



# Biosorption of Heavy Metals in Dumpsite Leachate by Metal-resistant Bacteria Isolated from Abule-egba Dumpsite, Lagos State, Nigeria

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## Authors' contributions

This work was carried out in collaboration between both authors. Authors HAS and AOA designed the study, performed the statistical analysis and wrote the protocol. Author AOA wrote the first draft of the manuscript and managed literature searches. Authors HAS and AOA managed the analyses of the study. Both authors read and approved the final manuscript.

## Article Information

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## ABSTRACT

**Aim:** We studied the potentials of four metal-resistant bacterial strains to reduce the concentration of selected metals in dumpsite leachate.

**Methods:** The bacteria which were obtained from the culture collection of the Environmental Microbiology and Biotechnology laboratory were subjected to increasing concentration of metals in a metal-supplemented medium. The bacteria were then introduced into a batch culture biosorption set-up containing the culture medium and tyndallized leachate and the residual metal concentration was determined after a 14-day incubation period using the atomic absorption spectrophotometer (AAS), after the bacterial cells had been separated from the leachate by centrifugation at 10000 rpm for 15 min.

**Results:** The bacterial strains demonstrated high resistance to the four selected heavy metals [lead (Pb), chromium (Cr), cadmium (Cd) and nickel (Ni)] and their combination. The Minimum Inhibitory Concentration (MIC) value for the strains on the metal-incorporated medium for all the selected

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metals ranged from 700-1500 µg/ml. The resistance to the metals was in the order: Pb > Ni > Cr > Cd. *Pseudomonas aeruginosa* had the highest MIC to the metal combination (1300 µg/ml) while the lowest was *Proteus mirabilis* (800 µg/ml); *Paenalcigenes faecalis* and *Bordetella petrii* had MIC values of 1000 µg/ml and 1200 µg/ml respectively. The biosorption set up showed that *Paenalcigenes hominis* had a higher percentage reduction for Pb in the leachate with a reduction of 35.77%, while *Bordetella petrii* removed the highest concentration of Cd and Ni in the leachate with values of 32.81% and 34.91% respectively. However the highest percentage reduction for Cr (32.54%) was observed when the leachate was treated with a consortium of the four bacterial strains.

**Conclusion:** This study revealed that these metal-resistant bacteria could be very useful in the biological treatment of metal-containing wastewater.

**Keywords:** Biosorption of metals; dumpsite leachate; minimum inhibitory concentration (MIC); metal combination.

## 1. INTRODUCTION

Environmental pollution with toxic heavy metals is increasing worldwide as a result of rapid industrialization in the fields of metallurgy, agriculture, mining, petro-chemicals, electroplating, among others. Heavy metal pollution of soil and aquatic ecosystems is a significant environmental challenge [1,2]. Heavy metals are elements with specific gravity usually greater than 5.0 g/cm<sup>3</sup>, which is five times the density of water. Of the ninety naturally occurring elements, fifty-three (including arsenic) are classified as heavy metals. Heavy metals are transition elements which are characterized by the possession of incompletely filled d-orbitals, which provides them with the ability to form complexes. Thus, metal cations are vital as trace elements in sophisticated biochemical reactions [3].

Metals play an integral role in the life processes of living organisms. Some metals serve as microelements, components of various enzymes; and are essential in many redox-processes; while many others have no known biological roles and are non-essential. Virtually, all metals whether essential or non-essential can exhibit toxicity above a certain threshold, which for highly toxic metal species may be extremely low [4].

Various techniques have been employed for the treatment of metal-bearing wastewater, and they include adsorption, precipitation and electrochemical technologies; but these techniques are expensive, not environment-friendly and usually dependent on the concentration of the waste especially in much diluted solutions. Therefore, the search for effective, cost effective and environmentally-friendly remedies for wastewater treatment has

been initiated. It was only in the 1990s that a new scientific area was developed to recover heavy metals and it was bioremediation, which is the use of biological agents; microorganisms (fungi, bacteria, algae), green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Heavy metal bioremediation involves the removal of heavy metals from wastewater and soil through metabolically-mediated or physico-chemical pathways. Microorganisms have proved to have great potentials in metal removal from wastewaters [5].

The process of biosorption is mainly used to treat wastewater where more than one type of metal ions are present; especially in cases where the removal of one metal ion is not influenced by the presence of other metal ions. For example: the uptake of uranium by biomass of bacteria, fungi and yeasts was unaffected by the presence of other metals in the same solution as reported by Petrisor et al. [6]. The advantages of biological technologies for the removal of toxic pollutants are that they can be carried out in situ at the contaminated site, they are cost effective and usually environmentally benign (no secondary pollution). They have also been demonstrated to possess good potentials in replacing conventional methods for the removal of metal contaminants [7,8]. Therefore, this study is aimed at employing bacterial species in the removal/reduction of selected metals in leachate samples obtained from a refuse dumpsite in Lagos state, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Bacterial Strains Used for the Study

The bacterial strains used for this study: *Proteus mirabilis* (GenBank Accession Number

ATCC 29906), *Pseudomonas aeruginosa* (GenBank Accession Number LMG 1242), *Paenacaligenes hominis* (GenBank Accession Number CCUG 53761A), *Bordetella pertussis* (GenBank Accession Number DSM 12804) were obtained from the microbial culture collection of the Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Nigeria. They were isolated from solid waste dumpsites in Lagos, Nigeria and have previously been reported by Sanuth et al. [9].

## 2.2 Sample Collection

Leachate samples were collected from a solid waste disposal dumpsite at Abule-Egba, Lagos, Nigeria. The samples were collected in pre-sterilized bottles and transported in ice packs to the Environmental Microbiology and Biotechnology laboratory, Department of Microbiology, University of Ibadan, Nigeria. The samples were kept under refrigeration condition prior to their use.

## 2.3 Metal Resistance Assay

The resistance of each bacterial strain to metal ions was determined as the minimum inhibitory concentration (MIC) on nutrient agar (Oxoid, UK) plates supplemented with metals as described by Aleem et al. [10]. Graded concentrations of membrane filter-sterilized soluble salts of the respective heavy metals ( $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{Pb}(\text{CH}_3\text{COO})_2$  and  $\text{CdCl}_2$ ) was incorporated into the medium; with 100  $\mu\text{g/ml}$  as the starting concentration for each metal. The concentration was thereafter increased by 50  $\mu\text{g/ml}$  at a time. The plates were incubated at 35°C for 72 hours and observed for growth. The culture growing on one concentration was transferred to a higher concentration and the MIC was noted when the bacteria failed to grow on the metal-incorporated medium.

## 2.4 Sample Preparation

The dumpsite leachate was filtered using Whatman filter paper No 4 and tyndallized in a water bath to eliminate the initial microbial flora present. The tyndallized leachate was cultured on both Nutrient agar and Potato Dextrose Agar to ascertain the sterility of the leachate, and no growth was observed.

## 2.5 Metal Biosorption Setup

The metal biosorption set up was done in 150 ml Erlenmeyer flasks containing 100 ml of the

tyndallized leachate samples each and into which nutrient medium has been incorporated to support the growth of the organisms according to the modified method of Murthy et al. [11]. The set up was inoculated with bacterial cells (wet weight) separated from nutrient broth by centrifugation at 10000 rpm for 10 min. The individual strains were inoculated with  $10 \pm 1$  mg cells, while the consortium was combined in the ratio 1:1:1:1, making up 10 mg of cells. The set up was subsequently incubated inside a rotary shaker (G24 Environmental Incubator shaker) at 35°C for 14 days at a revolution of 200 rpm. The experiment was conducted in triplicates. After the experimental duration, the set up was centrifuged at 10000 rpm for 15 min at 4°C to remove the bacterial cells and this was done using a Hitachi High Speed Refrigerated Centrifuge (HIMAC CR21GII).

### 2.5.1 Determination of the metal composition of the leachate

The metal composition in the treated and control leachate samples was determined using an Atomic Absorption Spectrometer (Perkin Elmer AAnalyst 200 Atomic Absorption Spectrometer), after the sample has been digested using concentrated acids (HCl and  $\text{HNO}_3$  in the ratio 3:1). Standards of chromium, cadmium, lead and nickel solutions, e.g., 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l were made from 1000 mg/l stock solutions analytes of the respective heavy metals. The set of standard solutions were analyzed using the Atomic Absorption Spectrophotometer (AAS) (UNICAM 929, London Atomic Absorption Spectrophotometer powered by SOLAAR software) for calibration. The detection limit of the metals in the samples was 0.0001 mg/l. The cathode lamp of each metal was used for the analysis of the respective mineral oils in the standards and the metal concentration in the sample filtrates. Gas mixtures were used to generate the flame [12].

## 2.6 Metal Reduction in the Leachate

The ability of the bacterial strains to reduce the metal composition of the dumpsite leachate was calculated as shown below:

$$\text{Metal reduction (\%)} = [(F_0 - F_1) / F_0] \times 100$$

where:

$F_0$ : Metal concentration in the control sample

$F_1$ : Metal concentration after treatment

## 2.7 Statistical Analysis

The analysis of the metal concentration was done using the SPSS version 20, while the Duncan Multiple Range Test (DMRT) was used for the mean separation.

## 3. RESULTS AND DISCUSSION

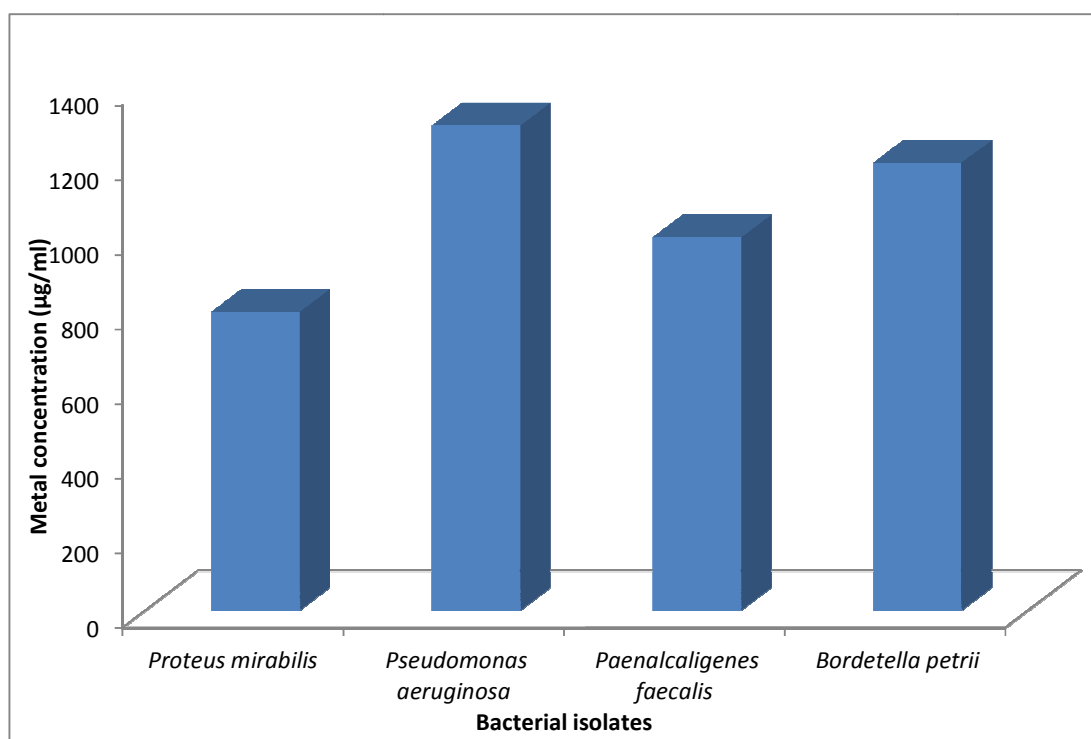
### 3.1 Heavy Metal Resistance

The bacteria demonstrated the ability to tolerate the four selected metals used for this study. The MIC of all the isolates on  $Pb^{2+}$  amended medium was considerably higher than the values for the other metals, and these values are slightly higher than the MIC value (1100  $\mu\text{g/ml}$ ) for  $Pb^{2+}$  by *Acinetobacter calcoaceticus* and *Kocuria varians* isolated from spent oil contaminated site and reported by Oriomah et al. [13]. The MIC values for  $Pb^{2+}$  by the strains used in this study ranged from 1200-1500  $\mu\text{g/ml}$ . The MIC for Cr ranged from 800-1000  $\mu\text{g/ml}$ , and this is higher than the MIC for  $Cr^{2+}$  (490  $\mu\text{g/ml}$ ) for some bacterial strains isolated from sewage as reported by Narasimhulu et al. [14]. The MIC values for the

bacteria for  $Ni^{2+}$  and  $Cd^{2+}$  on the amended medium in this study ranged from 900-1200  $\mu\text{g/ml}$  and 700-900  $\mu\text{g/ml}$  for the two metals respectively (Table 1). The metal resistance pattern of the bacterial isolates on the metal combination is shown in Fig. 1, the MIC ranged from 800-1300  $\mu\text{g/ml}$  with *Pseudomonas aeruginosa* having the highest MIC value (1300  $\mu\text{g/ml}$ ) while the lowest MIC was observed for *Proteus mirabilis* (800  $\mu\text{g/ml}$ ). *Paenacaligenes faecalis* and *Bordetella petrii* had MIC values of 1000  $\mu\text{g/ml}$  and 1200  $\mu\text{g/ml}$  on the metal combination respectively.

### 3.2 Concentration of Metals in the Leachate

The concentration of the selected metals in the treated and control dumpsite leachate is shown in Table 2. The Pb concentration in the uninoculated leachate which served as the control was 60.00 mg/l; Cr, 40.53 mg/l; Ni, 32.20 mg/l while that of Cd was 30.20 mg/l. These values are considerably higher than values reported by other authors who have worked on the biosorption of metals in liquid and solid



**Fig. 1. Minimum inhibitory concentration of the bacteria on the combination of the four heavy metals used in this study**

Note: The metal combination contains a mixture of the four metals in equal concentration (1:1:1:1)

**Table 1. Minimum inhibitory concentration (MIC) of the bacteria on the heavy-metal amended medium in comparison with other studies ( $\mu\text{g/ml}$ )**

Bacterial isolates	Lead	Chromium	Nickel	Cadmium	References
<i>Proteus mirabilis</i> ATCC 29906	1200	900	1000	700	Present study
<i>Pseudomonas aeruginosa</i> LMG 1242	1500	1000	1200	900	Present study
<i>Paenaltcaligenes hominis</i> CCUG 53761A	1300	800	900	800	Present study
<i>Bordetella petrii</i> DSM 12804	1400	1000	1100	900	Present study
<i>Acinetobacter calcoaceticus</i>	1100	-	-	-	Oriomah et al. [13]
<i>Kocuria varians</i>	1100	-	-	-	Oriomah et al. [13]
Unidentified strain 1	-	490	-	-	Narasimhulu et al. [14]
Unidentified strain 7	-	-	190	-	Narasimhulu et al. [14]
Unidentified strain 5	-	-	-	310	Narasimhulu et al. [14]

**Table 2. Concentration of metals in the treated and control (un-inoculated) dumpsite leachate (mg/l)**

Bacterial strain	Pb	Cr	Ni	Cd
<i>Proteus mirabilis</i> ATCC 29906	40.35 <sup>cd</sup>	28.30 <sup>b</sup>	24.63 <sup>b</sup>	21.84 <sup>b</sup>
<i>Pseudomonas aeruginosa</i> LMG 1242	42.59 <sup>c</sup>	28.09 <sup>b</sup>	21.43 <sup>e</sup>	21.51 <sup>c</sup>
<i>Paenaltcaligenes hominis</i> CCUG 53761A	38.54 <sup>d</sup>	25.58 <sup>b</sup>	22.41 <sup>d</sup>	21.20 <sup>cd</sup>
<i>Bordetella petrii</i> DSM 12804	41.31 <sup>c</sup>	29.49 <sup>b</sup>	20.96 <sup>f</sup>	20.29 <sup>e</sup>
Consortium	48.58 <sup>b</sup>	27.34 <sup>b</sup>	23.00 <sup>c</sup>	21.13 <sup>d</sup>
Control (Uninoculated) leachate	60.00 <sup>a</sup>	40.53 <sup>a</sup>	32.20 <sup>a</sup>	30.20 <sup>a</sup>

Note: Means with the same alphabet on the same column are not significantly different ( $p \leq 0.05$ ) from one another. (Each value is a mean of three replicates)

wastes; Notable among them was Kumar et al. [15] who reported values range of 0.010-0.865 mg/l of Pb in wastes collected from landfills and wastewater from selected locations in India. They reported a range of 0.032-7.60 mg/l for Ni, 0.018-8.56 mg/l for Cr while the Cd concentration was 0.001-0.523 mg/l. This suggests that the leachate employed in this study is heavily laden with metal, which is of serious environmental and public health concern, as this can easily permeate into underground water table thus causing contamination of aquifers. For all the metals analysed, the values in the control, were significantly higher at  $p \leq 0.05$  than for all the treated samples, which suggests various degree of metal reduction by the bacterial strains employed in this study.

### 3.3 Metal Removal by the Bacterial Strains

The bacteria were able to reduce the concentration of metals present in the leachate at different rate. The highest percentage reduction in the Pb concentration was 35.77% by *Paenaltcaligenes hominis* followed by *Proteus*

*mirabilis* at a value of 32.75%. These values were higher compared to 37.40% and 30.08% Pb uptake obtained by Petrisor et al. [6], using waste biomass of *Aspergillus niger* and *Penicillium* sp. respectively in the pre-screening of biosorbents for biosorption of metals in leachate samples generated from two Romanian mine waste disposal sites. This is not however in accordance with a study carried out by Kumar et al. [15], who reported a Pb reduction of 93% by heavy metal acclimated *Staphylococcus* sp. isolated from soil and sludge and 100% Pb removal by *Staphylococcus saprophyticus* from a study carried out by Ilhan et al. [16]. Worthy of note is the lowered percentage reduction in the concentration of Pb when the dumpsite leachate was treated with the consortium of all the bacterial strains (Fig. 2). This might be due to competition for nutrient and antagonistic activities among the strains when combined.

The consortium containing all the bacteria employed in this study showed a capacity of reducing Cr from the leachate sample used in this study (Fig. 3). A reduction of 32.54% was recorded by the consortium for Cr; this value is

comparatively higher than the 7.01% and 6.68% Cr reduction in Bauxite from a mining site leachate by *Thiobacillus* sp. and *Pseudomonas* sp. respectively [17]. The highest percentage Cr reduction in this study was by *Paenalcaligenes hominis* (36.89%), although the value in this study is comparatively lower than the percentage

reduction of Cr in magnesite reported by the same authors for *Pseudomonas* sp. (40.01%).

The highest percentage reduction for Ni was 34.91% by *Bordetella petrii* (Fig. 4), and this value was higher than the value recorded by Petrisor et al. [6], who reported a reduction of

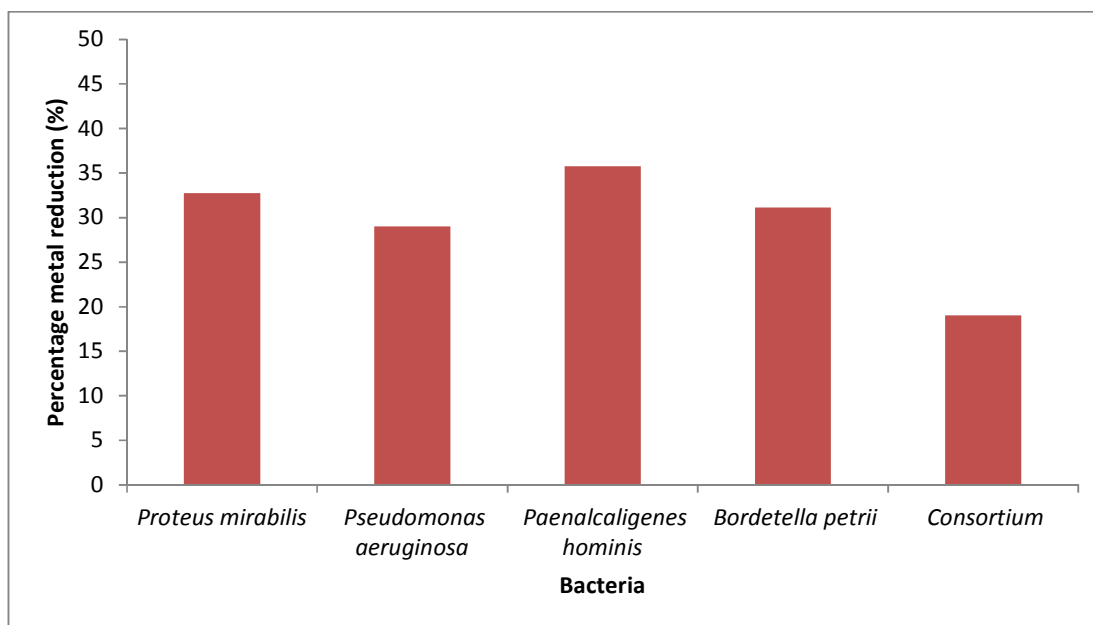


Fig. 2. Percentage reduction in the concentration of Lead after treatment with the bacteria

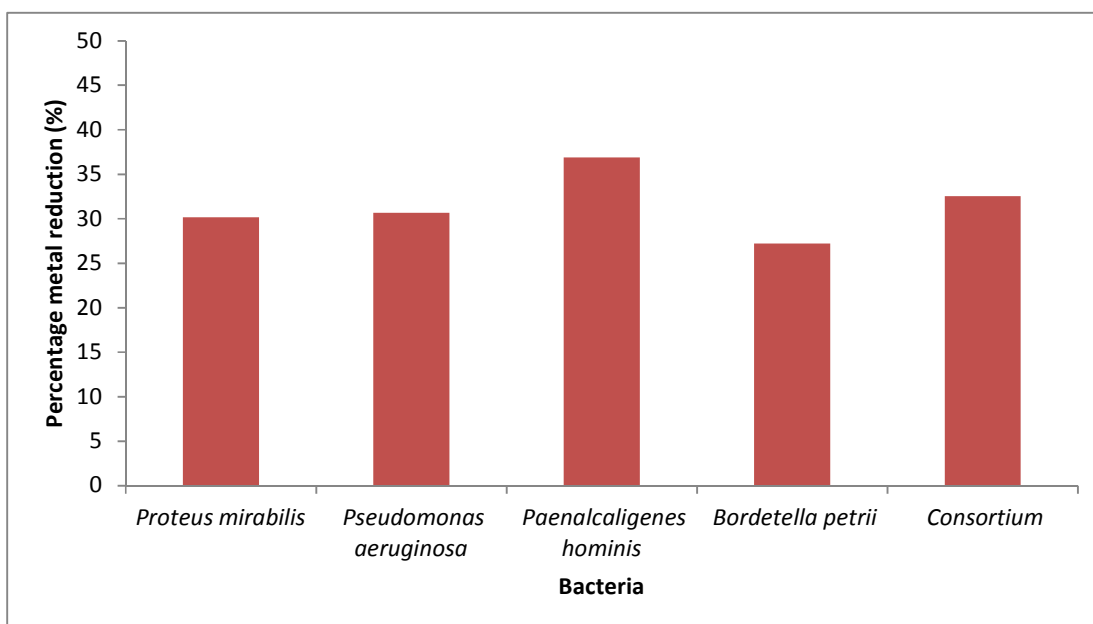
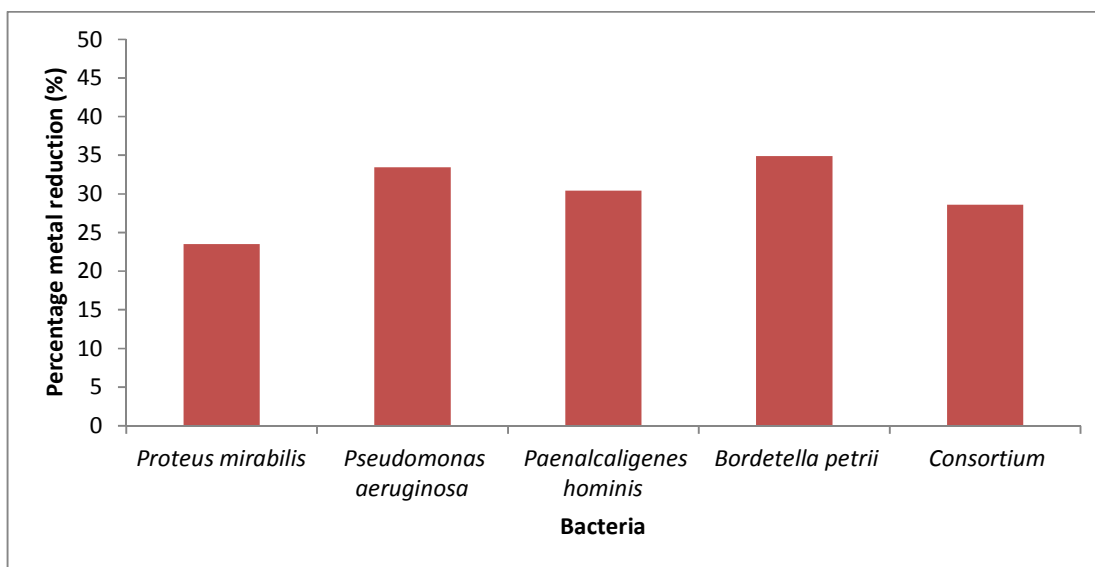
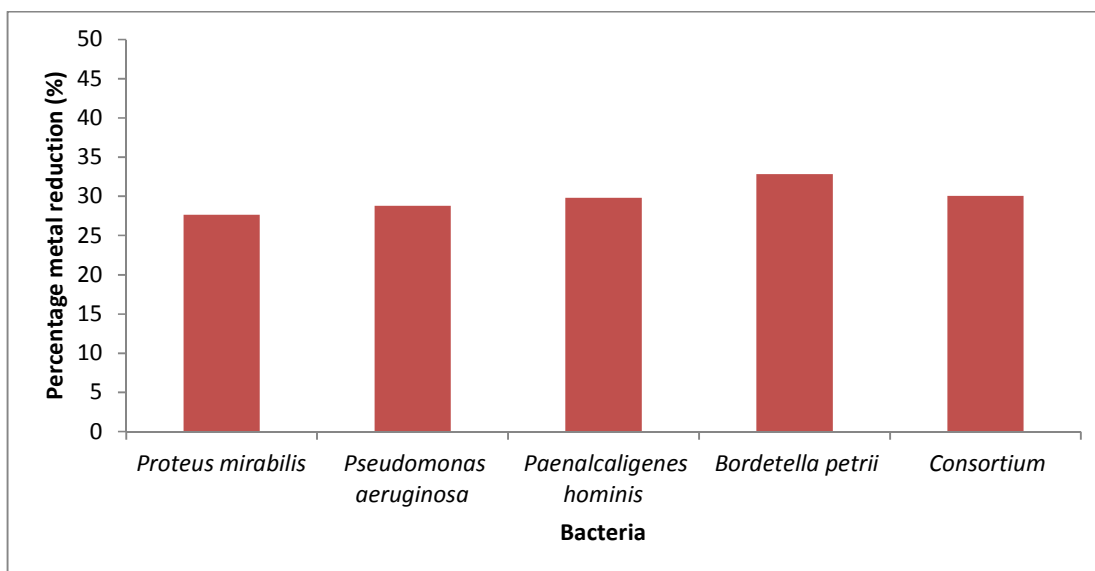


Fig. 3. Percentage reduction in the concentration of Chromium after treatment with the bacteria



**Fig. 4. Percentage reduction in the concentration of Nickel after treatment with the bacteria**



**Fig. 5. Percentage reduction in the concentration of Cadmium after treatment with the bacteria**

8.2% and 3.0% for a mine leachate sample obtained from Rosia Poieni; Although it should be stressed that their study was carried out using immobilized bacterial cells consisting of a mixture of heterotrophic and acidophilic microorganisms. The percentage reduction of Ni in the leachate was not enhanced when the consortium of all the bacterial isolates was utilised in this biosorption study.

The lowest Cd removal was for the leachate treated with *Proteus mirabilis* (27.68%), while the highest reduction was recorded with *Bordetella*

*petrii* (32.81%) as shown in Fig. 5. The reduction rate from this study was lower compared to the report of a study carried out by Kumar et al. [15], who reported a Cd reduction of 50% in a liquid waste by *Aspergillus niger*, a fungus isolated from the soil environment.

#### 4. CONCLUSION

The bacterial strains employed in this present study possess the ability to remove metals from the leachate generated from the refuse disposal site. Hence will be useful candidates for the

biological clean-up of environments contaminated with toxic heavy metals. However further studies need to be carried out on the genetics of heavy metal resistance by these bacterial strains.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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