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Sustainable remediation of heavy metal polluted soil: A biotechnical interaction with selected bacteria species

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ABSTRACT

When the inevitable nature of waste generation is considered detrimental to the environment, it becomes imperative to develop waste management options that do not only take care of disposal, but will ensure sustainability and environmental safety. Due to the persistent nature of heavy metals in landfill leachate contaminated soil, resident microbes need bioengineering with the aim of evaluating a biotechnical approach suitable for the bioremoval and/or immobilization of heavy metals in contaminated soil. Utilized bacterial strains optimized the reduction of extractable Al (72%), Cu (88%), Cd (41%), Mn (65%) and Pb (71%) ions from leachate-contaminated soil.

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1. Introduction

Waste generation is a common phenomenon associated with human and capital development, industrialization and socio-economic dynamism. When the inevitable nature of waste generation is considered detrimental to the environment, it becomes imperative to develop waste management options that do not only take care of disposal, but will ensure sustainability and environmental safety. In as much as some waste disposal/management options exist, especially incineration and composting, yet the use of landfills remain the most widely adopted option. In fact, in some parts of Asia, especially Malaysia, more than three hundred and six landfills are available as against the very few incinerating plants around, which in most cases fail to perform optimally. Landfills are known to accommodate almost every material in the solid waste stream especially among the developing nations (Agamuthu and Tanaka, 2014), and the municipal solid waste (MSW) is the most significantly disposed waste to landfills.

However, one of the major issues associated with MSW landfilling is the generation of leachate. The presence of this liquid substance, leachate, is often a subject of concern to both landfill managers and the environmental protectionists due to the impact of leachate on the environment, especially, on ground water, surface water and soil (when not properly handled). Leachate composition can vary across landfills regardless of the status/condition of operation, yet its characterization commonly shows the presence of inorganic macro-

* Corresponding author. E-mail addresses: emenike@um.edu.my, emenikecu@gmail.com (C.U. Emenike). compounds, dissolved organic matter, high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) loads (Ludwig et al., 2003; Emenike et al., 2012). Furthermore, heavy metals detected show higher concentrations in discharged leachate (Fauziah et al., 2013) and leachate contaminated soils.

Therefore, when the negative impact of heavy metal is reviewed in terms of adverse effect on human physiology and biological systems (Kobya et al., 2005), it becomes necessary to identify option(s) for its removal and/or immobilization within leachate contaminated soil. In most developing nations, where the landfill types are either mere dumpsites or non-sanitary landfills, leachate percolation of soil profile is inevitable and prevalent due to the vertical and lateral migratory nature of leachate (Jaffar et al., 2009). Most heavy metals have high affinity for other elements like sulphur, thereby disrupting enzyme functions of living cells via formation of bond, or even the use of ions to bind cell membranes that initiate interference within the cell transport processes (Manahan, 2004).

Due to the foregoing, the use of a biotechnical and environmentally safe approach is necessary for the remediation of heavy metal contaminated soil, especially in pollution induced by leachate, because it is a heterogeneous liquid. There is no doubt that the adoption of biotechnical approaches such as bioremediation is most welcome due to its sustainability potential, yet many biological techniques are not only relatively new but are inherently difficult to standardize most times due to the involvement of living organisms, especially microbes. Microbes relatively survive in landfill environment and such may suggest that favourable condition for metabolism exists. However, it is still necessary to optimize the impact of bacterial species on the bioremediation

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Lysinibacillus sp.

Acinetobacter sp.

Brevundimonas sp.

Microbacterium sp.

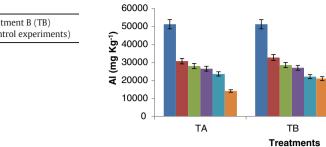
Rhodococcus sp.

able I solated microbes and the distribution in the microcosms.						
Isolated bacteria	Treatment A (TA)	Treatment E (Control exp				
Bacillus sp.	Bacillus sp.	NU				
Psuedomonas sp.	NU	NU				
Stenotrophomonas sp.	NU	NU				
Flavimonas sp.	NU	NU				

NU

NU

NU



"NU" means not used (meaning that the isolated bacteria species was not part of a particular treatment)

Lysinibacillus sp.

Rhodococcus sp.

NH

NU

NU

NU

NU

of soil contaminated with metals. Sometimes, metals distinctively perturb soil microbial biomass and activity and even reduce the composition and diversity of the microbial community of soil (Xu et al., 2015). Hence, this study evaluated the potentials of landfill resident microbes towards the bioremoval and/or immobilization of heavy metals in contaminated soil.

2. Materials & methods

2.1. Soil samples collection and characterization

The experiment undertaken at a laboratory scale involved the use of two soil sources; leachate soaked soil from landfill (3°13.78'N, 101°39.72"E) and non-contaminated garden soil (3°7'724.15'N, 101°39′16.79″E). While the first soil source was required for microbial isolations, the later was utilized for the setup of the bioremediation microcosm. Soil samples collected were in accordance to 2004 ASTM E-1197 standard guidelines for conducting terrestrial soil-core microcosm test (Sprocati et al., 2011). Samples were adequately replicated to accommodate variability and ensure homogeneity.

2.2. Microbial isolation

In order to identify the possibility of microbial survival in leachatecontaminated environment, microbial isolation was carried out prior to the study presented here. This is because the presence of microbes in the landfill environment may imply the persistent nature of the microbes, hence the potential involvement in some biological processes taking place within the contaminated soil. Hence, 1 g of soil was previously mixed with 0.9% NaCl and the suspension vortexed for 2 h at 150 rpm using Lab-line 3521 orbit shaker. Serial dilutions were plated (Kauppi et al., 2011) on nutrient agar (NA) and subsequently incubated for 48 h at 33 °C. Single colonies were grown separately on freshly prepared NA to obtain discrete pure cultures that were eventually identified using Biolog GEN III MicroPlate protocol (Bochner, 1989a; Bochner, 1989b).

Table 2

Initial and residual mean concentrations of heavy metals from the bioremediation of leachate contaminated soil.

Heavy metals	Initial concentrations (mg/kg)	Mean residual concentrations (mg/kg) and level of reduction (%)			
		Treatme	nt A	Treatme	nt B
Al	51,200	14,143	72%	20,967	59%
Cd	1.70	1.00	41%	1.00	41%
Cu	24.10	3.00	88%	11.00	54%
Mn	129	45.00	65%	98.00	24%
Pb	206.8	60	71%	121	41%



Fig. 1. Reduction of Al across experimental duration (100 days).

■0 ■20 ■40 ■60 ■80 ■100 days

2.3. Microbial formulation and bioaugmentation set-up

Bioaugmentation was the preferred method of bioremediation adopted in this study considering that sometimes microbes require some manipulation in order to optimally metabolize in presence of pollutants. The formula used in the bioaugmentation experiment contained three strains of bacteria isolated from the leachate soaked soil (contaminated landfill site). Each strain was re-grown as a pure culture and discrete suspensions at the same physiological phase (1.3 ABS at 600 nm) were then pooled in equal proportions to set-up inoculum for bioaugmentation. Soil microcosms of two treatments (A & B) were prepared by introducing 10% v/w of leachate concentration into the non-contaminated garden soil. Treatment A was inoculated with the three bacterial strains, whereas treatment B had no microbial addition in order to serve as a control. Portions of soil microcosms were sacrificed every 20 days (until 100 days) for onward metal analysis. Reported duration was to capture the most active period of the microbes (Emenike et al., 2016). Each soil sample taken for analysis was acid-digested (Hseu et al., 2002) using Multiwave 3000 microwave digester, while Optima 530,00 DV was used to obtain the elemental concentrations of Al, Cd, Cu, Mn and Pb according to USEPA 3050 B.

Data obtained were further processed to calculate the percentage of heavy metals removal using;

% of heavy metal removal =
$$\left(\frac{C_{0(x)} - C_{F(x)}}{C_{0(x)}}\right) \times 100\%$$
 (1)

where

 $C_{0(x)}$ = initial concentration of metal "x" (Al, Cd, Cu, Mn or Pb) in the soil at the start of experiment

 $C_{F(x)}$ = final concentration of metal "x" (Al, Cd, Cu, Mn or Pb) in the soil at the end of experiment.

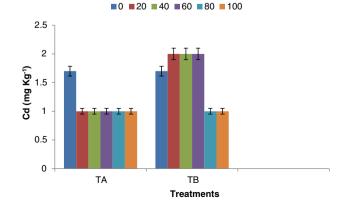


Fig. 2. Reduction of Cd across experimental duration (100 days).

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