ARID ZONE JOURNAL OF ENGINEERING, TECHNOLOGY & ENVIRONMENT
AZOJETE June 2020. Vol. 16(2):387-394
Published by the Faculty of Engineering, University of Maiduguri, Maiduguri, Nigeria.
Print ISSN: 1596-2490, Electronic ISSN: 2545-5818
www.azojete.com.ng

ORIGINAL RESEARCH ARTICLE

EVALUATING THE POTENTIAL OF MICROORGANISMS IN BIOREMEDIATION OF LEAD POLLUTED SOIL

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ARTICLE INFORMATION

Submitted 08 Feb., 2020
Revised 24 May, 2020
Accepted 30 May, 2020

Keywords:
Lead
Soils
Contamination
Bioremediation
removal kinetics

ABSTRACT

This study evaluated and compared the abilities of indigenous Bacillus sp. and Micrococcus sp. for treating lead polluted soil. The organisms were inoculated into the contaminated soil samples prepared with the optimum values of screened determinant factors and experimented on 8, 16, 24, 32, 40, 48, and 56 days respectively for lead ion residual concentration using Atomic Absorption Spectrophotometer. Bacillus sp. showed greater remediation strength than Micrococcus sp. The results show that Bacillus sp. attenuated the Pb initial concentration from 150.74 mg/kg to control level in 40 days while Micrococcus sp. achieved that in 48 days. The 56th day residual concentrations and removal efficiencies were 81.43 mg/kg and 46% for Bacillus sp., and 86.91 mg/kg and 42.34% for Micrococcus sp. The removal rate was higher for Bacillus sp. (-0.0715 d^{-1}) than for Micrococcus sp. (-0.0744d^{-1}); and the remediation process was transport controlled. This information will be useful for remediation programs; and it will serve as a reference material for future research.

1.0 Introduction

Human activities like mining, agriculture and manufacturing have kept the environment at a high risk of pollution through heavy metals (Gupta et al., 2016; Kure et al., 2018). Heavy metals have been reported to be very injurious to plants and animals (Vijaya et al., 2010; Girma, 2015). Metals are important for the performances of living organisms, but at concentrations above the required; they could be very injurious to lives (Musa et al., 2017). Lead (Pb) is classified among hazardous metals negatively affecting soil quality, crops yields; and public health (Gupta et al., 2016; Upadhyay et al., 2017). At concentration above the threshold (100 mg/kg) in soils (Chiroma et al., 2014), it can be injurious to plants; it can limit plant growth as a result of hampered photosynthetic activities, nutrients, and performances of necessary enzymes (Kabata-Pendias, 2010; Namatian et al., 2013). Metals are capable of bioaccumulation in food chain and in this manner; their effects on lives and associated risk are wider (Tak et al., 2013). Effects of lead include mental retardation, anemia, renal dysfunction, palsy, reproductive systems disorder (Athar and Vohora, 2006).

The removal of metals from the environment requires effective and affordable techniques that are better than the physical and chemical techniques with associated devastating post remediation toxicity, unwanted sludge, very high cost, and under performance at very low concentration (Ayangbenro and Babalola, 2017). These disadvantages of physical and chemical techniques of remediation are the reasons for a push for further remediation alternatives, which include bioremediation that involves the use of organism for the removal of contaminants from the environment. The capacities of organisms to effect bioremediation is tied to some vital factors (suitable pH, temperature, and moisture) which promote their metabolism and growth (Verma and Jaiswal, 2016).

Beside the use of organisms that are genetically engineered, the use of indigenous micro organisms is efficient for bioremediation (Gupta et al., 2016).

Human activities during mining in Amita agricultural forest in Ebonyi State of Nigeria has contaminated the forest soils (Ezeh and Chukwu, 2011) and some crops (tubers and vegetable)

(Onyedika and Nwosu, 2008) with some heavy metals including lead. These soils need effective and affordable remediation for their restoration for meaningful agricultural practice.

Microorganisms (Fungi, Bacteria and Algae) are known to be effective for removing heavy metals from water and soils (Kure et al., 2018). Dixit et al. (2015) documented that microbes like Pseudomonas, Escherichia, Bacillus, Yeasts and Enterobacter are useful for the removal of heavy metals from soils and water.

This study focused on determining the potential application of indigenous Bacillus sp. and Micrococcus sp. acquired from Amita forest soils for tackling the Pb pollution through the process of bioremediation for safe food and commercial supply purpose. The work also examined the processes controlling the bioremediation through rate-limiting steps established by some kinetics models.

2.0 Materials and Methods

2.1 Materials and Reagents

The materials and reagents used in this study include: 1. soil samples collected from Amita forest, Ebonyi State in Nigeria; 2. MacCartney bottles; 3. oxidase reagent; 4. Incubator; 5. Kovac's reagent; 6. cotton wool; 7. whatman filter papers; 8. sodium hydroxide; 9. measuring cylinder; 10. atomic absorption spectrophotometer; 11. perchloric acid; 12. wire loops; 13. refrigerator; 14. inoculating needles; 15. perchloric acid; 16. pipettes; 17.sulphuric acid; 18. crystal violent; 19. hot plate; 20. methylene blue; 21. Lugo's iodine; 22. Autoclave; 23. Ethanol; 24. hydrogen peroxide; 25. petri dishes; 26. hydrochloric acid; 27. Beakers; 28. Safranin; 29. Microscope; 30. nitric acid; and 31. conical flasks.

2.2 Nutrients Preparation

To prepare the nutrients needed for isolation of organisms and biostimulation of the organisms for effective bioremediation, 15g of peptone water powder; 28 g of nutrient ager; 65 g of triple sugar iron agar; 24 g of Simon Citrate agar; and 52 g of powered MacConkey agar were dissolved in 1000 cm^3 each of water for 10 minutes according to Cheesebrough (2000) and manufacturer's manual. The formulated solutions were autoclaved at 10342.14 N/m^2 for 15 minutes at 121 °C and cooled to 45 °C for use.

2.3 Acquisition of Microorganisms

At a microbiology laboratory of Delta State University in Abraka, Delta State of Nigeria, bacteriological evaluation of the collected soil sample was conducted. The soil was serial diluted by dissolving 1g of the soil in 10 ml of distilled water. One (1) ml of the resultant solution was diluted with 9 ml of distilled water and the resultant solution was serial diluted from step 10^{-1} to 10^{-5}. Using the pure plate method, 0.1ml from 10^{-1}, 10^{-3} and 10^{-5} serial delusion was measured into several petrri-dishes and cover with nutrient and MacConkey agars respectively (Baron et al., 1994); and incubated at 37 °C for 24 h. Developed colonies of the organisms were sub-cultured and recognized using the techniques in (Holt, 1994; Cheesebrough, 2000).

2.4 Selection of Optimal Factors

Bioremediation is very effective when carried out with optimum values of determinant factors (Murthy et al., 2012; Atikpo and Michael, 2018). This makes it imperative to select the optimum values of these determinant (pH, temperature, stirring frequency, mass of organisms and nutrient dosage) experimentally (Atikpo, 2016; Atikpo and Ezugwu, 2017).

The experiments were conducted by adopting the method in (Atikpo and Micheal, 2018). Five grams of soil samples were measured to have 39 pairs of 5g of the soil. From the 39 pairs of 5g of soil samples, 8 pairs were conditioned with nutrient dosage of 4, 5, 6, 7, 8, 9, 10 and 11ml; nine pairs were conditioned with 1, 2, 3, 4, 5, 6, 7, 8 and 9g of organisms' masses; seven pairs were conditioned with 10, 15, 20, 25, 30, 35 and 40 °C of temperature; eight pairs were conditioned with 4, 5, 6, 7, 8, 9, 10 and 11 of pH; and seven pairs were conditioned with 0, 1, 2, 3, 4, 5 and 6 per week (pw) of stirring frequency. One out of each pair was for inoculation with Bacillus sp. while the second one out of each pair was for inoculation with Micrococcus sp.

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These pairs of soil samples were inoculated with respective 24hr old Bacillus sp. and Micrococcus sp. that were isolated and sub-cultured and identified with the methods in (Baron et al., 1994; Holt, 1994; Cheesebrough, 2000). Adopting the least residual Pb ion content of soil as the criteria for identifying the optimum values of pH, temperature, stirring frequency, mass of organisms and nutrient dosage, their optimum values were then determined and recorded by evaluating the residual lead (Pb) ion content of the pairs of soil samples with Atomic Absorption Spectrophotometer (GBC SensAA Model no. A6358) on the 24th day.

2.5 Remediation Studies

The bioremediation studies were conducted following the method reported by (Atikpo and Micheal, 2018). The optimum masses selected for the organisms were inoculated in 5g each of soil sample in 50ml beaker and conditioned with the selected optimum values of pH, temperature, stirring frequency, mass of organisms and nutrient dosage. The remediation abilities of the organisms were determined at time 8, 16, 24 and 32, 40, 48 and 56 days by analyzing the residual Pb ion in the soils using Atomic Absorption Spectrophotometer (GBC SensAA Model no. A6358) after separating the organisms from the sample with a centrifuge.

The Pb ion removed with time; Pb ion removed at equilibrium; and the ionic removal efficiencies were determined using Equations (1), (2) and (3) respectively (Badmus et al., 2007).

$$q_t = \frac{(C_o - C_t)}{m}.V \quad (1)$$

$$q_e = \frac{(C_o - C_e)}{m}.V \quad (2)$$

$$\text{Efficiency} = \frac{(C_o - C_f)}{C_o}.100 \quad (3)$$

where: q_t (mg/kg) is Pb ion removed with time t; q_e (mg/kg) is Pb ion removed at equilibrium; C_o (mg/kg) is the initial lead ion brought in contact with the organisms; C_f (mg/kg) is the final Pb ion; V(m^3) is the volume of soil used; and m (g) is the optimum mass of organism.

Difference in the organisms' removal proficiencies was evaluated with Microsoft Excel 2016 at ($P < 0.05$); and the rates of Pb ion removal by organisms were assessed with pseudo-first order kinetics model (Owamah, 2014; Atikpo, 2016).

2.6 Removal Kinetics

This study was conducted to deduce the rate-limiting steps (Chen and Wang, 2007; Atikpo, 2016) useful for establishing process controlling the bio-removal of the metal ion from the soil (Atikpo, 2016); and for establishing the rate of the ion removal by the bio-sorbents (Owamah, 2014). The kinetics were evaluated by fitting the elovich, pseudo-first order, intra-particle diffusion, and pseudo-second order models with the batched experimental data using Equations (4), (5), (6) and (7) (Chien and Clayton, 1980; Mckay and Poots, 1980; Ho et al., 2000;); and the models' coefficient of determination, R^2 was engaged in determining the rate-limiting steps.

$$\frac{dq_t}{dt} = \alpha \exp\left(-\beta q_t\right) \quad (4)$$

$$\frac{dq_t}{dt} = k\left(q_e - q_t\right) \quad (5)$$

$$q_t = K_2 t^{\frac{1}{2}} + X \quad (6)$$

$$\frac{dq_t}{dt} = k_1\left(q_e - q_t\right)^2 \quad (7)$$

where: q_t in mg/kg is the ion removal capacity with time t in days; k is pseudo first order model rate constant per day (d^{-1}); q_e in mg/kg is equilibrium ionic removal capacity; k_1 is pseudo second order model rate constant in kg. $mg^{-1}d^{-1}$; α in mg/kg is elovich model initial ion removal rate; β in mg/kg is elovich model ion desorption rate constant; and k_2 is intra-particle diffusion model rate constant.

3.0 Results and Discussion

3.1 Organisms and Bioremediation Optimum Factors

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The developed and sub-cultured colony (3.2 x 10^2 cfu/ml) of microorganisms was found to contain Bacillus sp. and Micrococcus sp. through various microbiology analyses.

Figure 1 shows the influences of pH values and the corresponding residual Pb concentration. pH is very sensitive to bioremediation process. It affects negatives charges on organisms' cells and physiochemistry of metals (Lopez et al., 2000; Samarth et al., 2012). The organisms were influenced differently by the pH. The optimum values were 6 for Micrococcus sp., and 8 for Bacillus sp. The residual concentrations at these values were 83.91 mg/kg for Bacillus sp. and 103.81 mg/kg for Micrococcus sp.

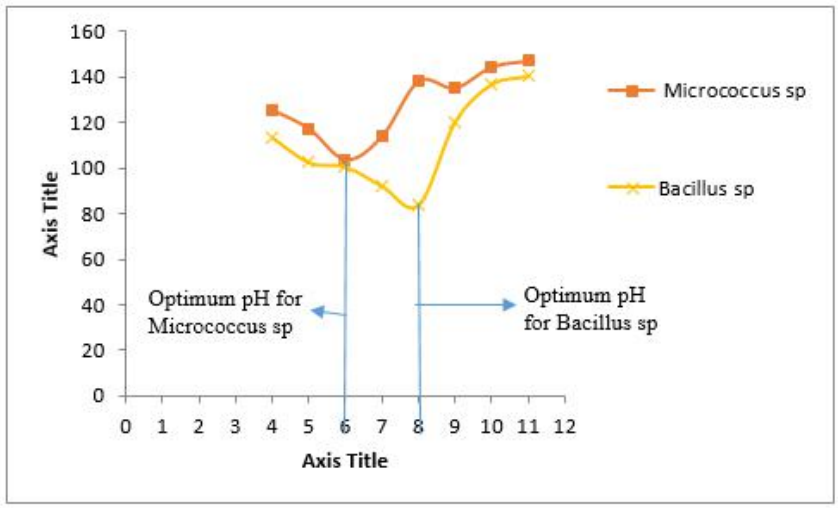

Figure 1: Performance of Organisms under Varied pH Values

The performances of these organisms observed under varied temperature values of 10, 15, 20, 25, 30, 35 and 40 °C were evaluated. The organisms performed differently under these temperatures. Figure 2 showed a common optimum temperature value of 30 °C for the organism; but their removal potential differs markedly. Bacillus sp. was more sensitive to temperature change than Micrococcus sp. This was evident from the residual concentrations of 85.47 mg/kg and 119.11 mg/kg relative to their performances. The optimum value is important because temperature influences remediation process (Ajaykumar et al., 2009; Murthy et al., 2012).

The influence of 4, 5, 6, 7, 8, 9, 10 and11ml of nutrients dosage; 2, 3, 4, 5, 6, 7, 8 and 9 g of organisms and 0, 1, 2, 3, 4, 5 and 6 (pw) of stirring frequencies were studied. The observed optimum values of these factors were 8ml for Bacillus sp. and 9 ml for Micrococcus sp.; 5 g for Bacillus sp. and 4 g for Micrococcus sp.; and 6 pw for the organisms.

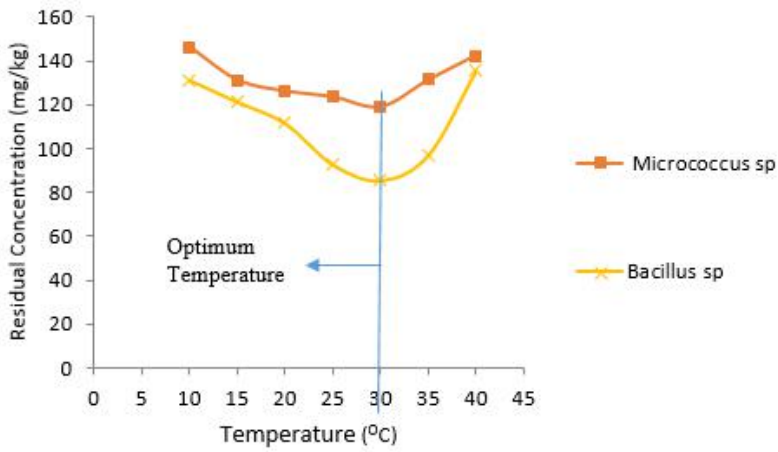

Figure 2: Performances of Organisms under Varied Temperature Values

3.2 Bioremediation

The bioremediation abilities of the organisms were experimented using the batched techniques in (Lin and Lin, 2005; Atikpo and Michael, 2018) for 8, 16, 24, 32, 40, 48 and 56 days. Their abilities to bring the concentration of Pb from 150.74 mg/kg to below the maximum allowable concentration value of 100 mg/kg set for agricultural soils by standards in (Chiroma et al., 2014) were assessed. The difference between 150.74 mg/kg and 100 mg/kg showed that the forest soil was unhealthy and needed remediation.

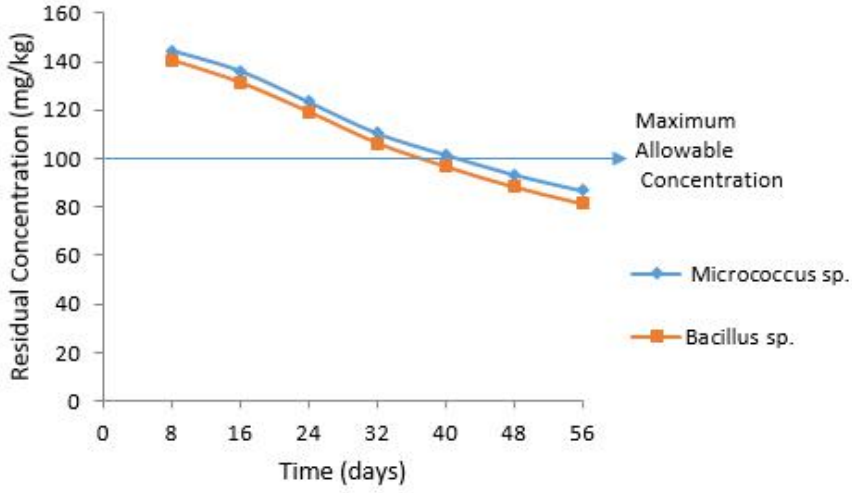

Figure 3: Comparative Removal by the Organisms

Figure 3 shows the organisms to be effectively useful for treating the lead contaminated soil. They are capable of reducing the concentration from 150.74 mg/kg to below 100 mg/kg at different rates and times. Removal by Bacillus sp. was more rapid than removal by Micrococcus sp. Bacillus sp. attenuated the Pb concentration to control level in 40 days while Micrococcus sp. did in 48 days. The respective residual concentrations and efficiencies on the initial safe concentration dates were 96.78 mg/kg and 35.80% for Bacillus sp., 93.45 mg/kg and 38.01% for Micrococcus sp. These safe concentration values were barely below the maximum allowable concentration. The 56th day residual concentrations and removal efficiencies were 81.43 mg/kg and 45.99% for Bacillus sp., 86.91 mg/kg and 42.34% for Micrococcus sp.
Two-ways ANOVA at ($P < 0.05$) conducted with Microsoft Excel, 2016 revealed the possibility of greater sorption by a consortium of the organisms because there was a significance difference between the organisms' sorption capacities. The ANOVA also revealed a possibility of relevant performances with time intervals shorter than the one adopted in this study. This was evident from the significant difference in sorption with experimental times adopted.

From descriptive statistics, the mean residual ion is 109.18 mg/kg for Bacillus sp., and 113.82 mg/kg for Micrococcus sp. The maximum residual ion (MAXRI), minimum residual ion (MINRI), and ion removed (IR56) on 56 day are shown in Table 1.

Table 1. Descriptive Parameters

Organism	MINRI (mg/kg)	MARI (mg/kg)	IR56 (mg/kg)
Micrococcus sp.	86.91	144.53	63.83
Bacillus sp.	81.43	140.57	69.31

3.3 Kinetics of Removal

Deducible rate-limiting steps from kinetics models (Chen and Wang, 2007; Atikpo, 2016) are useful for establishing whether biosorption process is reaction or transport controlled (Atikpo, 2016). Kinetics models are useful for establishing the rate of ions removal (Owamah, 2014). Kinetics models: elovich, pseudo first order, pseudo second order, and intraparticle diffusion were fitted with the experimental data to deduce the rate-limiting steps.

The relationships of $\ln(q_e-q_t)$ and t; t/q_t and t; q_t and $\ln(t)$; and q_t and $t^{1/2}$ from the linear version of models in equations (4), (5), (6), and (7) respectively were plotted as shown in

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Figures 4, 5, 6 and 7 reflecting the regression models and R^2. The high values of models' R^2 indicated that the experimental data were describable by the fitted kinetics models. The order of models fit was the fit for Micrococcus sp. before the fit for Bacillus sp. using pseudo-first and second order, intraparticle diffusion; and Bacillus sp. before Micrococcus sp. using elovich model. The removal rate of pseudo first order was higher for Bacillus sp. (-0.0715 d^{-1}) than for Micrococcus sp. (-0.0744d^{-1}).

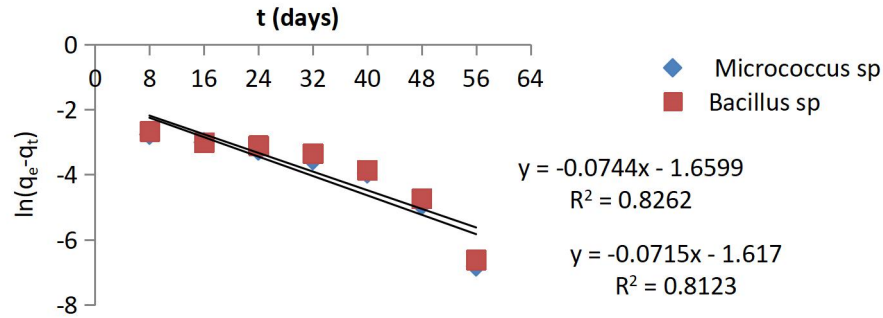

Figure 4: Fit of Pseudo First Order Model

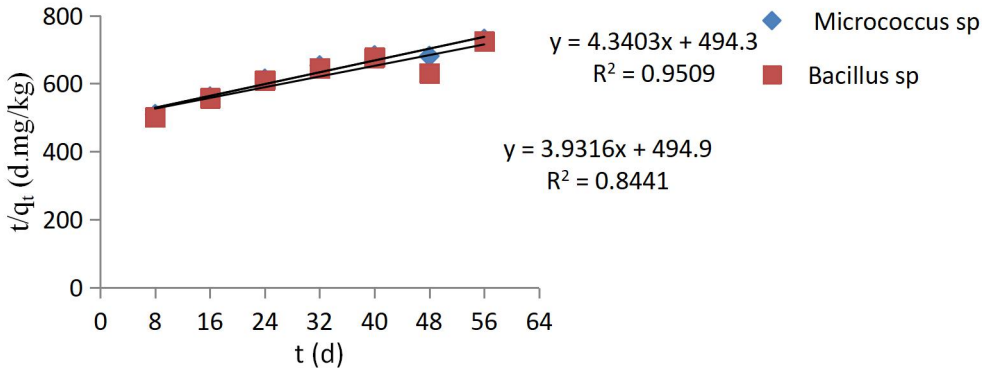

Figure 5: Fit of Pseudo Second Order Model

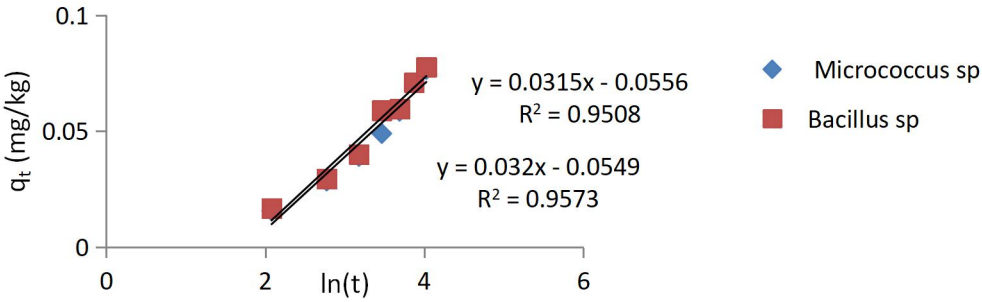

Figure 6: Fit of Elovich Model

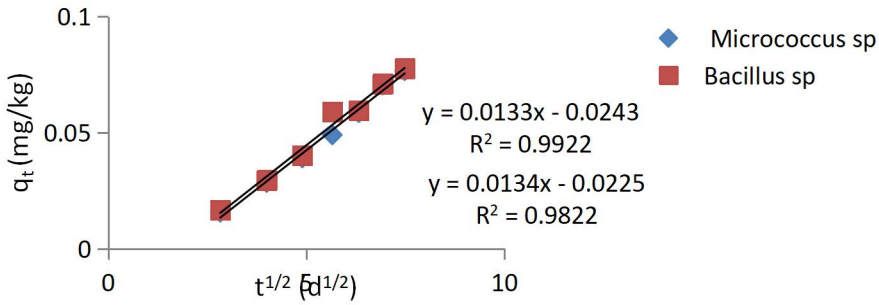

Figure 7: Fit of Intraparticle Diffusion Model

The laboratory data fitted well with tested kinetics models. The R^2 values comparisons in Table 2 displayed the intraparticle diffusion model as the best for describing the removals by the

organisms, Micrococcus sp. with R^2 of 0.992 and Bacillus sp. with R^2 of 0.982. Therefore, the diffusion process was the rate-limiting step for the removals by these organisms (Chen and Wang, 2007) and the processes were transport controlled (Atikpo, 2016).

Table 2. Kinetic Parameters for the Fitted Kinetic Models

Organism	Kinetic models and their parameters							
	Pseudo - first order		Pseudo - second order		Elovich		Intraparticle diffusion	
	k (d^{-1})	R^2	k_1 ($kg.mg^{-1}.d^{-1}$)	R^2	α ($mg.kg^{-1}$)	R^2	k_2 ($mg/kg.d^{1/2}$)	R^2
Micrococcus sp.	-0.0744	0.8262	0.0088	0.950	0.0054	0.950	0.0133	0.992
Bacillus sp.	-0.0715	0.8123	0.0079	0.844	0.0058	0.957	0.0134	0.982

Conclusion

This study is on bioremediation of lead contaminated soil in Amita forest in Ebonyi State of Nigeria using indigenous and biostimulated Bacillus sp. and Micrococcus sp.

The organisms were effective for treating the lead contaminated soil. Bacillus sp. showed greater remediation potential than the Micrococcus sp.; removal rate of (-0.0715 d^{-1}) for Bacillus sp. was higher than (-0.0744d^{-1}) for removal with Micrococcus sp.; and the remediation processes was transport controlled.
This study furnishes relevant insight that will be helpful for soil cleaning programs and further research.

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