



## Bioremediation of Lead Contaminated Agricultural Soil using *Klebsiella pneumoniae*

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**ABSTRACT:** This study focused on the use of *Klebsiella pneumoniae* for bioremediation of lead (Pb) contaminated agricultural soil used for sustainable farming. Atomic Absorption Spectrophotometer (GBC SensAAS, Model no. A6358) was used to measure the concentration of Pb in the soil. Results showed that the organism first reduced the initial concentration of lead from 181.41 mg/kg to below the maximum allowable limit of 100 mg/kg in 14 days. Analysis of variance (ANOVA) at  $P < 0.05$  shows the significance for only single factors in the order of temperature and stirring frequency with P value of 0.0015, volume of nutrient and mass of organism with P value of 0.0016 and pH with P value of 0.0018. The factors were ranked in the order of stirring frequency, temperature, volume of nutrient, mass of organism and pH with their respective percentage contributions of 18.20%, 17.97%, 17.57%, 17.52% and 16.88%.

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Soil plays an important part in the scheme of life. Hence, it is vital resource for life development on earth (Narayanan, 2007). Soil serves several functions in human society (Nwaugo *et al.*, 2008), and can be regarded as a sit for national economic development (Atikpo, 2016). Soil is a resource for food and energy crops for human society as well as for the animal world needed for game reserves. It is a resource for diverse agricultural entrepreneurial activities require for sustainable development. Heavy metals are highly dangerous because they bioaccumulate in living things. Once ingested into the environment by any source, may spread to various ecological components (Njoku and Ngene, 2012). They find their ways to plants via their growth media - soil, air, nutrients by their roots or foliage (Okoronkwo *et al.*, 2005). Growing plants in a polluted soil can accumulate the toxic metals at high concentration causing serious human health when consumed (Voustal *et al.*, 1996; Alloway, 1990). Many heavy metals are biological poisons even at very low concentration. The toxic metals accumulate in organic matter component of soils and are taken-up, translocated by growing plants (Mukesh, 2008). Long term exposure to heavy metal leads to chronic problems like toxicological effect on kidney, mental lapse, liver and gastrointestinal tract, skin poisoning and harmful effect on the central nervous system (Lennotech, 2009). The threat that

heavy metals pose to human, animal health and ultimately economic advancement is aggravated by their low environmental mobility and their long term persistence in the environment (Chamannejadian, 2011). Heavy metals alteration of soil is a major health concern needing preventive and remediation response to avoid its ecological consequences (Begun *et al.*, 2009; Galiulin *et al.*, 2002) which leads to developmental drawback. Therefore, this study focused on the use of *Klebsiella pneumoniae* for bioremediation of lead contaminated agricultural soil used for sustainable farming.

### MATERIALS AND METHOD

**Materials:** Soil, McCartney bottles, hot plate, autoclave, measuring cylinder, microscope, wire loops, pipette, conical flasks, inoculating needles, refrigerator, whatman filter paper, petri dishes, beakers, cotton wool, incubator, Atomic Absorption Spectrophotometer.

**Reagents:** These include oxidase reagent, Lugo's iodine, hydrochloric acid, nitric acid, ethanol, Kovac's reagent, perchloric acid and sulphuric acid; methylene blue, crystal violet, hydrogen peroxide, sodium hydroxide and safranin.

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**Soil Sampling:** Soil samples were collected from heavy metals contaminated site at Amaonye forest in Ishiagu clan, Ebonyi State of Nigeria by using spatula and stored in polythene bag. The samples were aseptically transported to the laboratory at the Department of Micro Biology of the Delta State University, Nigeria for bacteriological analysis.

**Agars/Nutrients Preparation:** Sticking to manufacturers' design and the technique in Cheesebrough (2000), 28, 15, 24, and 65 grams of powered nutrient agar, Simon citrate agar, peptone water powder and triple sugar iron agar were respectively dissolved for ten minutes in one liter of distilled water. For 15 minutes, and at temperature of 121°C and pressure of 1.5 psi, the solutions were autoclaved and cooled to 45°C for use.

**Characterization of Bio-sorbent:** Using the pure plate method, 0.1ml from serial dilution of 10<sup>-1</sup>, 10<sup>-3</sup> and 10<sup>-5</sup> was inoculated into sterile Petri-dishes; and topped with nutrient agar (Cowan, 1993; Baron *et al.*, 1994). The inoculated dishes were inverted and incubated at 37°C for 24 hours (Cheesebrough, 2000) and growth that developed were counted, recorded and sub cultured. The isolates were characterized and recognized using (Cowan and Steel, 1990; Holt *et al.*, 1994) methods; and biochemical tests were conducted by following the methods in Cheesebrough (2000).

**Factors screening for 2<sup>n</sup> factorial Design of Experiment:** Factorial design of experiment requires the imputation of lowest and highest values of factors into the requisite software. These values were obtained through initial screening of factors. To achieve this, harvested 24 hours old *Klebsiella pneumoniae* was inoculated into soil samples conditioned with varied values of factors: 2, 4, 6, 8, 10 and 12 ml dosage of nutrient, 1, 2, 3, 4, 5, and 6g of organism's mass, 10°C, 20°C, 30°C, 40°C, 50°C and 60°C of temperature, 4, 5, 6, 7, 8, and 9 of pH and 0, 1, 2, 3, 4 and 5 per week (pw) of stirring frequency in thirty 50 ml beaker containing 3g of soil each (Atikpo, 2016). These experiments were conducted in triplicate making a total of ninety (90) experiments and in accordance to the method in (Lima *et al.*, 2007); the average values were determined from their impact on the residual lead

ion in soil with an Atomic Absorption Spectrophotometer (GBC SensAA, Model no A6358) at time 14 days.

**Determination of Effects of Factors by Statistical Design of Experiment:** The treatment of lead ions from the soil followed the batch experiment procedure in (Lima *et al.*, 2007). The Design Expert version 7.0 was engaged in an experimental design of two levels, 2<sup>n</sup> factorial. Where n is the number of factors and were set at five (5) in this study. The factors were utilized to design a total of 32 experiments. These factors set as five independent variables were coded as -1 (low) and +1 (high) as shown in Table 1 and utilized as the experimental conditioners in thirty-two 50 ml capacity beakers stipulated by the Design Expert 7.0 and each beaker containing 3g of the soil samples. 24 hours old *Klebsiella pneumoniae* was harvested and inoculated in soils contained in each beaker for a period of 35 days (Atikpo, 2016). By this period, the bacterium was centrifuged from the soil, and the residual lead ion content determined with Atomic Absorption Spectrophotometer (GBC SensAA, Model no. A6358). The amount of the metal ion removed, removed with time, and the percentage removals were evaluated, using Equations (1), (2) and (3) respectively (Cleiton *et al.*, 2011, Badmus *et al.*, 2007).

$$q = \frac{(c_o - c_f)}{m} \cdot V \quad (1)$$

$$q_t = \frac{(c_o - c_t)}{m} \cdot V \quad (2)$$

$$\% \text{ Removal} = \frac{(c_o - c_f)}{c_o} \cdot 100 \quad (3)$$

Where q is lead ion (mg/kg) removed; q<sub>t</sub> is lead ion (mg/kg) removed with time t, C<sub>o</sub> is the initial concentration (mg/kg) of lead ion in contact with the bacterium, C<sub>f</sub> is the final concentration (mg/kg) of lead ion, V is soil volume (m<sup>3</sup>) in contact with the bacterium; and m is the bacterium's mass expressed in (g).

The resultant laboratory data was analyzed with Design Expert version 7.0, to generate the requisite information for decision making.

**Table 1.** The High and Low Levels of Factors for Design

Levels	Factors				
	Nutrient (A) (ml)	Mass of Organism (B) (g)	Temperature (C) (°C)	pH (D)	Stirring Frequency (E) (pw)
Low (-1)	8	1	30	7	4
High (+1)	10	4	40	9	5

## RESULTS AND DISCUSSION

**Characterization:** Microbiological study carried out for the isolation and identification of organism

revealed the selected organism from the biochemical characterization of the colony of 2.6 x 10<sup>2</sup> cfu/ml which developed after incubation. The bacterium was

distinguished with the respective biochemical indications of positive gram stain, negative catalase, negative oxidase, positive indole, positive citrate, negative glucose, negative lactose, H<sub>2</sub>S and motility as *Klebsiella pneumoniae*.

*Statistical Study of Factors for Optimal Bioremediation:* The experimental results of the percentage removal of lead by the organism as obtained based on the statistical design were recorded as shown in Table 2 and analyzed with Design Expert 7.0.

**Table 2.** Experimental Result on Statistical Study of Factors

Run	1	2	3	4	5	6	7	8	9	10	11
% Removal	50.43	70.44	64.23	70.43	70.23	76.32	70.32	70.54	70.42	50.01	64.38
Run	12	13	14	15	16	17	18	19	20	21	22
% Removal	73.35	64.23	73.57	73.93	73.67	70.32	64.21	70.11	50.64	50.86	64.37
Run	23	24	25	26	27	28	29	30	31	32	
% Removal	45.52	64.78	70.43	64.78	70.65	64.32	64.78	64.56	73.24	50.43	

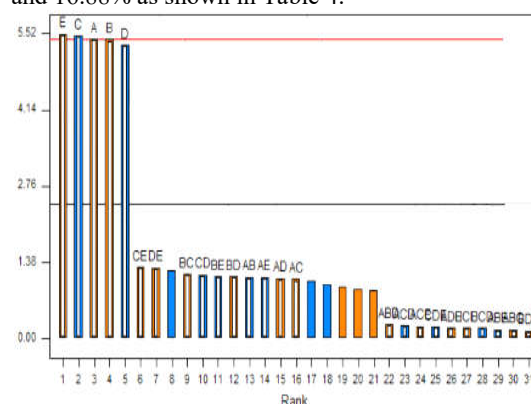
The useful analytical informative derivations are the goodness of fit parameters, analysis of variance (ANOVA), Pareto charts and factors' effects list. The ANOVA informed on the factors and their interactions significance through the displayed probability values. Reliability of model was confirmed by the model's coefficient of determination (R<sup>2</sup>) and the adjusted R<sup>2</sup>. The respective high values of R<sup>2</sup> and the adjusted R<sup>2</sup> confirmed the model as adequate. Relying on the adequacy of model, the factors' effects lists and Pareto chart were generated. The relevance of the effects list is the revelation of the standardized effects and the percentage contributions of both the factors and their combinations. The Pareto chart permitted the detection of the factors and interaction effects of more relevance to the removal process by displaying the absolute values of the effects and drawn reference line. Any factor effect that extends beyond the reference line is significant. The placement of the significance of every factor and interaction depends on the value of the coefficient of each factor or its interaction (Pavan *et al.*, 2007). The analysis indicated the model to be reliable. This was abundantly confirmed by the model's coefficient of determination (R<sup>2</sup>) and the adjusted R<sup>2</sup> with their respective values of 0.9642 and 0.8149 shown in Table 3.

**Table 3.** Parameters for Model Adequacy

Standard Deviation	3.64	R-Squared	0.9642
Mean	65.33	Adjusted R-Squared	0.8149
C.V %	5.57	Predicted R-Squared	-
			0.0192
PRESS	2262.29	Adequacy of Precision	10.101

The ANOVA conducted showed the single factors in the order of temperature and stirring frequency with P values of 0.0015, volume of nutrient and mass of organism with P value of 0.0016 and pH with P value of 0.0018 significant at 95% confidence level (P<0.05); while none of the combined factors had significant effects since the probability value was greater than 0.05 (P>0.05). The ANOVA analysis showed that only the single factors were significant.

The Pareto chart revealed the ranking of these factors with their contributory values as shown in Figure 1. Factors favoring *Klebsiella pneumoniae* remediation capability were ranked in the order of stirring frequency, temperature, volume of nutrient, mass of organism and pH with their respective percentage contributions of 18.20%, 17.97%, 17.57%, 17.52% and 16.88% as shown in Table 4.



**Fig 1.** Pareto Chart Ranking of Selected Factors

**Table 4.** Summary Parameters

R <sup>2</sup> =0.9642 Adj. R <sup>2</sup> =0.8149		
Ranking	P < 0-05 at 95% confidence level	% Contribution
SF	0.0015	18.20
T	0.0015	17.97
VN	0.0016	17.57
MO	0.0016	17.52
pH	0.0018	16.88

VN: Volume of Nutrient, MO: Mass of Organism, T: Temperature, SF: Stirring Frequency.

*Conclusion:* Contaminated soil leads to contaminated food and ultimately sickly society. Therefore, a way of making contaminated soil uncontaminated by applying *Klebsiella pneumoniae* for soil remediation was the focus of this study. The organism was found to reduce the initial concentration of lead from 181.41 mg/kg to below the allowable concentration of 100 mg/kg. This concentration reduction first occurred on the 14<sup>th</sup> day and further reduced with time. This Knowledge is vital for soil remediation planning and practice.

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