Original article

Assessment of heavy metal bioremediation potential of bacterial isolates from landfill soils

O. Oziegbe, A.O. Oluduro, E.J. Oziegb, E.F. Ahuekwe, S.J. Olorunsola

Department of Biological Sciences, Covenant University, Ota, Nigeria
Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria
Department of Geosciences, University of Lagos, Akoka, Nigeria

Abstract

Indiscriminate disposal of wastes on landfills has led to increase in heavy metal contamination in landfill soils. However, the ability of the indigenous microorganisms to remediate the polluted environment can be of great influence in reclamation of such soils. The objectives of this study were to assess the bioremediation potential of the screened indigenous bacteria and evaluate the effects of carbon source and pH in the enhancement of the bioremediation process. Bacterial isolates from landfill sites were screened for their capability to utilize heavy metal (Cd and Pb). Nutrient Agar was supplemented with five different concentrations of each metal (25 to 600 mg L⁻¹). Viable counts of the isolates were taken four times at two days interval. Pseudomonas aeruginosa, Klebsiella edwardsii and Enterobacter cloacae were selected based on their tolerance to heavy metal for remediation process. Peptone broth was also supplemented using different concentrations of heavy metals. The remediation process was assessed by monitoring the growth of biomass using UV spectrophotometer at 600 nm and the residual heavy metal was evaluated after 8 days of incubation using AAS. Pseudomonas aeruginosa exhibited the highest bioremediation potential among the bacterial isolates with 58.80 and 33.67 remediation percentage in 50 mg Cd L⁻¹ and 300 mg Pb L⁻¹. However, higher remediation percentage (79.87 and 92.41) was observed by Klebsiella edwardsii through addition of carbon source (5 g/L) and varying the pH (6) of the media in the heavy metal contaminated medium. The results of this study indicate that the effectiveness of the indigenous bacteria in remediation process can be enhanced through the addition of carbon source and increase pH for effective reclamation of contaminated soil.

Keywords:
Landfill soil
Heavy metal contamination
Cadmium
Lead
Indigenous bacteria
Soil reclamation

1. Introduction

Generally considered to be the most available economic means of waste disposal, landfills are estimated to constitute about 95% of solid waste generated worldwide (Scott et al., 2005). The World Bank reports of Hoornweg and Bhada-Tata, (2012) posted a cumulative 1,300 million tonnes per year (1.2 kg/capita/day) of wastes generated from urban centres of the world, which is expected to rise to 2,200 million tonnes per year by 2025. Within the sub-Saharan Africa, Nigeria’s waste generation is approximately 62 million tonnes a year with each person generating an average of 0.65 kg/day, and is projected to rise to 161.27 million tonnes by 2025, at about 0.85 kg of waste/capita/day.

In developing countries, landfilling is virtually the only approach of waste management (Rubinos and Spagnoli, 2018). Unfortunately, in major cities of Nigeria, these largely unsegregated and heterogeneous in composition wastes are dumped in open grounds which are not engineered to prevent serious contamination of the underlying soil, ground and surface waters by toxic materials leached from the wastes (Misra et al., 2018). The submission of Karak et al. (2012) is that whatsoever be the source of these wastes, its impact on environment and quality of life is mainly related to air, water, and soil contaminations, in addition to space consumption, odours and aesthetic prejudice.

Furthermore, with the 2012 World Bank report also posting a 5% presence of metals in the wastes generated in Nigeria, several authors have documented the presence of heavy metals like, arsenic, cadmium, chromium, nickel, lead copper, mercury and zinc (Kamunda et al., 2016; Simalo, 2017; Gbadamosi et al.,...
2. Materials and methods

2.1. Geographical location of the study area

The study covered three landfills in Lagos State, Nigeria (Fig. 1). They were Olusosun (Latitude 6° 35'50"N- 6° 36'30"N; Longitude 3° 22'45"E – 3° 23'30"E), Solous II (Latitude 6° 34'15"N- 6° 34'24"N; Longitude 3°15'02"E – 3°15'12"E) and Solous III (Latitude 6° 33'42"N- 6° 33'58"N; Longitude 3°15'02"E – 3°15'15"E) (Fig. 2).

2.2. Geology of the study

Lagos is made up of the sedimentary terrain which is an extension of the Dahomey Basin. The Dahomey Basin is a Basin that stretches from southeastern Ghana through Togo, Benin Republic to southwestern Nigeria (Fig. 3). Lagos State is one of the three states located in the Dahomey basin part of Nigeria (Fig. 4), the other States are Ogun and Ondo. Olusosun landfill receives approximately 40% of the total waste from Lagos. The size is 47.2 ha and it has a residual life span of 20 years (Olufunfemi, 2011). Solous II is on 7.8 ha of land with average life span of 5 years, while Solous III is on approximately 5 ha of land with average life span of 5 years. Each of the Solous sites receives an average of about 2,250 m³ of waste per day.

2.3. Soil samples

Soil samples were collected at 0–15 cm depth randomly from the landfill site divided into different plots to obtain representative samples. The control soil sample was obtained from the soil of undisturbed site within the radius of about 3 km from the landfill site. The soil samples were air-dried, mixed thoroughly and passed through a 2 mm sieve to remove debris. The bacterial isolates were isolated and biochemically characterized. API kit 20E was used for the identification and the organisms were screened for heavy metal (Cd and Pb) tolerance (Oladipo et al., 2018).

2.4. Bioremediation experiment

Subsequently, P. aeruginosa, K. edwardsii and E. cloacae isolated from the landfill soil were reactivated. Peptone supplemented with different concentrations of Cd (0, 25, 50, 75, and 100 mgL⁻¹) and Pb (0, 150, 300, 450 and 600 mgL⁻¹) were used for this experiment. The broth without the heavy metal served as the control. The broth was autoclaved at 121 °C for 15 min and allowed to cool. Each of the test organisms (Pseudomonas aeruginosa, Klebsiella edwardsii and Enterobacter cloacae) was inoculated into the heavy metal supplemented and control broth. It was then incubated at 35 °C. The growth and the remediation potential were monitored at two days interval for 8 days. The remediation potential of the selected organisms was evaluated by monitoring the growth of biomass using ultra violet (UV) spectrophotometer at 600 nm. The residual heavy metal in the broth was determined using atomic absorption spectrophotometer (AAS). Remediation percentage was calculated using the formula according to Nandish (2005) and Kaczorek (2012):

\[
\text{Remediation percentage} = \left( \frac{\text{Initial heavy metal concentration} - \text{Residual heavy metal concentration}}{\text{Initial heavy metal concentration}} \right) \times 100
\]

2.5. Maximum remediation enhancement

Different levels of carbon source and pH were used to assess the maximum remediation of the heavy metals by the selected organisms. The broth medium with low remediation efficiency was supplemented with different levels of carbon source of 0, 1, 2, 3, 4, 5 and 6 (g/l) while the pH was varied (unadjusted pH, 5, 6, 7, 8 and 9). All the supplemented broth was inoculated with the efficient heavy metal degrader and incubated for 8 days (Loh and Wang, 1998). The growth and remediation potential was monitored at two days interval for 8 days. The optimum carbon source and pH value were determined based on their remediation percentage.
Fig. 1. Map of Olusosun landfill site.

Fig. 2. Map of Solous II and III landfill sites.
Fig. 3. Regional map of the Gulf of Guinea showing the location of Benin (Dahomey) Basin in relation to other basins (Brownfield and Charpentier, 2006).

Fig. 4. Geological map of Dahomey Basin in the Nigerian sector and the states located on the basin (Olabode, and Mohammed, 2016).
2.6. Statistical Analysis

Analysis of variance (ANOVA) and separation of means were done using Duncan’s New Multiple Range Test (p < 0.05).

3. Results and discussion

3.1. Bioremediation process

The bioremediation capabilities of three indigenous gram-negative bacteria isolates (Pseudomonas aeruginosa, Klebsiella edwardsii and Enterobacter cloacae) were evaluated using Cd and Pb supplemented medium (Table 1). Bennisse et al., 2004; Mounaouer et al., 2014; Giovanella et al., 2017 also, indicated in their studies that Gram-negative bacteria are more tolerant to heavy metals than Gram-positive. However, the study conducted by Ndeddy Aka and Babalola (2017), discovered that most of the heavy metal tolerant organisms were gram positive, especially Bacillus. In addition, the isolated indigenous organisms showed different response to heavy metal type and concentrations in this study as confirmed by Bennisse et al., 2004; Ndeddy Aka and Babalola, 2017, Abioye et al. (2018). Mostly, organisms that are able to survive and remain active in extreme environments can be identified and potentially targeted for bioremediation purposes (Akob et al., 2007; Jain et al., 2011).

Optical Density (OD) was used to monitor the microbial growth using pH and carbon source to ascertain the bioremediation potential of the test organisms (Nwinyi et al., 2014). The OD of the control medium throughout the days of incubation was significantly higher (p < 0.05) than Cd supplemented medium at all levels of concentration except at 75 mg Cd L⁻¹ in P. aeruginosa inoculated medium (Table 1). Pseudomonas aeruginosa has been reported several times on its ability to adapt to polluted environment as well as its tolerance to heavy metal (Singh et al., 2013; Das et al., 2016; Rivzi and Khan 2017; Tang et al., 2018; Pourfadakari et al., 2019; Jia et al., 2020; Varjani et al., 2020; Chen et al., 2021) due to its biosorption capability (Al-Dhabi et al., 2019). In Cd supplemented medium, P. aeruginosa has the highest remediation percentage of 58.80% at 50 mg Cd L⁻¹ and highest optical density (0.759) at 75 mg Cd L⁻¹. The variation of the influence of P. aeruginosa at 50 mg Cd L⁻¹ and 75 mg Cd L⁻¹ in this study might be due to the difference enzymatic activity at each of the Cd concentrations. Some studies have shown that P. aeruginosa exhibits some enzymatic pathways through which pollutant are attacked and converted to harmless products. (Choudhary and Sar, 2011; Nwinyi et al., 2014; Yin et al., 2016; Giovanella et al., 2017; Ojewunmi, 2018; Zhang et al., 2020). Basically, the rate of bioremediation process depends on the concentration of the contaminant and the expression of some specific enzymes by the organisms.

The effect of bacterial isolates on the remediation of Pb as shown in Table 1. E. cloacae had the lowest remediation percentage at all levels of Pb supplementation except at 600 mg Pb L⁻¹ which exhibited high significance (p < 0.05) in remediation percentage of 25.63. This shows that the tolerance and utilization of the heavy metal was not only dependent on the heavy metal concentration but the days of incubation of the organisms. Similar findings on the ability of Bacillus megaterium and Rhizopus stolonifer to enhance the removal of Pb, Cd and Ni from contaminated broths with increase in incubation time were reported by Njoku et al., 2020. Nikhil et al. (2013) also confirmed that incubation period of the test organisms increases the rate of degradation of diesel engine oil in soil indicating that, the organisms could still be at the exponential phase of their growth. The genera Klebsiella and Pseudomonas were most tolerant to Pb and were able to grow in all Pb concentrations investigated. Although, Pseudomonas aeruginosa had the highest remediation percentage of 33.67. The variability of the test organisms towards heavy metal tolerance in this study confirmed that the test organisms exhibited several mechanisms to reduce the elevated concentrations of heavy metals which could be specific for one or a few metals (Nies, 2003; Piddock, 2006). In addition, the concentration of heavy metal with the highest biomass of the test organisms as shown by the OD did not correspond to the concentration at which the maximum remediation occurs except at 450 mg Pb L⁻¹. This could be as a result of different mechanisms used by the organisms to remove heavy metal as well as the ability of the heavy metal to induce oxidative damage which denatures and reduce remediation potential of the organisms.

| Isolate Code | Optical density | Remediation % | Optical density | Remediation % |
|--------------|----------------|---------------|----------------|---------------|
| KE           | 0.562          | NA            | 0.562          | NA            |
| EC           | 0.594          | NA            | 0.594          | NA            |
| PA           | 0.744          | NA            | 0.744          | NA            |
| KEA1         | 0.409          | 36.40         | 1.013          | 21.36         |
| ECA1         | 0.504          | 22.90         | 0.544          | 16.75         |
| PAA1         | 0.350          | 46.49         | 1.440          | 20.54         |
| KEA2         | 0.409          | 34.89         | 1.032          | 25.17         |
| ECA2         | 0.506          | 37.83         | 1.161          | 17.58         |
| PAA2         | 0.195          | 58.80         | 1.093          | 33.67         |
| KEA3         | 0.405          | 33.67         | 0.578          | 17.50         |
| ECA3         | 0.515          | 38.70         | 0.663          | 15.14         |
| PAA3         | 0.759          | 37.26         | 1.036          | 25.99         |
| KEA4         | 0.403          | 31.57         | 0.891          | 16.13         |
| ECA4         | 0.400          | 12.52         | 0.573          | 25.63         |
| PAA4         | 0.206          | 39.90         | 1.147          | 18.73         |

Values in the same group followed by the same letter did not differ significantly at p < 0.05 according to Duncan’s New Multiple Range Test
KE- Klebsiella edwardsii, EC- Enterobacter cloacae
PA- Pseudomonas aeruginosa
A1- 25 mg L⁻¹ (Cd), 150 mg L⁻¹ (Pb)
A2- 50 mg L⁻¹ (Cd), 300 mg L⁻¹ (Pb)
A3- 75 mg L⁻¹ (Cd), 450 mg L⁻¹ (Pb)
A4- 100 mg L⁻¹ (Cd), 600 mg L⁻¹ (Pb)
NA- Not Applicable
(Liu et al., 2017; Priyadarshane and Das 2020; Tarekegn et al., 2020; Shuaib et al., 2021). However, this study shows that most of the heavy metal with the highest concentration (100 mg Cd L⁻¹ and 600 mg Pb L⁻¹) had low remediation percentage. To enhance the remediation potential of the test organisms, further study with these organisms was carried out.

### 3.2. Bioremediation enhancement

The use of the indigenous microorganisms alone in bioremediation could limit their potential as a result of competition and elevated heavy metal concentrations. Bioremediation process could be improved using different approaches, depending on the state of the contaminated environment. One of these approaches is biostimulation, which involves promoting the growth of microorganisms at the contaminated site, in order to introduce pH-correction substances, nutrients, surfactants and oxygen (Randelović et al., 2015; Raimondo et al., 2020). Thus, nutrient addition and modification of environmental parameters allow microbial growth and accelerate bioremediation processes (Mehrzad et al., 2015; Nwinyi and Akinmulewo, 2019). The result of this study revealed varying effect of the carbon source concentration and pH on the microbial biomass and remediation potential. Considering, the importance of nutrients in growth and cultivation of microorganisms, the quantity of the nutrient is also imperative. In this study, the Optical Density and remediation percentage were higher for all the test organisms in the media supplemented with 1 and 2 g L⁻¹ carbon source than the control medium of 100 mg Cd L⁻¹ (Table 2). *Klebsiella edwarsii* Optical Density was significantly (p < 0.05) higher than other selected bacteria in 3 and 6 g L⁻¹ of carbon source supplemented media all through the days of incubation. The minimum Optical Density (0.206) and remediation percentage (8.29%) were exhibited by *Pseudomonas aeruginosa* at 0 and 6 g L⁻¹ carbon source supplementation in 100 mg Cd L⁻¹ medium respectively (Table 2). In contrast, the maximum Optical Density (1.176) was by *Klebsiella edwarsii* while maximum remediation percentage (53.18%) was exhibited by *P. aeruginosa* in 100 mg Cd L⁻¹ medium as shown in Table 2. This confirms that the microbial biomass and specie possess varied biosorptive capabilities which depend on treatments and experimental conditions (Malik, 2004; Fomina and Gadd 2014). Also, levels of carbon source in 600 mg Pb L⁻¹ media did not have any positive effect on the Optical Density of the investigated isolates. Hence, the organisms exhibited a lower Optical Density at all levels of carbon supplementation which is not in support of the findings of Teng et al., 2010 in the use of carbon as supplement in the bioremediation of PAH contaminated soil. Although, the same study (Teng et al., 2010) supports this study as the bacterial isolates exhibited increase in remediation percentage especially at 4 and 5 g L⁻¹ carbon supplement. *K. edwarsii* has the highest remediation percentage of 79.87 at 5 g L⁻¹ carbon supplement. The solubility of heavy metal in bioremediation could be specific as heavy metals used in this study exhibit varied effect on the test microorganisms. Zahoor and Rehman (2009) reported reduction of hexavalent chromium by Bacillus sp. JDM-2-1 and Staphylococcus capitis, to trivalent form as aided by the solubility of Cr (VI). Furthermore, Jin et al. (2018) describe the variation in the solubility of different heavy metal as Zn, Ni, and Cu dissolved easily while Pb and Cr are less soluble.

The pH also has significant effect on heavy metal ions solubility and cell surface charge, which affects the heavy metal removal processes (Guibal et al., 1994; Jin et al., 2018). Table 3 showed the effect of varied pH levels on the remediation of 100 mg L⁻¹ Cd and 600 mg L⁻¹ Pb by bacterial isolates. The maximum optical density (0.414) was by *E. cloacae* at pH 8 while the maximum remediation percentage (81%) was by *K. edwarsii* at pH 5. In addition, the test organisms had the least optical density (0.026, 0.079 and 0.060) and remediation percentage (8.47%, 9.05% and 4.42%) value at pH 9 in 100 mg L⁻¹ Cd remediation. The adjusted pH did not have significant (P < 0.05) improvement on the optical density of the bacterial isolates for both heavy metals. There was a remarkable improvement in the remediation percentage at pH 5 and 7 in 600 mg L⁻¹ Pb supplemented medium (Table 3). Moreover, *K. edwarsii* had the highest (92.41%) and lowest (1.38%) remediation percentage at pH 6 and 9 respectively. It was observed in this study, that the remediation potential and biomass of the organisms can be optimized within the pH range of 5 to 8. This shows that microbial biosorption, and optimum pH usually vary for different

### Table 2

Effect of carbon source levels of the medium on the remediation of 100 mg Cd L⁻¹ and 600 mg Pb L⁻¹ by bacterial isolates.

| Isolate Code | Optical density | Remediation % | Optical density | Remediation % |
|--------------|-----------------|---------------|-----------------|---------------|
| KEA₁₀        | 0.717           | 8.29³         | 0.422           | 68.64²        |
| KEA₈         | 0.630           | 53.18⁴        | 0.461¹         | 66.42¹        |
| KEA₆         | 0.574           | 13.70⁵        | 0.435⁴         | 76.62²        |
| KEA₄         | 1.176           | 18.01²        | 0.429          | 75.42²        |
| KEA₂         | 0.627           | 14.53³        | 0.512          | 23.51²        |
| KEA₁         | 0.319           | 40.18⁴        | 0.511          | 55.16²        |
| PAA₁₀        | 0.630           | 14.40⁴        | 0.468¹         | 70.87⁴        |
| PAA₈         | 0.554           | 16.14⁴        | 0.506²         | 60.17⁴        |
| PAA₆         | 0.574           | 13.70⁵        | 0.435⁴         | 76.62²        |
| PAA₄         | 0.630           | 8.01³         | 0.531¹         | 79.39³        |
| PAA₂         | 0.631           | 53.18⁴        | 0.461¹         | 66.42¹        |
| PAA₁         | 0.638           | 15.03³        | 0.345          | 79.87³        |
| PAA₆         | 0.680           | 16.04⁴        | 0.431          | 63.03³        |
| PAA₄         | 0.630           | 16.14⁴        | 0.358          | 57.42²        |
| KE₁₀         | 1.176           | 18.01²        | 0.429          | 75.42²        |
| KE₈          | 0.630           | 16.14⁴        | 0.358          | 57.42²        |
| KE₆          | 0.574           | 13.70⁵        | 0.435⁴         | 76.62²        |
| KE₄          | 0.630           | 8.01³         | 0.531¹         | 78.39³        |
| KE₂          | 0.631           | 53.18⁴        | 0.461¹         | 66.42¹        |
| KE₁          | 0.638           | 15.03³        | 0.345          | 79.87³        |
| PAA₁₀        | 0.618           | 12.73³        | 0.552          | 70.44⁴        |
| PAA₈         | 0.717           | 8.29³         | 0.422          | 68.64²        |

Values in the same group followed by the same letter did not differ significantly at p < 0.05 according to Duncan’s New Multiple Range Test

Legend: KE – *Klebsiella edwarsii*, EC – *Enterobacter cloacae*, PA – *Pseudomonas aeruginosa*, Aₙ – Unadjusted carbon source (Control), A₁ – 1 g/L carbon source, A₂ – 2 g/L carbon source, A₃ – 3 g/L carbon source, A₄ – 4 g/L of carbon source, A₅ – 5 g/L carbon source, A₁₀ – 6 g/L carbon source

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4. Conclusion

The environmental and economic impacts of heavy metal pollution on soils are enormous; eliciting changes capable of affecting nutrient cycling, impeding nutrient uptake by plant roots and leading to reduction in crop yield. This causes serious damages to vegetation, soil fertility and soil-borne microorganisms. In the present study, the results showed that 4-6 g/L carbon source supplement at pH 5–8 enhanced the heavy metal bioremediation potential of all types of biomass. Ashokkumar et al. (2017) recorded 48%, 75% and 52% for removal of Cu, Pb, and Cr respectively by Sphaerotilus natans in at pH 7. Also, Joshi and Lee (1995) indicated that bacteria can efficiently degrade majority of the soil contaminants within the pH range of 5.0 and 9.0. Ultimately, the study reveals that all the test organisms had the lowest remediation potential and biomass at pH 9. This confirms that heavy metals tend to be insoluble at high pH and soluble at low pH. A study conducted by Ameen et al. (2020) recorded maximum uptake of Cd²⁺ and Pb²⁺ by Lactobacillus plantarum at pH 2 while Franklin et al. (2000) and Olaniran et al. (2013) reported that heavy metals are more toxic at high pH. Hence, the rate of heavy metal remediation by microorganisms increases with increase in pH levels and decreases as it gets to its limit.

Table 3

Effect of pH of the medium on the remediation of 100 mg Cd L⁻¹ and 600 mg Pb L⁻¹ by bacterial isolates.

| Isolate Code | Optical density | Remediation % | Optical density | Remediation % |
|--------------|-----------------|---------------|----------------|---------------|
| KEA4         | 0.403a          | 31.57b        | 0.891b         | 16.13c        |
| ECA4         | 0.400a          | 12.52a        | 0.573a         | 25.63a        |
| PAA4         | 0.206a          | 39.90f        | 1.470          | 18.73g        |
| KEA14        | 0.236b          | 81.50a        | 0.459f         | 43.54f        |
| ECA14        | 0.232b          | 25.62c        | 0.650f         | 14.87c        |
| PAA14        | 0.263a          | 31.48b        | 0.620a         | 86.38c        |
| KEA13        | 0.147b          | 9.81l         | 0.511b         | 92.41l        |
| ECA13        | 0.180b          | 17.22b        | 0.698b         | 70.15b        |
| PAA13        | 0.272a          | 18.31a        | 0.495b         | 72.93b        |
| KEA12        | 0.060b          | 14.40b        | 0.482b         | 82.61b        |
| ECA12        | 0.125c          | 59.03a        | 0.666b         | 85.43c        |
| PAA12        | 0.150a          | 12.60c        | 0.494b         | 73.39c        |
| KEA14        | 0.172c          | 24.19c        | 0.501c         | 12.26c        |
| ECA14        | 0.414c          | 25.85b        | 0.707c         | 15.57b        |
| PAA14        | 0.240b          | 40.56a        | 0.580c         | 35.42c        |
| KEA15        | 0.026b          | 8.47c         | 0.158c         | 1.38c         |
| ECA15        | 0.079a          | 9.05c         | 0.206b         | 3.89c         |
| PAA15        | 0.060c          | 4.42c         | 0.127c         | 5.40c         |

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Authors’ Contribution

OO and OАО conceived and designed the study. OO, OАО, OEJ, AЕF and OJS performed experiments, drafted and edited the manuscript. All authors read and approved the final manuscript.

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