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# Comparative Assessment of Heavy Metal Removal by Immobilized and Dead Bacterial Cells: A Biosorption Approach

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**Abstract:** 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 the effluent samples of an electroplating industry and the biosorption of copper (Cu), cadmium (Cd) and lead (Pb). These isolates were characterized to evaluate their applicability for heavy metal removal from industrial wastewaters. Initially the samples physico-chemical parameters were 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 dried biomass and immobilized bacterial isolates. The biosorption capacity of immobilized bacterial isolates like *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. were 69.34, 90.41 and 84.27% of Cu, Cd and Pb respectively. But the dried biomass of the same species adsorbed only 44.73, 86.66 and 79.22% of Cu, Cd and Pb respectively. Experimental results reveal that all the immobilized strains have potential application for the removal of Cu, Cd and Pb from industrial wastewater than the dried biomass.

**Key words:** Biosorption · Bacteria · pH · Heavy metal · Dried biomass · Immobilization

## INTRODUCTION

Developmental progress of agriculture and industry are taken as a major criterion in any country. Industrial creation have emerged as one of the world most dynamic economic sectors, offering vast opportunities for cultural, social and economic development. Today, India is one of the top ten industrialized countries of the world, where volume of factory output catches the 14$^{th}$ place. However, in industrial production expansion is outstripping global growth from one side and several highly polluting industries are also growing more rapidly in another side. Because of the rapid growth in urbanization and industrialization the impact of foreign direct investment in environmental terms has largely been negative. The current pattern of industrial activity allows the natural flow of materials and introduces novel toxic chemicals into the environment [1]. Toxic chemicals like heavy metals include cadmium, lead, chromium, copper, nickel, etc., that contaminate the soils, ground water, sediments and surface waters are present in soluble form. Most of them are extremely toxic to biological systems and ecological activity is disturbed. 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 preservative, pulp processing, steel manufacturing, etc. and the concentration levels of these heavy metals varies widely in the environment. Several methods have been devised for the treatment and removal of heavy metals. Conventional physico-chemical methods being economically expensive have disadvantages like incomplete metal removal, higher reagent, energy requirements and generation of toxic sludge's and other waste products that require disposal. As a result, many new technologies are required to mitigate heavy metal concentrations to environmentally acceptable levels. Biological approach has the great potential that contributes for the achievement of this goal. Biosorption is proven to be quite effective for the removal of metal ions from contaminated solution in a low cost and environment friendly manner [2]. In this present study, the ability of isolated native microbial strains towards biosorption of copper, cadmium and lead were evaluated. Effect of temperature, pH, biomass and tolerance to the heavy metals by the microbial isolates were carried out.

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## MATERIALS AND METHODS

**Sampling:** Effluent sample were collected from the electroplating industry at Tiruchchirappalli district, Tamil Nadu, India that uses copper (Cu), cadmium (Cd) and lead (Pb) 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 as per the method of Saxena *et al.,* [3]. Heavy metal concentrations were analyzed using 400/HGA 900/AS 800–Perkin Elmer Atomic Adsorption Spectrometer (AAS).

**Isolation and Identification of Heavy Metal-resistant Bacteria from the Effluents:** Cu, Cd and Pb-resistant bacterial strains 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 and pour plate was performed in NA medium and was incubated at 37°C for 24h. Morphological, physiological and biochemical characteristics of the isolates were determined by adopting standard methods [4].

**Determination of Heavy Metal-resistant Bacterial Isolates**

**Broth Method:** Heavy metal resistance capacities of the bacterial strains were determined as described by Konopka and Zakharova [5]. The bacterial isolates were raised in nutrient broth containing 10, 20, 40, 60, 80 and 100 mg/L of Cu, Cd and Pb. Broth tubes were inoculated with 0.1 ml of appropriate organisms grown to an $OD_{620}$ of 0.8-1.0 and then incubated at 37°C for 24h. After incubation, $OD_{620}$ was measured using spectrophotometer (Geneys 20). A culture having an $OD_{620}$ of greater than 0.1 was considered as resistant strain.

**Plate Diffusion Method:** Heavy metal resistant bacteria were determined by plate diffusion method [6]. 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 24h. After incubation, the zone of inhibition was measured. A zone size less than 1 mm scored as resistance strain.

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) | 151.2a (±1.86)b |
| Organic carbon (%) | 3.5 (±0.59) |
| Organic matter (%) | 6.13 (±0.74) |
| Heavy metals (mg/L) | |
| Copper | 2.8860 (±2.75) |
| Cadmium | 1.9820 (±5.40) |
| Lead | 1.2720 (±2.37) |

a Values represent average of two samples except pH and EC.
b Values in parentheses represent standard deviation.

**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 equi-distances. Zones of inhibition were measured and were classified as resistance or sensitive strains [7].

**Optimization for Heavy Metal Removal:** Temperature, pH, biomass, heavy metal concentrations are the factors which affects the biosorption process. Particularly, pH [8], biomass concentration [9] and heavy metal concentration [10] on biosorption experiments were investigated by optimization process.

**Heavy Metal Adsorption by Dried Cells:** The dried cells of bacteria (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 dried 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 24h incubation [8]. The supernatants of the samples were analyzed and the percentage of each metal removal was measured using AAS.

**Heavy Metal Adsorption by the I mmobilized Cells:** The immobilized bacterial beads were prepared according to the procedure of Leung *et al*. [11] and were maintained in the conical flask containing 50 ml of samples for incubation, after which the samples were with drawn for heavy metal analysis by using 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 organisms as heavy metal-resistant or as sensitive strains to heavy metals [12]. Heavy metal-resistant strains show no inhibition of growth for higher concentration of heavy metals, whereas heavy metal-sensitive strains 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 the Fig. 1.

In antibiotic resistance test, metal resistance capacities of the microbes are mainly associated with antibiotic resistance. The selected three bacterial strains 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 [13]. Metal tolerance and antibiotic resistance are often closely associated that are found in many clinical isolates [14].

Table 2: Antibiotic susceptibility of heavy metal resistant bacteria

| | Antibiotic susceptibility pattern | | | |
|---|---|---|---|---|
| Antibiotics | Disc potency (mcg) | 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

**Optimization for Heavy Metal Removal:** Optimization of the pH range studied (5 to 9) all the heavy metal resistant bacterial strains growth were increased gradually at initial pH 7 and decreased at increased pH (Fig. 2). The biosorption capacity of the cell was sensitive to pH [15]. 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 [16]. The increase of pH resulted in an increased negative charge on the surface of the cell which favored electrochemical attraction and adsorption of metal [8]. *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

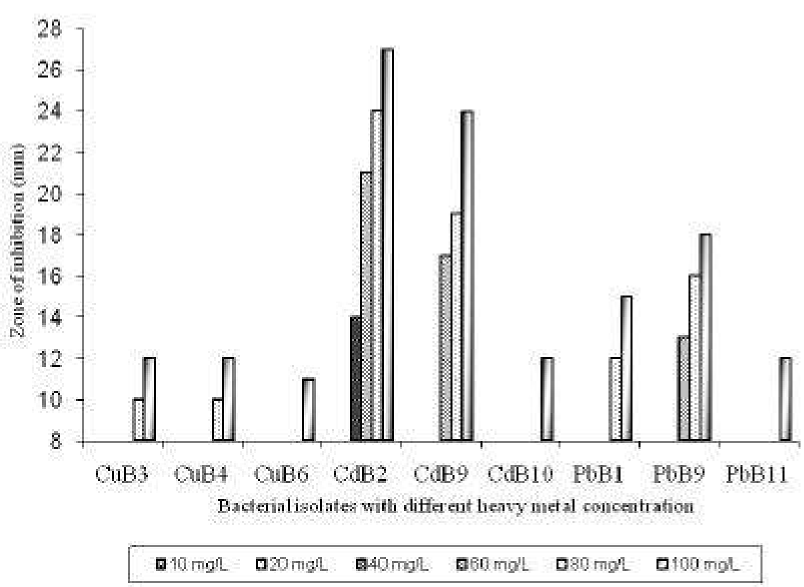


Fig. 1: Growth of bacteria in different concentrations of heavy metals by plate diffusion method

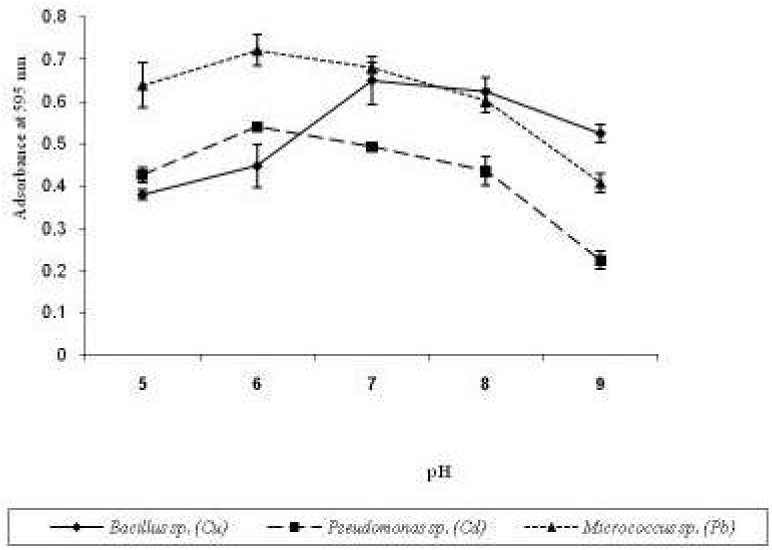


Fig. 2: Growth of bacterial isolates in response to various pH

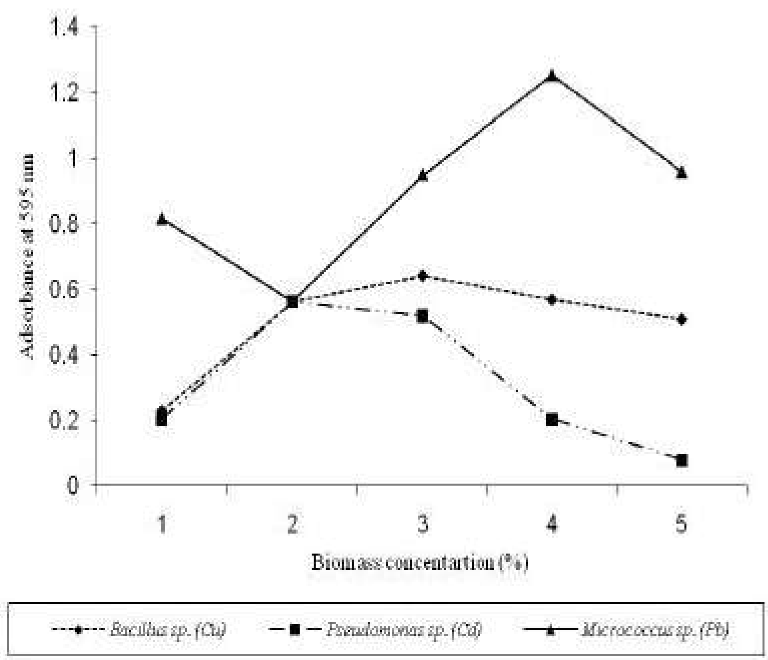


Fig. 3: Growth of bacterial isolates in response to various biomass concentrations

similar to the results of Wang and Chen [17] and Blackwell *et al.* [18]. 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 (Fig. 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* [9]. From this study, it indicates that 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 [19]. This result indicates a ‘screen

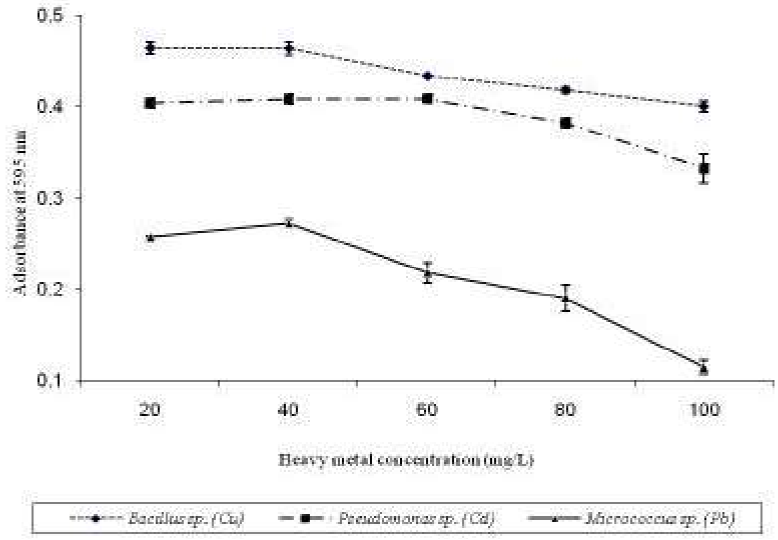


Fig. 4: Growth of bacterial isolates in response to various heavy metal concentrations

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 biosorbents concentration), as this condition ensures optimal electrostatic interaction between cells with a significant factor of biosorption [20].

The maximum growth of *Bacillus* sp. in the presence of Cu attained at 20 mg/L and it gradually decreases up to 100 mg/L (Fig. 4). This result was similar to *Klebsiella aerogenes* in which the adsorption of both metals gradually increased at initial concentration; moderately high adsorption yield was obtained. *Pseudomonas* sp. showed the maximum growth in the presence of Cd at 60 mg/L. However, this organism has the capacity to withstand up to 60 mg/L. This result was similar to maximum adsorption of Cd (II) ion at 60 mg/L on the dried cells of *Zoogloea ramigera* reported by Norberg [21]. Maximum growth of *Micrococcus* sp. in the presence of Pb was observed at 40 mg/L. Similar report performed by *Neurospora crassa* during studies on the Pb biosorption from its synthetic aqueous solutions [10]. It was explained at low metal concentration the biosorption capacity of the biosorbents is not fully utilized [22]. Several previous studies were confirmed in the case of *Trametes vesicolor* for Cu, Pb and Zn removal [23], *Bacillus* sp. for chromium removal [24] and *Bacillus firmus* also having less ability to remove Pb, Cu and Zn [25]. All these results clearly reveal the existence of infinite heavy metal reduction capacity possibly due to heavy metal toxicity towards the cells.

**Biosorption of Heavy Metal by Dried Biomass:** The biosorption is basically at lab scale inspite of its development for decades [17]. The mechanisms involved in biosorption are metal-microbe interactions should be further studied with great efforts by utilizing various techniques and the combination of them [2]. In the present study, the dried biomass 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 (Fig. 5). It was proved that *Bacillus* sp. can grow in significant levels of heavy metal media and have high capacity for heavy metal adsorption [26]. *Bacillus* sp. has the ability to adsorb the Cu at a maximum level of 400 mg/L. *Bacillus* sp. has 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 the maximum adsorption of heavy metals that reached up to 88% by *Pseudomonas* sp. [27] 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 [28].

**Biosorption of Heavy Metal by Immobilized Beads:** The biosorption capacity of immobilized beads of bacterial isolates like *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. adsorbed Cu (69.34%), Cd (90.41%) and Pb (84.27%) respectively (Fig. 5). The paired samples

Table 3: Paired Samples Test

| | | Paired Differences | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 95% Confidence Interval of the Difference | | | | |
| | | Mean | Std. Deviation | Std. Error Mean | Lower | Upper | t | df | Sig. (2-tailed) |
| Pair 1(Bacillus sp.) | Immobilize beads-dried biomass | 24.6067 | 1.66377 | 0.96058 | 20.4736 | 28.7397 | 25.617 | 2 | 0.002 |
| Pair 2(Pseudomonas sp.) | Immobilize beads-dried biomass | 3.7500 | 0.99292 | 0.57327 | 1.2834 | 6.2166 | 6.541 | 2 | 0.023 |
| Pair 3(Micrococcus sp.) | Immobilize beads-dried biomass | 5.0500 | 0.54249 | 0.31321 | 3.7024 | 6.3976 | 16.123 | 2 | 0.004 |

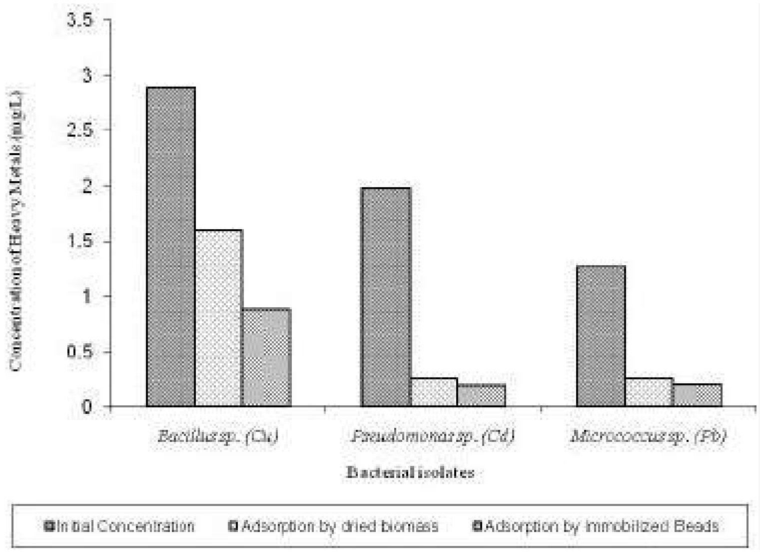


Fig. 5: Heavy metal biosorption by bacterial isolates

test shows a significant difference between the immobilized beads and dried biomass used for the biosorption process (Table 3).

All these results reveal that the adsorption capacity of the immobilized microbial isolates was greater than that of dried biomass by.002,.023 and.004 in *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. respectively. It was reported because of the dried biomass that consists small particles with low density, poor mechanical strength and little rigidity [29] immobilized beads has greater adsorption capacity than the dried biomass. 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 [30].

## CONCLUSIONS

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

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