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Potential of *Arthrobacter nicotiniae* to Preferentially Remove Lead, Cadmium, Silver and Zinc from Contaminated Soil from Amaonye-Ishiagu Agricultural Forest, Ebonyi State, Nigeria

***[1]ETUKE, JO; [2]IHIMEKPEN, NI**

*[1]*Department of Civil and Environmental Engineering, Faculty of Engineering, Delta State University, Oleh Campus, PMB 1, Abraka, Delta State, Nigeria.*
[2]*Department of Civil Engineering, Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria*

**Corresponding Author Email: juliusetuke@yahoo.com*
Co-Author Email: n.ihimekpen@uniben.edu

ABSTRACT This study evaluates the potential of *Arthrobacter nicotiniae* to preferentially remove lead, cadmium, silver and zinc from contaminated soil from Amaonye-ishiagu Agricultural Forest, Ebonyi State, Nigeria using standard methods. The study revealed optimum values of factor as 8ml of nutrient volume, 5g of the organism, 30ºC of temperature, pH of 8 and stirring frequency of 4pw. The study showed 79.78% removal of lead, 61.50% removal of cadmium, 48.59% removal of arsenic and 7.04% removal of zinc on the 35th day. The organism is thus good for lead and cadmium pollution control, arsenic pollution abatement but it is neither good for zinc pollution control, nor abatement.

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Soil is a crucial component of environments (Atikpo, 2016). It fulfills a wide range of important and inter-related functions (Narayanan, 2007; Atikpo et al., 2017a) and is unfortunately a repository for anthropogenic wastes. These wastes, through biochemical processes, can pollute water bodies and impact food chains (Adigun and Kayode, 2019; Atikpo et al., 2021). Therefore, the sustainable use of soil is necessary to prevent damage to it and keep it in a good state to support a wide range of functions (Narayanan, 2007). It is impossible to visualize a soil without trace levels of heavy metals (HMs) (Atikpo, 2016, Atikpo et al., 2017b). HMs occur naturally in rocks and soils, but chiefly in forms that are not available to living organisms, such as constituents and replacement in rock and soil minerals (Atikpo, 2016). Increasingly higher quantities of heavy metals are being released into the environment by anthropogenic activities, primarily associated with industrial processes, manufacturing and disposal of industrial and domestic refuse and waste materials (Dixit, 2015). HMs contamination of soil is a worldwide problem that affects a large number of sites (Gray et al., 2006; Dixit, 2015).

HMs are of major concern because of their toxicity and threat to human life and the environment (Kure et al., 2018; Atikpo and Ihimekpen, 2020) and they can enter the ecosystem, leading to geoaccumulation and bioaccumulation. Due to the worldwide experience of the serious ecological consequences of soils contaminated with heavy metals, there is a need to take

**Corresponding Author Email: juliusetuke@yahoo.com*

preventive and treatment measures (Salawu et al., 2014). Conventional treatment approaches like physico-chemical methods of remediating site polluted by heavy metals may present a complex situation and may also lead to the formation of unknown toxic intermediates and more toxic end-products in the soil (Kure et al., 2018; Atikpo and Eboibi, 2020). In addition, physical-chemical methods are usually not effective and very expensive when the concentrations of the metals are very low (Kure et al., 2018).

Therefore, bioremediation is an attractive alternative to physic-chemical methods. Previous works reported that some microorganisms such as bacteria, algae, yeast, fungi, and cellulosic materials have capabilities to remove a large amount of metal ions from soils and water (Sulaimon et al., 2014, Ihimekpen et al., 2020). This works seeks to explore the potential of *Arthrobacter nicotiniae* (*A. nicotiniae)* for removing Pb, Cd, As and Zn from the contaminated agricultural forest soil in Amaonye-shiagu, Ebonyi State, Nigeria. Cases of heavy metals pollution have documented for the community as a result of mining activities (Ezeh and Chukwu, 2011; Atikpo and Ihimekpen, 2018). And the community is known for farming of good quantities of yam, rice, okro and leafy vegetables for consumption and supplies to other parts of Nigeria (Atikpo, 2016).

Therefore, the soils require remediation to make it fit for crop production in line with millennium development goals. Hence, the objective of this research work is to evaluate the potential of *A. nicotiniae* to preferentially remove lead, cadmium, silver and zinc from contaminated soil from Amaonye-ishiagu Agricultural Forest, Ebonyi State, Nigeria.

MATERIALS AND METHODS

*Identification of Microorganism***:** Contaminated soils collected from Ishiagu agricultural forest were transported to the Microbiology Laboratory at the Delta State University, Abraka, Delta State in Nigeria where microbiology analysis was carried out.

Following the procedure in (Chessebrough, 2000) and the manufacturer's directive, nutrient agar was prepared by dispensing 28g of powder nutrient agar in a liter of distilled water. The solution was left for 10 minutes, and swirled to allow for proper dissolution. The resultant solution was sterilized for 15minutes at 1.5 psi and 121^0C; and was cooled to 45^0C, mixed again. Aliquot (0.1ml) of serial diluted soil samples was inoculated into the sterile Petri-dishes. The media was distributed into the respective petri-dishes (Baron et al., 1994; Atikpo and Micheal, 2018). The inverted inoculated plates were incubated at 37^{o}C for 24 hours; and then examined for growth (Chessebrough, 2000). Developed Colonies were counted, recorded in colony forming unit per millimeter (CFU/ml) (Baron et al., 1994; Atikpo et al, 2019); and sub-cultured on freshly prepared media. The pure cultures were characterized using the biochemical tests: gram stain, catalase, oxidase, citrate, triple sugar iron, indole and motility test (Holt, 1994). The resultant pure and identified cultures were stored as a slant for use.

*Selection of Optimum Factors for Bioremediation***:** Harvested twenty-four hours old *A. nicotiniae* was inoculated into soil samples prepared for bioremediation experiments prepared in triplicate (Atikpo, 2016; Atikpo and Eboibi, 2020). Nutrient volumes (NV) [2, 4, 6, 8, and 10 ml], mass of organism (MO) [1, 2, 3, 4 and 5g], temperature (T) [10^o, 20^o, 30^o, 40^o and 50^o], pH (5, 6, 7, 8, and 9) and stirring frequencies (SF) [0, 1, 2, 3, and 4 per week (pw)] were used to condition 3g of soil inoculated with *A, nicotiniae*. The optimum values of the factors (NV, MO, T, pH, and SF) were identified and selected using the index of least concentration of heavy metals analyzed at 7 days using Atomic Absorption Spectrophotometer (GBC SensAA, Model No. A6358).

Bioremediation Studies: **t**he capability of *A. nicotiniae* to remove the selected HMs from soil was carried out in triplicate using the batch procedure in (Atikpo, 2016; Atikpo and Ihimekpen, 2020). Three grams each of soil in 50ml beakers were inoculated with 24 hours *A. nicotiniae* and conditioned with the optimum values of the selected remediation factors. The residual metals contents of the soil samples were analyzed on 7, 14, 21, 28 and 35 days with Atomic Absorption Spectrophotometer (GBC SensAA, Model No. A6358) centrifuging the samples to eliminate the organism from the soil. The ions removed, ions removed with time and percentage removal of ions by *A. nicotiniae* were determined using Equations (1), (2) and (3) respectively (Chen and Wang, 2007; Atikpo and Ezugwu, 2017a; Atikpo and Michael, 2018).

$$q = \frac{(C_o - C_f)}{m}.V \quad (1)$$

$$q_t = \frac{(C_o - C_t)}{m}.V \quad (2)$$

$$\%\, Removal = \frac{(C_o - C_f)}{C_o}.100 \quad (3)$$

Where q denotes ion (mg/kg) removed; q_t denotes (mg/kg) removed at time t, C_o denotes the initial ion

concentration (mg/kg) in contact with *A. nicotiniae*, C_f denotes final concentration (mg/kg) after the experiment, *V* denotes the volume of soil (m^3) used and m denotes the mass (g) of *A. nicotiniae*.

RESULTS AND DISCUSSION

*Identification of Organism***:** For this purpose, microbiology analysis conducted on the soil sample and the developed Colonies were counted and recorded as 4.1 x 10^1 cfu/ml; and the organism was identified as *A. nicotiniae* with GPR gram catalase, oxidase glucose, lactose, motility results, indole, citrate and H_2S results displayed in Table 1.

Optimal Factors: Some factors are known to influence bioremediation of HMs contaminated soils (Murthy et al., 2012; Atikpo and Ezugwu, 2017). The identification and selection of optimum values of these factors require a careful pre-remediation study and screening to achieve their scientific significant influence on remediation experiments (Atikpo, 2016; Ihimekpen et al., 2020). The least residual concentration was the criterion used for selecting the optimum values of the factors utilized for the study (Atikpo, 2016) as 8ml of NV, 5g of the MO, 30 ^oC of T, pH of 8 and SF of 4pw on the 7th day. The impacts of 2, 4, 6, 8, and 10 ml of NV on the metals removal by *A. nicotiniae* was studied for 7 days. The influence was in the other of 8 ml > 10 ml > 6 ml > 2 ml > 4 ml. the emerged optimal was 8 ml and was adopted for the bioremediation study. The MO relate directly with organism's population in contact with contaminated soil. Population of organisms have effect on the activities and effectiveness of organisms use for bioremediation (Atikpo, 2016; Ihimekpen et al., 2020).

Table 1. Identification of Organism

Cultural Morphology	Biochemical Tests									Organism Isolate
	Gram reaction	Catalase	Oxidase	Indole	Citrate	Glucose	Lactose	H_2S	Motility	
Creamy flat irregular entire on agar olate	GPR	+	-	-	+	+	+	-	-	Arthrobacter nicotiniae

GPR: Gram Positive Rods (Bacilli)

For this purpose, the optimum of MO was screened from 1, 2, 3, 4, 5 g of organisms used for conditioning the soil in the pre-remediation study. The removal of the metals decreased with the selected masses of the organism in the order of 5 > 4 > 2 > 3 >1; and 5 gram was adopted for the study. Temperature is documented as the dominant influencer of bioremediation of HMs (Murthy, 2012; Atikpo, 2016). Its variation impacts on the other factors which stimulate bioremediation (Ajaykumar et al., 2009; Ihimekepen et al., 2020); thus, the degree of heat supplied for the process is a vital determinant of the removal efficient removal (Murthy, 2012; Atikpo, 2016). A study of the various experimented degrees of temperatures revealed an optimum of 30^oC for the effective removal of the metals' ions by the organisms. The impact of pH on the removal of the metals by the organism was studied, and its effectiveness was in the order of 8 > 7 > 9 > 6 > 5. This guided on the selection of value 8 as the optimum pH value. This was necessary because pH is the most influential factor for bioremediation; it affects cells charges connectivity, wall chemistry, hydrolysis, and physiochemistry of metals necessary for efficient and effective bioremediation (Samarth et al., 2012). The impacts of stirring frequency values of 1, 2, 3, and 4pw on the removal of the metals were accessed in other to select the optima value. An optimal value of 4pw was selected for the study. This study of SF was necessary to promote oxygen diffusivity needed for aerobic activities of the organism. The study showed that the highest frequency of stirring (4pw) was more favorable to the bio - removal of the metals. This is in line with the findings in similar studies by (Atikpo, 2016, Atikpo and Micheal, 2018, Ihimekpen et al., 2020).

Preferential Removal of the Selected Metals: Figure 1 shows the performances of *A. nicotiniae* in the removal of the metals from the soils. The organism was able to reduce Pb, Cd, and As concentrations from their respective initial values of 182,11 mg/kg, 90.05 mg/kg and 72.91 mg/kg to their respective maximum allowable concentrations (MACs) of 100 mg/kg, 3 kg/mg and 20 mg/kg in (Chiroma, 2014); but could not reduce Zn concentration from initial value of 885.19 mk/kg to below the MAC of 300 mg/kg in (Chiroma

et al., 2014 The organism showed an excellent performance of 79.78% in the removal of lead at time 35 days, a very good performance of 61.50% in the removal of cadmium at time 35 days, a fair performance of 48.59% in the removal of arsenic at time 35 days and a very poor performance of 7.04% in the removal of zinc at time 35 days. This order was consistent with time, but with much incremental variation in the removal lead than the other metals. This is similar to the findings of (Atikpo and Ezugwu, 2017). In this dimension of removal, the concentration of lead was brought below the maximum allowable limit for lead. This was not so in the cases of the other metals, but there is a great potential of the organism to achieve this in the cases of cadmium and arsenic if their concentration is lower in the soil. As shown in Figure 1, the organism with poor percentage removal for zinc is neither fit for pollution control, nor pollution abatement of zinc ions in the soils.

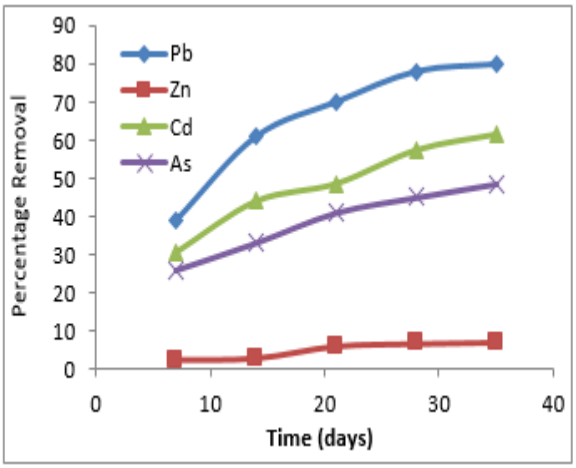

Fig 1: Comparative Removal of Heavy Metals by Arthrobacter nicotiniae

*Conclusion***:** The study looked into the performance of *A. nicotiniae* for removal of Pb, Cd and Zn from contaminated soils in Amaonye forest. It identified optimum factors of temperature, pH, organism's mass, and dosage of nutrient and stirring frequency as 30^{o}C, 8, 5g, 8 ml and 4pw for effective removal of the metals. The study showed an excellent ability of the organism for Pb removal; very good ability for Cd removal, fair ability for As removal and poor ability for Zn removal. These indicate that organism can be used to clean-off the current concentrations of Pb and Cd from the soils, clean-off low concentration of As, but cannot handle Zn in the soil. It is thus good for Pb and Cd pollution control, As pollution abatement but not good for Zn removal.

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