



Performance Evaluation of Six Microorganisms Utilized for the Treatment of Lead Contaminated Agricultural Soil

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ABSTRACT: This work focused on the ability of six indigenous microorganisms (*Arthrobacter nicotinae*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Trichophyton interdigitale* and *Onychocola canadensis*) isolated from contaminated agricultural soil obtained from Ishiagu forest in Ebonyi State, Nigeria for the removal of lead (Pb) ion from the soil. The weights of organisms, nutrient dosage, temperature, pH and stirring frequency were optimized for the removal of the metal ion from the soil. The data revealed that *Bacillus subtilis*, *Klebsiella pneumoniae* and *Arthrobacter nicotinae* were found to reduce the metal concentration below recommended standard at time 14 days with respective efficiencies of 58.25 %, 60.64 % and 60.80 % at the 14 days; and 78.25 %, 76.19 % and 79.78 % at time 35 days; while *Proteus mirabilis*, *Onychocola canadensis* and *Trichophyton interdigitale* were found to reduce the metal concentration at time 21 days with respective efficiencies of 51.74 %, 44.65 % and 48.08 % at time 21 days; and 59.67 %, 51.13 % and 55.27 % at time 35 days. The study showed that the organisms can be utilized to remove Pb ion from the contaminated soils. *Arthrobacter nicotinae* showed the greatest removal capacity with time, followed by *Bacillus subtilis*, and then *Klebsiella pneumoniae*; before *Proteus mirabilis*, *Trichophyton interdigitale* and *Onychocola canadensis* in that order.

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Pollution through heavy metals has emerged a serious environmental issue these days (Vargas – Garcia *et al.*, 2012); and pollution of arable land was turning intense in the world (Shu *et al.*, 1998). This has also become the case of Amaonye – Ishiagu forest soils in Ebonyi State of Nigeria. The soils, as reported by Atikpo (2016); and Ezeh and Chukwu (2011) are highly polluted by lead. This pollution status was linked with metals mining in the forest (Atikpo, 2016; Ezeh and Chukwu, 2011; Nwanguo, 2008). Soils, if polluted, transmit pollution to plants, air and water bodies; and further pose a serious threat to plants, animals and humans (Atikpo, 2016). Lead pollution is to be resisted because of its dangerous effects on the ecosystem. Lead, an environmental contaminant, lacks biological role but toxic to microorganisms (Sobolev and Begonia, 2008). At low concentration even, it is highly toxic and goes with high health implications (Begum *et al.*, 2009) such as intelligence reduction in children (Badawy *et al.*, 2013), anemia, encephalopathy, renal dysfunction and palsy (Athar and Vohora, 2006). This has necessitated the protection of our soils from lead pollution. And in situations where soils are polluted by this metal, treatment option should not be handled with levity.

Different treatments methods are available. These include physical, chemical and biological approaches. Bioremediation, a biological approach to lead removal from soil has proven to be the most cost effective and eco - suitable method. The findings of previous research works on the edge of bioremediation over other remediation approaches are the reasons for the selection of indigenous microorganisms utilized in this work to bring treatment solution to Amaonye contaminated agricultural land – with the major aim of selecting the most effective organism through the process of comparative study. This work also seeks to provide contribution to the existing knowledge on the suitability of these organisms for lead removal from contaminated soils.

MATERIALS AND METHODS

Materials and Preparation: Materials: Soil samples collected from Amaonye forest in Ishiagu communities, Ebonyi State in Nigeria, Petris dishes, beakers, cotton wool, measuring cylinders, microscope, inoculating needles, refrigerator, McCartney bottles, hot plate, autoclave, whatman

filter papers, incubator, atomic absorption spectrophotometer, wire loops, pipette, conical flasks. **Reagents:** These include nitric, perchloric and sulphuric acids; methylene blue, crystal violet, Lugo's iodine, hydrogen peroxide, ethanol, oxidase reagent, hydrochloric acid, Kovac's reagent, sodium hydroxide and safranin.

Agars/Nutrients and Preparation: Adhering to manufacturers' directions and the method of Cheesebrough (2000), 28, 52, 39, 24, 65 and 15 grams of powered nutrient agar, MacConkey agar, potato dextrose agar, Simon Citrate agar, triple sugar iron agar and peptone water powder respectively were soaked and swirled in one liter each of distilled water for 10 minutes. The solutions were autoclaved for 15 minutes, at 121°C and 1.5 psi and cooled to 45°C before usage.

Characterization of Organisms: Bacteriological and mycological studies were conducted on the soil samples in Micro Biology Laboratories of the Delta State University, Abraka, Delta State, Nigeria. Applying the pure plate techniques, 0.1 ml each of serial diluted solution was inoculated into various sterile Petri-dishes labeled for nutrient, MacConkey and potato dextrose agars respectively from 10^{-1} , 10^{-3} and 10^{-5} diluents; and distributed on with media (Cowan, 1993 and Baron *et al.*, 1994). The respective inoculated plates with Nutrient agar and MacConkey agar, being inverted, were incubated at a temperature of 37°C for 24 hours, while the potato dextrose agar plates were placed at temperature of 28°C for 48 hours to 72 hours (Cheesebrough, 2000). Microorganisms Colonies that developed were counted, recorded and sub cultured. The bacterial isolates were characterized and identified with the methods given by (Holt *et al.*, 1994; Cowan and Steel, 1990). The fungi isolates were identified according to the procedure given by (Barnett and Hunter, 1972; Hanigan and MacCane, 2002). Biochemical tests for bacterial were performed in accordance with the methods of Cheesebrough (2000).

Optimal Factors for Remediation Study: The purpose of this is to select the optimal values of factors (dosage of nutrient, weights of organisms, temperature, pH and stirring frequency) necessary for lead removal capabilities evaluations. The evaluations were carried out in triplicate (Lima *et al.*, 2007; Atikpo, 2016) with varied nutrient dosage of 2, 4, 6, 8, 10, 12 and 14 ml introduced into eighteen 50 ml beakers with 3g each of soil and inoculated with 24 and 48 hours old bacteria and fungi respectively for 14 days. The soils were evaluated for lead concentration with Atomic Absorption Spectrophotometer (GBC SensAA, Model no. A6358) on the 14th day, following the elimination

of the organisms from the samples with a centrifuge. The same process was followed to determine the optimal values of organism' weights, temperature, pH and stirring frequency from 1, 2, 3, 4, 5, 6, and 7g organisms; 20, 25, 30, 35, 40 and 45°C temperature for bacteria and 10, 15, 20, 25, 30 and 35°C for fungi; 4, 5, 6, 7, 8, 9 and 10 of pH; and 0, 1, 2, 3, 4, 5 and 6 per week (pw) stirring frequency respectively. The optimal values of the studied factors were selected and recorded.

Determination of Removal Capabilities: This was carried out in triplicate with the six individual organisms to evaluate their performances on lead removal from the soil using the methods of Lima *et al.* (2007) and Atikpo (2016). The selected optimal weights of the organisms were inoculated into 3g each of soil in ninety 50 ml beakers (eighteen for each organism); and the samples (soils) conditions were adjusted to the selected optimal values of factors in the respective order of organisms' weights (g), dosage of nutrient (ml), temperature (°C), pH and stirring frequency (pw) of 1g, 8 ml, 30°C, 7 and 6 pw for *Proteus mirabilis*; 5g, 8 ml, 30°C, 8 and 6 pw for *Bacillus subtilis*; 5g, 12 ml, 30°C, 7 and 6 pw for *Klebsiella pneumoniae*; 1g, 10 ml, 25°C, 7 and 6 pw for *Onychocola canadensis*; 1g, 8 ml, 25°C, 6 and 6 pw for *Trichophyton interdigitatae*; and 1g, 6 ml, 30°C, 7 and 6 pw for *Arthrobacter nicotinae*. The soils residual lead ion was evaluated at times 7, 14, 21, 28 and 35 days with Atomic Absorption Spectrophotometer (GBC SensAA, Model no. A6358) after centrifuging to eliminate the organisms from the soil samples.

The lead ion removed with time in (mg/kg) and the efficiency of removal in (%) were determined from Equations (1) and (2) (Badmus *et al.*, 2007).

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

$$\varepsilon = \frac{(C_o - C_f)}{C_o} \cdot 100 \quad (2)$$

C_o , C_t , C_f , V and m are the initial lead ion in mg/kg present in soil; residual lead ion in soil with time in mg/kg, final lead ion (mg/kg) in soil, volume (m^3) of soil used and the mass (g) of organisms utilized.

RESULTS AND DISCUSSION

Microbiology Analysis: Microbiological analysis carried out towards the isolation and identification of organisms yielded the selected organisms from the biochemical distinguishing of characters among the colonies (2.8×10^4 cfu/ml and 1×10^3 sfu/ml) that developed after incubation. The bacteria were distinguished with the respective biochemical

indications of gram stain, catalase, oxidase, indole, citrate, glucose, lactose, H₂S and motility as *Arthrobacter nicotinae* (positive, negative, negative, positive, positive, positive, negative and negative); *Bacillus subtilis* (positive, positive, negative, negative, positive, positive, negative and positive); *Klebsiella pneumoniae* (positive, negative, negative, positive, positive, negative, negative and negative); *Proteus mirabilis* (positive, negative, negative, negative, positive, positive, positive and positive); and the fungi characterized as documented in Table 1.

Optimum Factors: Vital factors have been discovered to have significant influence on biosorption process and rate (Atikpo *et al.*, 2015; Murthy *et al.*, 2012). This has necessitated that these factors are studied with an aim to select their optimum values necessary for this biosorption study (Atikpo, 2016). The impacts of 2, 4, 6, 8, 10, 12 and 14 ml of nutrient; 1, 2, 3, 4, 5, 6, and 7g organisms; 20, 25, 30, 35, 40 and 45^oC temperature for bacteria and 10, 15, 20, 25, 30 and 35^oC for fungi; 4, 5, 6, 7, 8, 9 and 10 of pH; and 0, 1, 2, 3, 4, 5 and 6 per week (pw) of stirring frequency on Pb biosorption by the organisms were studied for 14 days to obtain optimum values through screening. A criterion of least concentration remaining was engaged in identifying these optimum factors as shown in Table 2. The respective optimum values of nutrients' volumes, organisms' weights, temperature, pH and stirring frequency were recognized and selected at the respective least concentration remaining of 101.11, 103.93, 93.51, 94.12 and 95.12 mg/kg for *Bacillus subtilis*; 123.42, 124.32, 106.12, 117.61 and 115.32 mg/kg for *Proteus mirabilis*; 99.28, 102.64, 96.11, 97.35 and 92.09 mg/kg for *Klebsiella pneumoniae*; 103.46, 119.45, 110.14, 117.43 and 120.31 mg/kg for *Onychocola canadensis*; 100.29, 117.37, 116.27, 120.32 and 119.11 mg/kg for *Trichophyton interdigitale*; and 88.51, 95.58, 92.42, 96.16 and 94.66 mg/kg for *Arthrobacter nicotinae*.

Removal Capabilities: Figure 1 is a graphical display of the influences exerted by the organisms for the removal of the metal. The organisms' order of removal influence is *Arthrobacter nicotinae*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Trichophyton interdigitale* and *Onychocola Canadensis*. And the removal of this metal by the microorganisms showed similar trend, and increased generally with time. These organisms were able to reduce this metal from the initial concentration of 181.41 mg/kg to a concentration below the maximum allowable concentration of 100 mg/kg specified in (World Health Organization – WHO, 2001; Pandias, 1992); and this occurred at different times for the organisms depending on their removal strengths. This

occurred for *Proteus mirabilis* at time 21 days, with a residual concentration of 87.54 mg/kg and concentration removed of 93.81 mg/kg and an efficiency of 51.74 %.

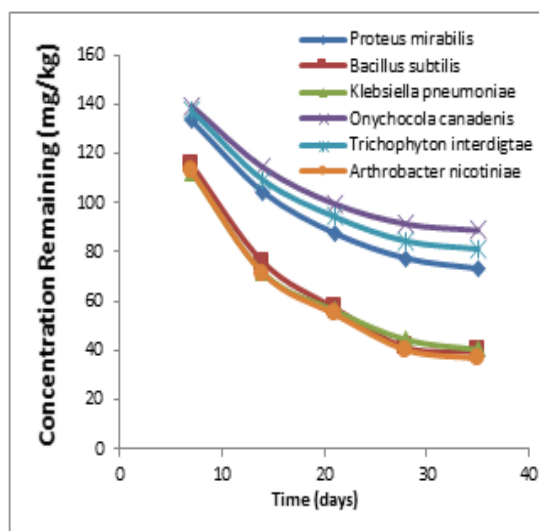


Fig 1. Comparative Removal of Lead by the Microorganisms

This residual concentration fell with time until it got to a concentration value of 73.17 mg/kg at time 35 days with an amount removed of 108.24 mg/kg and 59.67 % efficiency. For *Bacillus subtilis*, the soil Pb ion fell below the maximum allowable at time 14 days with a residual concentration of 75.74 mg/kg at an amount removed of 105.74 mg/kg, and efficiency of 58.25 %. This residual concentration fell until it reached 39.46 mg/kg on the 35th day with a removed concentration of 141.95 mg/kg and 78.25 % efficiency. *Klebsiella pneumoniae* removed the metal below the maximum allowable concentration at time 14 days. The concentration remaining at this time was 71.41 mg/kg with an amount removed of 102.26 mg/kg and 60.64 % efficiency. This concentration remaining further dropped with time until it got to a value 43.20 mg/kg at time 35 days, which is a concentration after a removal of 133.65 mg/kg with 76.19 % removal efficiency. Unlike the organisms in the two preceding paragraphs, *Onychocola canadensis* dropped the lead concentration below the maximum allowable limit at time 21 days with a concentration remaining of 99.42 mg/kg at an amount removed of 81.99 mg/kg and 44.65 % efficiency. This concentration remaining further fell with time until it hit a concentration value of 88.65 mg/kg at time 35 days with an amount removed of 92.76 mg/kg and a removal efficiency of 51.13 %. Similarly, *Trichophyton interdigitale* reduced the concentration below the maximum allowable concentration at time 21 days with a value of 94.21 mg/kg for the residual concentration at an amount removed of 87.20 mg/kg and 48.07 % removal

efficiency. This residual concentration dropped with time to a value of 81.14 mg/kg at time 35 days, with an amount removed of 100.27 mg/kg at a removal efficiency of 55.27 %. The concentration remaining under the influence of *Arthrobacter nicotinae* came below the maximum allowable at time 14 days with a residual concentration value of 71.12 mg/kg at an

amount removed of 110.29 mg/kg and efficiency of 60.80 %. There was a fall in the residual concentration with time until it hit a value of 36.68 mg/kg at time 35 days. This happened at an amount removed of 144.73 mg/kg and 79.78 % removal efficiency. These are summararily shown in Table 3.

Table 1. Identification of Fungal Isolates

Isolates	Cultural Characteristics	Morphological Characteristics	Identified Organism
Organism 1	Large fluffy white cottony colonies which later turned black as culture ages.	Non-septate hyphae with upright sporangiophore connected by stolon and rhizoids bearing dark peer-shaped sporangium on hemispherical columella.	Trichophyton interdigitatae
Organism 2	White mycelia which covered the surface of plate rapidly and turned with age.	Hyphae were non-septate, sporangiophore were long branched and bore terminal sporangia.	Onychocola canadensis

Table 2. Selected Optimal Factors for Biosorption Studies

Factors	Micro Organisms Utilized					
	Proteus Mirabilis	Bacillus Subtilis	Klebsiella Pneumoniae	Onychocola canadensis	Trichophyton Interdigitatae	Arthrobacter nicotinae
Weight of Organism (g)	1	5	5	1	1	1
Volume of Nutrient (ml)	8	8	12	10	8	6
Temperature (°C)	30	30	30	25	25	30
pH	7	8	7	7	6	7
Stirring Frequency (pw)	6	6	6	6	6	6

Table 3: Information on Lead Pollution Control with the Organisms

Organism	Time in (Days) to Reduce Lead Ion to Below Standards	Residual Lead Ion at initial time of Pollution Control (mg/kg)	Efficiency (%)	Residual Concentration at 35 Days in (mg/kg)	Efficiency (%) at 35 Days
Proteus mirabilis	21	87.54	51.74	73.17	59.67
Bacillus subtilis	14	75.74	58.25	39.46	78.25
Klebsiella pneumonia	14	71.41	60.64	43.20	76.19
Onychocola Canadensis	21	99.42	44.65	88.65	51.13
Trichophyton interdigitatae	21	94.21	48.07	81.14	55.27
Arthrobacter nicotinae	14	71.12	60.80	36.68	79.78

Conclusion: The study showed that these organisms can be utilized to remove Pb ion from *Amaonye – Ishiagu forest soils*. *Arthrobacter nicotinae* showed the greatest removal capacity with time, followed by *Bacillus subtilis*, and then *Klebsiella pneumoniae*; before *Proteus mirabilis*, *Trichophyton interdigitatae* and *Onychocola canadensis* in that order. The removal of this metal by the microorganisms followed similar trends; and closeness in removal abilities is seen in removals by *Arthrobacter nicotinae*, *Bacillus subtilis* and *Klebsiella pneumoniae*.

REFERENCES

Athar, M; Vohora, SB (2006). Heavy Metals and Environmental. New Age International (P)

Limited Publishers, 4835/24, Ansari Road, Daryaganji, New Delhi-110002.

Atikpo, E (2016). Spatial Distribution and Attenuation of Heavy Metals Pollution in Amaonye-Ishiagu Forest Soils. A PhD Thesis Submitted to the Department of Civil Engineering, University of Benin, Benin City, Nigeria.

Atikpo, E; Anyata, BU; Mokwenye, II (2015). Variation of Factors for Metals Removal: a Guide to Factors Selection for Bioremediation of Heavy Metals Polluted Soils in Amaonye-Ishiagu of Ebonyi State in Nigeria. *J. Civ. Environ. Sys. Engi.*13:172-181.

- Badawy, RK; El-Gawad, AMA; Osman, HE (2013). Health Risks Assessment of Heavy Metals and Microbial Contamination in Water, Soil and Agricultural Foodstuff from Waste Water Irrigation at Sahl El-Hessania area, Egypt. *J. App. Sci. Resea.* 9(4):3091-3107.
- Badmus, MAO; Audu, TOK; Anyata, BU (2007). Removal of Lead Ion from Industrial Wastewaters by Activated Carbon Prepared from Periwinkle Shells (*Typanotonusfuscatus*). *Turk. J. Envi. Engin. Sci.* 31: 251-263.
- Barnet, HL; Hunter, BB (1972). *Illustrated General of Imperfect Fungi*. Burgess Publishing Company, Minneapolis.
- Baron, EJ; Peterson, LR; Finegold, SM (1994). *Bailey and Scotts Diagnostic Microbiology*. 9th Edition. Mosby, Baltimore.
- Begum, A; Ramaiah, M; Harikrishna, O; Irfanulla, K; Veena, K (2009). Analysis of Heavy Metals Concentrations in Soil and Lichen from Various Localities of Hosur Road, Bangalore, India, CODEN ECJHAO, E-A. *J. Chem.* 6(1): 13-22.
- Chessebrough, M (2000). *District Laboratory Practice in Tropical Countries*. Part 2, Cambridge low Price Edition, Cambridge University Press, London.
- Cowan, ST (1993). *Cowan and Steels Manual for the Identification of Medical Bacteria*. Cambridge University Press, London.
- Cowan, ST; Steel, R (1990). *Manual for the Identification of Medical Bacteria*. Cambridge University Press, London.
- Ezeh, HN; Chukwu, E (2011). Small Scale Mining and Heavy Metals Pollution of Agricultural Soils: the Case of Ishiagu Mining District, South Eastern Nigeria. *J. Geo. Min. Rese.* 3(4): 87-104.
- Hanigan, WJ; McCane, EM (2002). *Laboratory Methods in Foods and Dairy Microbiology*. Fourth Edition, Academic Press, London, New York.
- Holt, JC (1994). *The Shorter Bergeys Manual of Determinative Bacteriology*. Eight Edition, Williams and Willkins Company, Baltimore.
- Lima, EC; Royer, B; Vaghetti, JCP; Brasil, JL; Simon, NM; Dos S Jr., AA; Pavan, FA; Dias, SLP; Benvenuto, EV; Da Silva, EA (2007). Adsorption of Cu (II) on *Araucaria Angustifolia* wastes: Determination of the Optimal Conditions by Statistic Design of Experiments. *J. Hazar. Mate.* 140: 211-220.
- Murthy, S; Bali, G; Sarangi, SK (2012). Biosorption of Lead by *Bacillus cereus* Isolated from Industrial Effluents. *Brit. Biotech. J.* 2(2): 73-84.
- Nwaugo, VO; Onyeagba, RA; Akubugwo, EI; Ugbogu, S (2008). Soil Bacterial Flora and Enzymatic Activities in Zinc and Lead Contaminated Soil. *BIOK.* 20(2): 77-84.
- Pendias, AK; Pendias, H (1992). Elements of Group VIII. In: *Trace Elements in Soils and Plants*. Boca Raton: CRC Press.
- Shu, JM; Wang, JJ; Liu, XC (1998). Ecological Restoration of Mining Wasted Lands, China Population. *Resou. Envir.* 8(3): 72-75.
- Sobolev, D; Begonia, MFT (2008). Effects of Heavy Metal Contamination upon Soil Microbes: Lead-Induced Changes in General and Denitrifying Microbial Communities as Evidenced by Molecular Markers. *Int. J. Environ. Res. Pub. Hea.* 5(5): 451.
- Vargas-Garcia, MDC; Lopez, MJ; Suarez-Estrella, F; Moreno, J (2012). Compost as a Source of Microbial Isolates for the Bioremediation of Heavy Metals: in Vitro Selection. *Sci. Tot. Envir.* 431: 62-67.
- World Health Organization (2001). *Health Guidelines for the Use of Waste Water in Agriculture and Aquaculture*. Report of a Scientific Group Meeting, Technical Report Series, No. 778, World Health Organisation, Geneva.