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Utilization of metal-resistant bacteria in the detoxification of contaminated dumpsites

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Abstract

The potentials of four metal-resistant bacterial strains to reduce the concentration of selected metals in dumpsite leachate were studied. The Environmental Microbiology and Biotechnology laboratory's culture collection of bacteria was used to extract the bacteria, which were then exposed to progressively higher metal concentrations in a medium enriched with metals. After the bacterial cells had been separated from the leachate by centrifugation at 10000 rpm for 15 min, the bacteria were then added to a batch culture biosorption set-up containing the culture medium and tyndallized leachate. The residual metal concentration was then determined after a 14-day incubation period using the atomic absorption spectrophotometer (AAS). 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 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); *Paenalcaligenes faecalis* and *Bordetella petrii* had MIC values of 1000 µg/ml and 1200 µg/ml respectively. *Bordetella petrii* removed the highest concentration of Cd and Ni from the leachate, with values of 32.81 percent and 34.91 percent, respectively, according to the biosorption setup, while *Paenalcaligenes hominis* had a higher percentage reduction for Pb in the leachate with a reduction of 35.77 percent. However, when the leachate was treated with a combination of the four bacterial strains, the largest percentage decrease for Cr (32.54 percent) was seen.

According to this research, these metal-resistant bacteria might be highly helpful in the biological treatment of wastewater that contains metals.

Keywords: Dumpsite leachate; Atomic absorption spectrophotometer (AAS).; Minimum Inhibitory Concentration (MIC); Biosorption; Strains; Metal-resistant

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. Heavy metals are elements with specific gravity usually greater than 5.0 g/cm^3, 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 [1].

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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 [2].

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 [3].

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. 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 [4]. 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 Onitsha, Anambra state.

2. Materials and methods

2.1. The study area

The study region is Onitsha, a town in Anambra state, southward eastward Nigeria. It is situated around within latitudes 6° 05' 39"N to 6° 12' 13"N of the equator and longitude 6° 45' 29"E to 6° 50' 07"E of the Greenwich Meridian. The study region (Onitsha Urban Area) overspread approximately 6366.851 sq km. It is located on the eastward bank of the river Niger.

2.2. Sample Collection

Leachate samples were collected from a solid waste disposal dumpsite at Onitsha, Anambra, Nigeria. The sampling area is characterized by wet and dry seasons with high annual rainfall in the range of 350 – 400mm, mangrove vegetation and run-off estimated to reach 90%. The waste dumpsite is managed by Anambra State Waste Manangement Agency (ASWAMA) and receives wastes on daily basis. Most of these disposed wastes are mainly domestic and market wastes. At the time of sampling, the waste dumpsite covered an area of about 3,265m^2 and is less than 500m away from a small stream that serves as source of domestic and irrigation water for many households within the study site.

The samples were collected in presterilized bottles and transported in ice packs to the Microbiology laboratory, Department of Microbiology, Anambra state University, Nigeria. The samples were kept under refrigeration condition prior to their use. Were obtained from the microbial culture collection of the Microbiology Laboratory, Nigeria. They were isolated from solid waste dumpsites in Onitsha, Nigeria.

2.3. Enumeration of Bacteria

Serially diluted samples were planted on Nutrient agar (NA) for the enumeration of aerobic heterotrophic bacteria. The colonies were counted and pure cultures were obtained by repeated sub-culturing and maintained on agar slants for further characterization and identification.

2.3.1. Purification of isolates

Bacterial colonies were picked at random and sub-cultured repeatedly into nutrient agar for purification. Purified isolates were stocked in nutrient agar slants for further studies.

2.3.2. Characterization and Identification of isolates

The bacteria isolates were characterized by colonial morphology and biochemical tests. These were compared with known taxa to identify the isolates. The biochemical tests are described below:

Gram staining

A clean and grease free slide was gotten and a drop of normal saline was place at the edge of the slide. A wire loop was then flamed and of the bacteria colony was picked and mixed homogeneously with normal saline. It was allowed to air dry and then it was heat fixed using a Bunsen. Crystal violet was flooded on the slide (primary stain) and allowed to stain for 1 minute. It was then placed with lugols iodine for 1 minute (this act as mordant). The iodine was then decolorized with absolute alcohol then washed with water until no more violet runs from slide.

The smear was then counter stain with safaranine (secondary stain) and allowed to dry and it was then observed under the microscope using x 100 oil immersion objective.

Catalase test

Using a glass slides placed flat on the surface of the table, a drop of 3% of hydrogen peroxide was placed on the center of the slide the using a sterile inoculating loop, a portion of the culture was transferred onto the drop of hydrogen peroxide. The immediate evolution of gas (oxygen) bubbles indicates a positive reaction although some cultures are slow catalase producers and had to be observed for few minutes before the positive result was obtained, while negative catalase producers did not liberate oxygen bubbles.

Oxidase test

1% solution of aqueous tetramethyl-p-phenylene Diamine hydrochloride. (the regent used) this test is particularly useful for differentiating pseudomonas from certain other enteric or gram negative bacteria. On a nutrient agar plate containing 24 hours culture streaking from a nutrient agar slant, a few drops the reagents was placed on the line of streaking of each culture. Oxidase positive colonies developed a pink colour which progressively became purple within 30 seconds, while oxidase negative colonies did not produce this purple colouration.

Citrate utilization

This test is used for differentiation of coliform bacteria by their ability to utilize citrate as sole of carbon. Using simmons citrate agar (oxoid), stab in test tube, the colony was stabbed into the agar and incubated at 370c for 24 hour. Colour change of the citrate agar indicator from green to blue, indicate positive citrate utilization while negative had no colour change.

Urease activity

The urea agar (oxoid) slant in tubes were inoculated with the pure cultures from the nutrient agar slants and incubated at 370c from 24 hours. A change in the colour of the urea agar form yellow to red, which was due to the release of urea, hydrolyzing urea to release ammonia that resulted in increased pH indicate a positive urease activity.

Triple sugar lron (T.S.I) test

The TSI agar (oxoid) slant in tubes were inoculated with pure culture from the nutrient agar slants and incubated at 370c for 24 hours. A change in the colour of the slant from red to yellow indicated utilization of glucose recored as A, while an absence of colour change was recorded as K. A colour change in the butt from red to yellow, indicated utilization of lactose and/or sucrose recorded as a, while absence of colour change was recorded as K. therefore, utilization of all sugars was recorded as A/A and the lack of utilization of any sugar was recorded as K/K. But, utilization of one of the sugar was recorded as K/A or A/K depending on which sugar was utilized, with result for glucose utilization as the numerator and the lactose or sucrose as denominator.

2.3.3. Molecular Characterisation

Molecular characterization was performed to identify the bacterial strains involved. This was done at Microbiology Laboratory, Nnamdi Azikiwe University, Awka.

2.4. 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*.,(2014), in which Graded concentrations of membrane filter-sterilized soluble salts of the respective heavy metals (NiSO4. 6H2O, K2Cr2O7, Pb(CH3COO)2 and CdCl2) was incorporated into the medium; with 100 µg/ml as the starting concentration for each metal. The concentration was thereafter increased by 50 µg/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.

2.5. 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.

2.6. Metal Bio sorption 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*., (2012). 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±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 at 35°C for 14 days. 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).

2.6.1. 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 HNO3 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 (hseu, 2004).

2.7. 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:

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

Where;
F_0: Metal concentration in the control sample
F_1: Metal concentration after treatment

2.8. 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

3.1. Morphological and molecular characterization of isolated bacteria

Four bacteria were isolated from *the leachate* and classified as Gram-positive and gram negative. The nucleotide sequences of the 16S rDNA enabled the identification at the species level of the strain, which were identified as *Proteus*

mirabilis (GenBank Accession Number ATCC 29906), *Pseudomonas aeruginosa* (GenBank Accession Number LMG 1242), *Paenalcaligenes hominis* (GenBank Accession Number CCUG 53761A), *and Bordetella petrii* (GenBank Accession Number DSM 12804)).

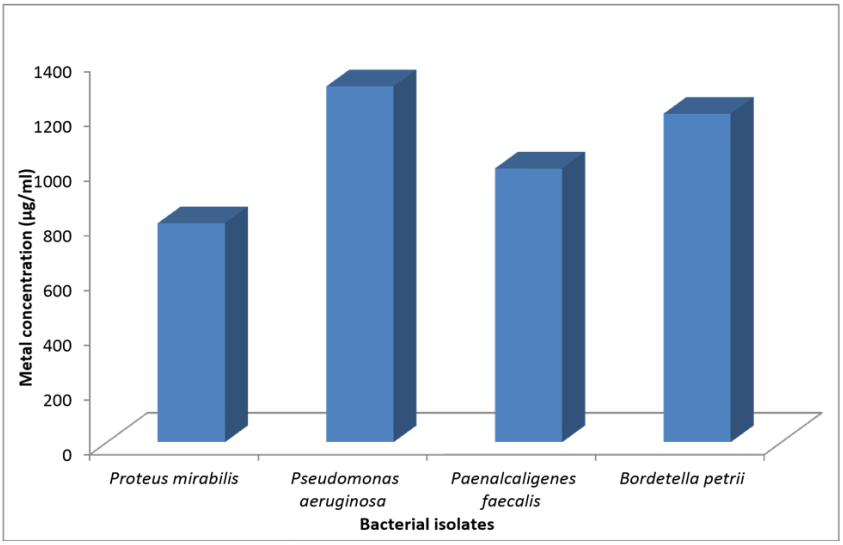

Figure 1 Minimum inhibitory concentration of the bacteria on the combination of the four heavy metals used in this study

Table 1 Minimum inhibitory concentration (MIC) of the bacteria on the heavy-metal amended medium in comparism with other studies (µg/ml)

Bacterial isolates	Lead	Chromium	Nickel	Cadmium	References
Proteus mirabilis	1200	900	1000	700	Present study
Pseudomonas aeruginosa	1500	1000	1200	900	Present study
Paenalcaligenes hominis	1300	800	900	800	Present study
Bordetella petrii	1400	1000	1100	900	Present study
Acinetobacter calcoaceticus	1100	-	-	-	[5]
Kocuria varians	1100	-	-	-	[5]
Unidentified strain 1	-	490	-	-	[6]
Unidentified strain 7	-	-	190	-	[6]
Unidentified strain 5	-	-	-	310	[6]

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

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

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)

Table 3 Permissible limits for FEPA, DPR and WHO

Trace Metals	FEPA µg/g	DPR µg/g	WHO µg/g
Cd	0.01	0.01	0.005
Cr	0.03	0.03	0.02
Pb	0.05	0.05	0.05
Ni	0.1	0.1	0.5

3.2. Metal Removal by the Bacterial Strains

The bacteria were able to reduce the concentration of metals present in the leachate at different rate

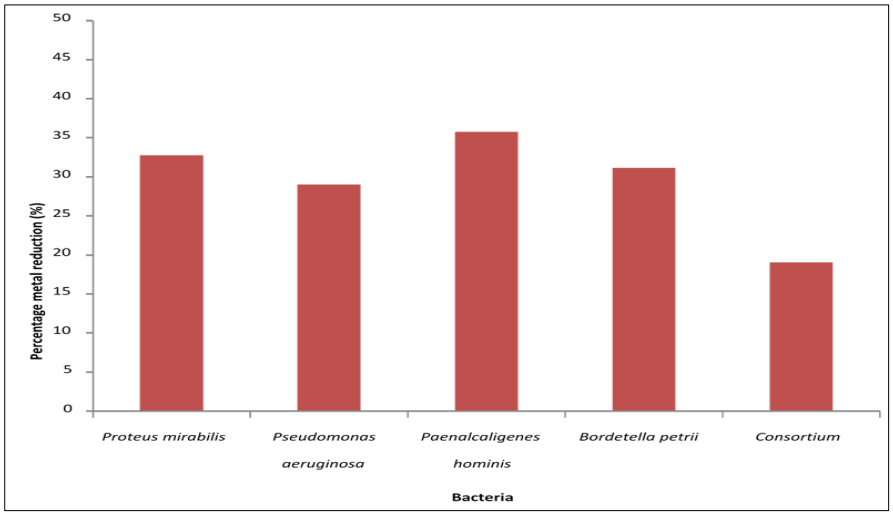

Figure 2 Percentage reduction in the concentration of Lead after treatment with the bacteria

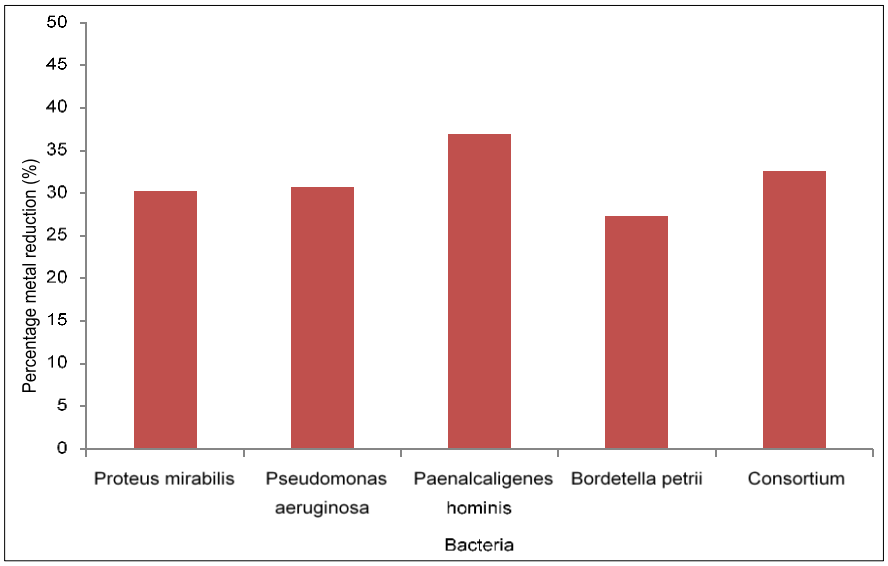

Figure 3 Percentage reduction in the concentration of Chromium after treatment with the bacteria

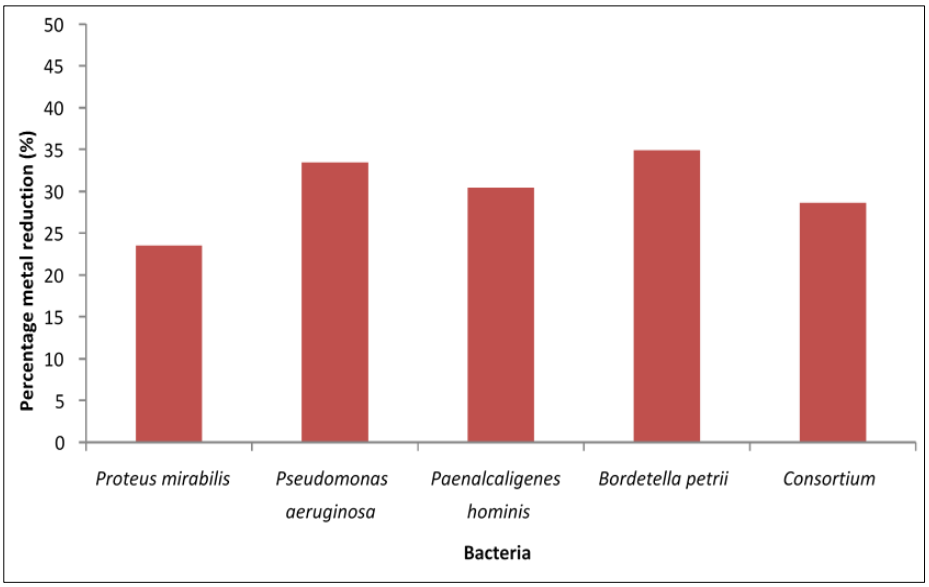

Figure 4 Percentage reduction in the concentration of Nickel after treatment with the bacteria

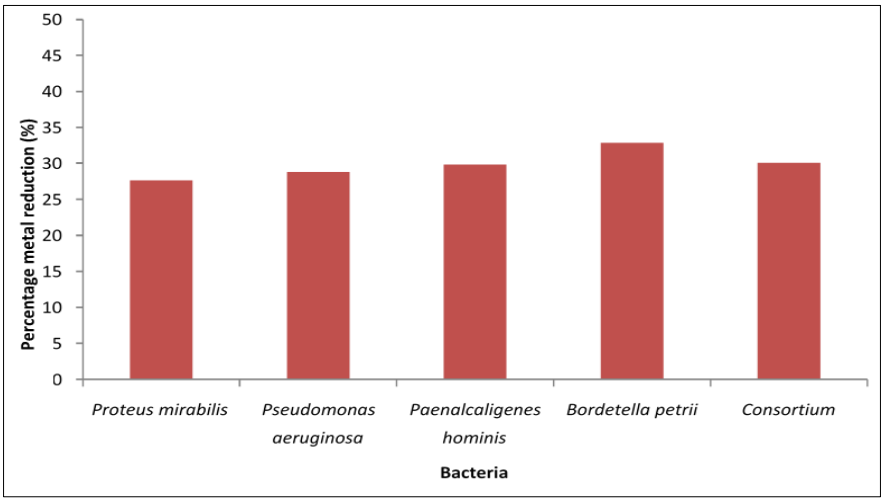

Figure 5 Percentage reduction in the concentration of Cadmium after treatment with the bacteria

4. Discussion

4.1. 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 Pb2+ amended medium was considerably higher than the values for the other metals, and these values are slightly higher than the MIC value (1100 µg/ml) for Pb2+ by *Acinetobacter calcoaceticus* and *Kocuria varians* isolated from spent oil contaminated site and reported by [6,7]. The MIC values for Pb^{2+} by the strains used in this study ranged from 1200-1500 µg/ml. The MIC for Cr ranged from 800-1000 µg/ml, and this is higher than the MIC for Cr^{2+} (490 µg/ml) for some bacterial strains isolated from sewage as reported by [5]. The MIC values for the bacteria for Ni2+ and Cd2+ on the amended medium in this study ranged from 900-1200 µg/ml and 700-900 µg/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 µg/ml with *Pseudomonas aeruginosa* having the highest MIC value (1300 µg/ml) while the lowest MIC was observed for Proteus mirabilis (800 µg/ml). *Bacillus thurengensis* and *Bordetella petrii* had MIC values of 1000 µg/ml and 1200 µg/ml on the metal combination respectively.

4.2. 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 wastes; Notable among them was [8], 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, 0.0188.56 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. This shows that the area around Onitsha and the discharged leachate have significant levels of heavy metal contamination and pose a risk to the ecosystem and human health.

In our analysis, the significant high concentration of heavy metals in Onitsha might be due to anthropogenic implication in the area. Cr, Pb and Ni indicated high level of concentration due to the maximum allowable effluent discharge limit from industrial channels and also to the limit set by FEPA, DPR and WHO.

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.

4.3. Metal Removal by the selected Bacteria

The highest percentage reduction in the Pb concentration was 35.77% by *Paenalcaligenes 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 [9], 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 [10,11], 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 [12]. 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. 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%).

It should be noted that [13], reported a reduction of 8.2 percent and 3.0 percent for a mine leachate sample obtained from Rosia Poieni. It should also be noted that their study was conducted using immobilised bacterial cells consisting of a mixture of heterotrophic and acidophilic microorganisms. The highest percentage reduction for Ni was 34.91 percent by Bordetella petrii (Fig. 4), and this value was higher than the value recorded by when the consortium of all the bacterial isolates was used in this biosorption investigation, the percentage decrease of Ni in the leachate was not improved.

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 [14, 15], who reported a Cd reduction of 50% in a liquid waste by *Aspergillus niger*, a fungus isolated from the soil environment.

5. Conclusion

This study indicates significantly high level of Heavy Metals (Cd, Cr, Pb and Ni) concentration in dumpsite leachate located at Onitsha, a town in Anambra state, southward eastward Nigeria as investigated. The results obtained showed that the leachate sample from the site have a high level of heavy metals concentration as compared to the permissible limits (Tables 2-3).

High persistence of metals in soil originating from indiscriminate discharge of wastewater indicates high possibilities of these toxins and high level in food chain. Long term toxicological and biochemical effects due to proliferation and passage through food chain. Therefore, waste management and treatment are recommended.

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.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares that there is no conflict of interests regarding the publication of this paper.

References

[1] Shun-hong, H., Bing, P., Zhi-hui, Y. Li-yuan, C., and Li-cheng, Z.Chromium accumulation, microorganism population and enzyme activities in soils around chromium- containing slag heap of steel alloy factory. Transactions of Nonferrous Metals Society of China. .2019;19: 241-248

[2] Mora, A.P., Calvo, J.J.O., Cabrera, F. and Madejon, E. Changes in enzyme activities and microbial biomass after ''in situ'' remediation of a heavy metal-contaminated soil. Applied Soil Ecology. 2018; 28: 125–137

[3] Umeaku, C. N. and Ozoh, C,N. Public health implication of ready-to drink soymilk yogurt sold in Onitsha urban Anambra state. Public health.2019; 3(8).

[4] Zorpas A.A., Vassilis, I. Loizidou, M. and Grigoropoulou H. Particle Size Effects on Uptake of Heavy Metals from Sewage Sludge Compost Using Natural Zeolite Clinoptilolite. Journal of Colloid and Interface Science. 2012; 250(9):1– 4

[5] Oriomah, C., Adelowo, O.O. and Adekanmbi, A.O. Bacteria from spent engine-oil contaminated soils possess dual tolerance to hydrocarbon and heavy metals, and degrade spent oil in the presence of copper, lead, zinc and combinations thereof. IOSR Journal of Environmental Science, Toxicology and Food Technology. 2015; 65(8):207-215.

[6] Narasimhulu, K., Rao, P.S. and Vino, A.V. Isolation and identification of bacterial strains and study of their resistance to heavy metals and antibiotics. Journal of Microbial Biochemistry and Technology. 2015; 2(2):074-076

[7] Mora, A.P., Calvo, J.J.O., Cabrera, F. and Madejon, E. Changes in enzyme activities and microbial biomass after ''in situ'' remediation of a heavy metal-contaminated soil. Applied Soil Ecology.2018; 28(5): 125–137

[8] Umeaku, C. N., Chris-Umeaku, C.L and Emmyegbe, I.O. Bacteriological and Physico- chemical Analysis of drinking water quality in Uli. International Journal of Recent Innovations in Academic Research.2019; 3(7): 77-88.

[9] Umeaku, C. N. Characterization of Rhizomicrobial isolates associated with crude oil pollution. African Journal of Education.2016;3(2):185.

[10] Petrisor, I.G., Komnitsas, K., Lazar, I., Voicu, A., Dobrota, S. and Stefanescu, M. Biosorption of heavy metals from leachates generated at mine waste disposal sites. European Journal of Mineral Products. 2018; 2(2):158-167.

[11] Umeaku, C. N., Orsla, E.L., Clarice, U.C., Emmanuel, E.S and Ijeoma, U.C.U. Biodegradation of crude oil-polluted Soil by Bacterial isolates from Nigeria. Frontiers in Environmental Microbiology.2019; 5(1):14.

[12] Umeaku, C. N. and Ozoh, C,N. Public health implication of ready-to drink soymilk yogurt sold in Onitsha urban Anambra state. Public health. 2014;3(8).

[13] Petrisor, I.G., Komnitsas, K., Lazar, I., Voicu, A., Dobrota, S. and Stefanescu, M. Biosorption of heavy metals from leachates generated at mine waste disposal sites. European Journal of Mineral Products. 2012; 2:158-167.