cd (90.41%) and Pb (84.27%) respectively (Figure 5). The paired 'T' test showed a significant difference between the immobilized and dead bacterial cells used for the biosorption process (Table 3).

All these results reveal that the adsorption capacity of the immobilized bacterial cells was greater than that of dead bacterial cells by .002, .023 and .004 in *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. respectively. It

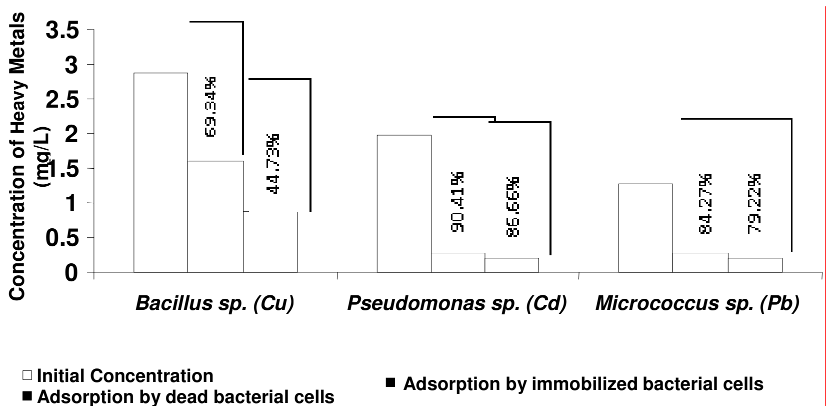


**Figure 5.** Heavy metal biosorption by bacterial isolates

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**Table 3.** Paired 'T' Test.

| | | Paired differences | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. deviation | Std. error mean | 95% Confidence interval of the difference | | T | df | Sig. (2-tailed) |
| | | | | | Lower | Upper | | | |
| Pair 1 (*Bacillus* sp.) | Immobilized and dead bacterial cells | 24.6067 | 1.66377 | 0.96058 | 20.4736 | 28.7397 | 25.617 | 2 | .002 |
| Pair 2 (*Pseudomonas* sp.) | Immobilized and dead bacterial cells | 3.7500 | 0.99292 | 0.57327 | 1.2834 | 6.2166 | 6.541 | 2 | .023 |
| Pair 3 (*Micrococcus* sp.) | Immobilized and dead bacterial cells | 5.0500 | 0.54249 | 0.31321 | 3.7024 | 6.3976 | 16.123 | 2 | .004 |

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was reported because of the dead bacterial cells that consists small particles with low density, poor mechanical strength and little rigidity (Leusch et al., 1995), the immobilized bacterial cells have greater biosorption than the dead bacterial cells. Hence the biomass is to be immobilized before being subjected to biosorption. The immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems (Holan and Volesky, 1994).

## Conclusion

The results of this study revealed that all the immobilized isolates have a greater potential application for the removal of Cu, Cd and Pb from industrial wastewater than the dead bacterial cells. Further research will be scoped to study the desorption process for the management of heavy metal fraught biomass as an environmental friendly method of disposal.

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