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 SensAA, 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 (Voustat 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 (Lenntech, 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; Galilin 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.
Soil Sampling: Soil samples were collected from heavy metals contaminated site at Amaonye forest in Ishiuagu clan, Ebenyi 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 10⁻¹, 10⁻² and 10⁻⁵ 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 2ⁿ 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, 2ⁿ 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).

\[
q = \frac{(c_0 - c_f)}{V} \quad (1)
\]

\[
q_t = \frac{(c_0 - c_f)}{m} \quad (2)
\]

\[
% \, \text{Removal} = \frac{(c_0 - c_f)}{c_0} \cdot 100 \quad (3)
\]

Where q is lead ion (mg/kg) removed; qₜ is lead ion (mg/kg) removed with time t, C₀ is the initial concentration (mg/kg) of lead ion in contact with the bacterium, C₈ is the final concentration (mg/kg) of lead ion, vis soil volume (m³) 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.

| Levels                  | 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 10² cfu/ml which developed after incubation. The bacterium was

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distinguished with the respective biochemical indications of positive gram stain, negative catalase, negative oxidase, positive indole, positive citrate, negative glucose, negative lactose, H₂S and motility as Klebsiella pneumoniae.

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

| Run | % Removal |
|-----|-----------|
| 1   | 50.43     |
| 2   | 70.44     |
| 3   | 64.23     |
| 4   | 70.43     |
| 5   | 70.23     |
| 6   | 76.32     |
| 7   | 70.32     |
| 8   | 70.54     |
| 9   | 70.42     |
| 10  | 50.01     |
| 11  | 64.38     |

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.

### Table 3. Parameters for Model Adequacy

| Standard Deviation | R-Squared | Adj. R-Squared | P < 0.05 at 95% confidence level | Contribution |
|--------------------|-----------|----------------|-------------------------------|--------------|
| 3.64               | 0.9642    | 0.9642         | 0.0015                        | 18.20        |
| Mean               | 65.33     | 81.14          | 0.0016                        | 17.97        |
| C.V %              | 57.57     | 0.0192         | 0.0016                        | 17.52        |
| PRESS              | 2262.29   | 10.101         | 0.0016                        | 16.88        |

### Table 4. Summary Parameters

- **SF**: 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 14th day and further reduced with time. This knowledge is vital for soil remediation planning and practice.
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