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# BIOREMEDIATION AND GROWTH PROMOTING EFFECTS OF IDENTIFIED BACTERIA FROM DIFFERENT SOIL LOCATIONS IN NIGERIA

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## ABSTRACT

Soil contains diverse bacteria that are vital for maintaining soil health and fertility. While many soil bacteria are known for their plant growth-promoting (PGP) abilities, combining this role with bioremediation is less common. The ability of the bacteria isolates to solubilize phosphate, fix nitrogen and bioremediate environmental pollutants were investigated in this study. Isolated organisms were further tested to know their ability to sequester toxicants such as heavy metals, pesticides and hydrocarbons while their capability to produce growth hormones were also confirmed. The bacteria were molecularly identified using the 16S rDNA sequencing. The identified isolates were: *Bacillus* species, *Enterobacter* species and *Vagococcus fluvialis*. Production of halo zones on the Pikovskaya's agar showed the proficiency of the organisms to solubilize phosphate, change of Salkowiski's reagent to pink displayed their ability to produce Indole acetic acid while their capability to produce siderophore was indicated by the change in the dark blue CAS indicator to orange. The heavy metal tolerance test conducted showed that the organisms could resist metals at maximum concentration of: chromium (1.0mM), lead (16.0mM), cadmium (1.6mM) and nickel (4.0mM). All the bacteria isolated and identified were found to have great biofertilizer potential. At the same time, they also serve as bioremediators of polluted environment as well as biofertilizers.

**Key words**: Biofertilizer, Phosphate solubilizers, Heavy metals, Siderophore.

## INTRODUCTION

Over the years, soil, has experienced a lot of degradation due to natural and anthropogenic forces. These challenges have great deleterious effects on not just the output of the agricultural soil due to nutrient depletion but also, on the general environment. Microorganisms, which are part of human existence come into play in solving the problems as there are known microorganisms that serve in providing soil with all required nutrients with improved and increased production without deleterious effects both on the soil and the environment. These microorganisms, generally known as growth promoting bacteria (GPB) come as phosphate solubilizers, nitrogen fixers and also as toxicant sequestering bacteria, acting, most often, not only as bio-fertilizer but also as bio-degraders of pesticide and heavy metals in soil. To sustain soil's production, problems of phosphorus (P) deficiency, nitrogen(N) fixation and toxicants pollution are needed to be arrested as soils may contain P pools several thousand times greater than the amount required for plant growth, but a less soluble fraction is available for plant transport (Sohrt *et al.*, 2017). In modern agriculture, fertilizers and pesticides are used indiscriminately in the hope of achieving high yields, which destroys the fertility of the soil and the quality of the crop. Also, the contamination of farm lands by toxicants such as heavy metals and hydrocarbon products that find their way to terrestrial ecosystem have great deleterious effects on lands thereby affecting soil performance and eventual crop productivity. The extraction of natural resources is accompanied by intrinsic restrictions, which is why they are classified as environmental pollutants of global concern (Odoh *et al.*, 2019b; Kumar *et al.*, 2019). Cities, sectors, and cultural contexts all influence their existence in ecosystems. (Odoh *et al.*, 2017; Sam *et al.*, 2017). Particularly in poorer nations, their primary means of obtaining access to land resources are unofficial pursuits like artisanal processing and exploration. It has also been linked to overuse of pesticides, fertilizers, power, biofuels, municipal waste, and careless trash disposal. Copper (Cu), zinc (Zn), nickel (Ni), lead (Pb), cadmium (Cd), cobalt (Co), mercury (Hg),

chromium (Cr), and arsenic (As) are a few heavy metals that are frequently found in soil. Due to their non-biodegradable nature, heavy metals frequently build up in the soil (Walker *et al.*, 2003; Eze *et al.*, 2018), posing a risk to soil fertility and the health of humans and animals. These heavy metals cause reduced production and financial losses in agriculture. Microbial communities are disrupted as a result of hydrocarbon pollution's effect on soil toxicity. It has detrimental effects on the climate, biodiversity loss, and the environment. The ecological balance and local health standards are drastically altered by this circumstance (Ite *et al.*, 2013). Due to technological errors, sabotage, and intentional actions, oil development in the tropics has put food security at risk and reduced soil fertility (Zabbey *et al.,* 2017; Odoh *et al.*, 2019b) Ajai (2010) stated that hydrocarbon stress directly affects the entire environment, especially food and other vital resources. Therefore, there is an increasing need for studies highlighting the various functions of soil microorganisms, particularly in the areas of environmental improvement (Sam *et al.*, 2017; Zabbey *et al.*, 2017; Eze *et al.*, 2018), food and agriculture (Compant *et al.*, 2010; Clark *et al.*, 2009; Odoh *et al.*, 2019a).

## METHODOLOGY

### Sampling

Soil samples were collected from four regions across Nigeria: Kebbi: 5.490398 Longitude, 11.398558 Latitude; Ebonyi: 6.265880 Longitude, 8.116588 Latitude; Kwara: 5.1605 Longitude, 9.08837 Latitude; and Ogun: 3.435797 Longitude, 7.235469 Latitude. Sterile paper bags were used for the collection and transportation of the samples. Samples were properly labelled and mixed to form a composite sample. The samples were properly stored in a fridge.

### Sterilization of Equipment and Culture Media

### Glassware

Glassware such as conical flasks, test tubes were sterilized in the autoclave at 121°C for 15 minutes. Inoculating loops, needles were sterilized by holding the Nichrome wire in the Bunsen flame until it glowed red. To reduce the incidence of contamination, work bench was always swabbed with 70% ethanol and incubator cleaned with a mixture of alcohol and phenol. Absolute sterility was maintained throughout the course of this research to avoid growth of contaminant and unwanted organism.

### Media and Composition

Enrichment culture system was set up. The culture system consisted: Phosphate Solubilizing organisms- PVK, Nitrogen fixers- Without nitrogen source, Heavy metals (HM) sequesters- addition of Heavy metals and Pesticide sequesters- Without carbon source + pesticide. The culture media used such as Pikovskaya's media (PVK), Jensen's media, Luria Bertani media (LB), Minimal Salt Media (MSM) and other composed media were prepared according to required compositions and sterilized in an autoclave at a temperature of 121°C for 15 minutes before use. Trace element solution SL-6 was also prepared with the required compositions.

### Isolation of Bacteria

### Isolation of Phosphate Solubilizing Bacteria (PSB)

Phosphate solubilizing bacteria (PSB) was isolated using Pikovskaya's (PVK) media. PVK broth was prepared and standardized to pH 7.0. The broth was homogenized using water bath and later sterilized. Ten gram each of the samples was dispensed into conical flask containing 90 mL each of the sterilized PVK broth. The labelled samples were put on rotary shaker at 130 rpm for 7 days. The broth was used to start an enrichment culture and four rounds of transfer was done after 7 days each by transferring 10 mL of the 100 mL (90 mL PVK broth + 10 g sample) to a freshly prepared PVK broth. After the enrichment, 0.1 mL of the fourth enrichment was inoculated on sterilized PVK agar plates for seven days using the spread plate method.

### Isolation of Nitrogen Fixing Bacteria

Jensen's media was used in isolating nitrogen fixing bacteria, 0.1 mL of enrichment culture was inoculated on sterilized Jensen's agar plates for 3-5 days using the spread plate technique.

### Isolation of Toxicant Sequestering Bacteria

Minimal Salt Media (MSM) was prepared. One millilitre of trace element and 0.05 g of yeast extract were added and pH was adjusted to 7.2.

The media was amended with pesticides: Paraquat di chloride (Dragon) and Glyphosate (Vinash) and sterilized. Upon cooling, the samples were placed on rotary shaker at 130 rpm for 21 days. Using the spread plate technique, 0.1 mL was spread on Luria-Bertani (LB) agar plate and incubated for growth.

**Analysis of Heavy Metals**

Heavy metal analysis for Maximum Inhibitory Concentration (MIC) was done by adopting the protocol reported by Oyetibo *et al.* (2010). Pure culture of isolates grown on overnight culture of LB broth was inoculated into 5 mL normal saline and the concentration of the isolates was brought to 0.5 McFarland standard. Luria-Bertani broth was prepared and amended with each heavy metal according to the working solution. The LB broth with the amended heavy metal was then sterilized and allowed to cool. The inoculum (100 µl) was then added to each LB-heavy metal amended broth.

**Hydrocarbon Degradation**

**Crude Oil Degradation**

Crude oil degradation ability was done following the protocol of Oyetibo *et al.* (2015). Minimal salt Media (MSM: 2.13 g $Na_2HPO_4$, 1.30 g $KH_2PO_4$, 0.5 g $NH_4(SO_4)_2$, 0.2 g $MgSO_4.7H_2O$) was prepared and pH adjusted to 7.0. Fifty (50) mL of MSM was dispensed into conical flasks and 0.5 mL (1% v/v) of crude oil was dispensed into each of the labelled conical flask and inoculum was added and incubated for 28 days for qualitative observation by formation of bolus.

**Phenanthrene Degradation**

This was carried out following the procedure of Oyetibo *et al.* (2015). Approximately 0.2 g of phenanthrene was dissolved in chloroform and 1mL each was dispensed into the sterilized conical flask and allowed to 'vent' overnight. Fifty (50) mL of MSM was then dispensed into each of the labelled 'vented' phenanthrene-chloroform flasks and inoculum added, and incubated for 28 days for qualitative observation.

**Production of other plant growth promoting enzymes and hormones**

**Siderophore Production**

The procedures of Schwyn and Neilands (Shin *et al.*, 2001) were modified for the preparation of CAS-blue agar. Fifty (50) mL of distilled, deionized water was used to dissolve 60.5 mg of Chrome Azurol S (CAS), which was then combined with 10 mL of an iron (III) solution (1 mmol/L $FeCl_3.6H_2O$, 10 mmol/L HCl). This solution was gradually added to 72.9 mg of dissolved hexadecyltrimethylammonium (HDTMA) in 40 mL of water while being stirred. After being diluted 20 times, the resulting dark blue fluid was autoclaved at 121°C for 15 minutes.

Basal agar medium was employed as a gelling agent, and the pH was carefully adjusted to 6.8 being very sensitive to pH changes and the basal median was autoclaved at 121°C for 15 minutes. The medium contained, in g/L, 0.1 $K_2HPO_4$, 3.0 $KH_2PO_4$, 0.2 $MgSO_4.7H_2O$, 1.0 $(NH_4)_2SO_4$, 4.0 succinic acid, and 20 g agar. Before beginning, every piece of glassware was cleaned (without exception) with 3 mol/L HCl to eliminate iron and rinsed with deionized water (Cabaj and Kosakowska, 2007). After cooling, the two solutions—the baseline agar medium and the CAS indicator solution—were carefully transferred onto plates. Fresh overnight cultures were streaked on those agar plates after they had solidified to check for siderophore production.

**Production of Indole-3-acetic acid (IAA)**

Indole-3-acetic acid (IAA) production was determined following the standard method (Patten and Glick, 1996). Luria Bertani (LB) was prepared and allowed to cool. Tryptophan (2 g/L) was then added and dispensed into already autoclaved test tubes. One milliliter of overnight grown culture was introduced into each of the test tubes and incubated for 3 days. Broth culture was taken (2 mL) and centrifuged at 8000 rpm for 2 minutes. Salkowiski's reagent (2 mL) was added to 1 mL of the supernatant and kept in the dark and observed after 30 minutes.

**Identification of Isolates with sequencing**

**Genomic DNA Extraction**

As directed by the manufacturer, the FAST DNA® Spin Kit for soil (MP Biomedicals) was used to extract genomic DNA from 0.5 g of soil sample from the composite sample. The FASTPREP® Cell Disruptor FP 120 (Qbiogene, Heidelberg, Germany) was used at 6.5 speed for 30 seconds. In accordance with the advice of

Takada and Matsumoto (2005), skim milk (40 mg) was added to the sample in the lysis matrix in order to eliminate any potential humic material interference with the DNA. DNA was extracted and observed in a 1% (w/v) agarose gel stained with ethidium bromide using UV trans-illumination, and quantification was done using UV-V spectrophotometry using an EpochTM spectrometer system (BioTek, Winooski, VT, USA).

**Pyrosequencing of bacterial 16S rDNA**

The genomic DNA at the V3–V4 regions of the 16s rDNA was amplified using the bacterial primers 27F and 1492R (ChunLab Inc., Seoul, South Korea). Illumina indices and adapters from a Nextera® XT Index Kit (Illumina, San Diego, CA, USA) were used to tag the PCR's purified amplicons. ChunLab Inc. (Seoul, South Korea) used the Illumina MiSeq platform to build libraries. The quality of the libraries was assessed using an Agilent 2100 Bioanalyzer system (Agilent Technologies, Palo Alto, CA, USA) using a DNA 7500 chip. The Quanti-iTTM PicoGreenTM dsDNA Assay Kit (Invitrogen) was then used to quantify the libraries in accordance with the manufacturer's instructions. Using CleanPCRTM (CleanNA, Netherlands), short DNA fragments were eliminated, and Illumina's MiSeq Reagent kit v2 was used for sequencing. Organisms were blasted on NCBI and deposited at the NCBI GenBank for accession number.

**Statistical Analysis**

According to Kemp and Aller, the estimated coverage of the created 16S rDNA gene libraries was determined as C = 1- (n/N) X 100, where n is the number of singletons following assembly and N is the total number of sequences in the original dataset.

## RESULTS

All the twenty-seven identified bacterial isolates screened for heavy metal tolerance (chromium, lead, cadmium and nickel), phosphate solubilization, nitrogen fixation and toxicant sequestration ability tested positive except for nickel. Ten (10) of the twenty-seven (27) bacterial isolate selected based on their stronger show of capabilities to produce plant growth hormones and sequester toxicants were molecularly identified and the identities of the selected bacterial isolates by sequencing is presented in table 1..

**Table 1**: Molecular identification of the bacterial isolates.

| ISOLATE NUMBER | ISOLATE CODE | IDENTIFIED BACTERIA | ACCESSSION NUMBER |
|---|---|---|---|
| 6 | PEST MFB (R) | *Bacillus anthracis* | PQ031261 |
| 8 | PRFA (R) | *Enterobacter cloacae* | PQ049136 |
| 12 | JMR2 | *Bacillus paranthracis* | PQ049135 |
| 16 | PMFB (S) | *Bacillus amyloliquefaciens* | PQ048030 |
| 22 | PMS2 | *Bacillus subtilis* | PQ048041 |
| 31 | JMB (S) | *Bacillus nitratireducens* | PQ047971 |
| 32 | JMB (R) | *Bacillus nitratireducens* | PQ048029 |
| 33 | PRR2 | *Bacillus thuringiensis* | PQ048096 |
| 35 | JMS2 | *Vagococcus fluvialis* | PQ094782 |
| 38 | PEST MA (R) | *Enterobacter hormaechei* | PQ048097 |

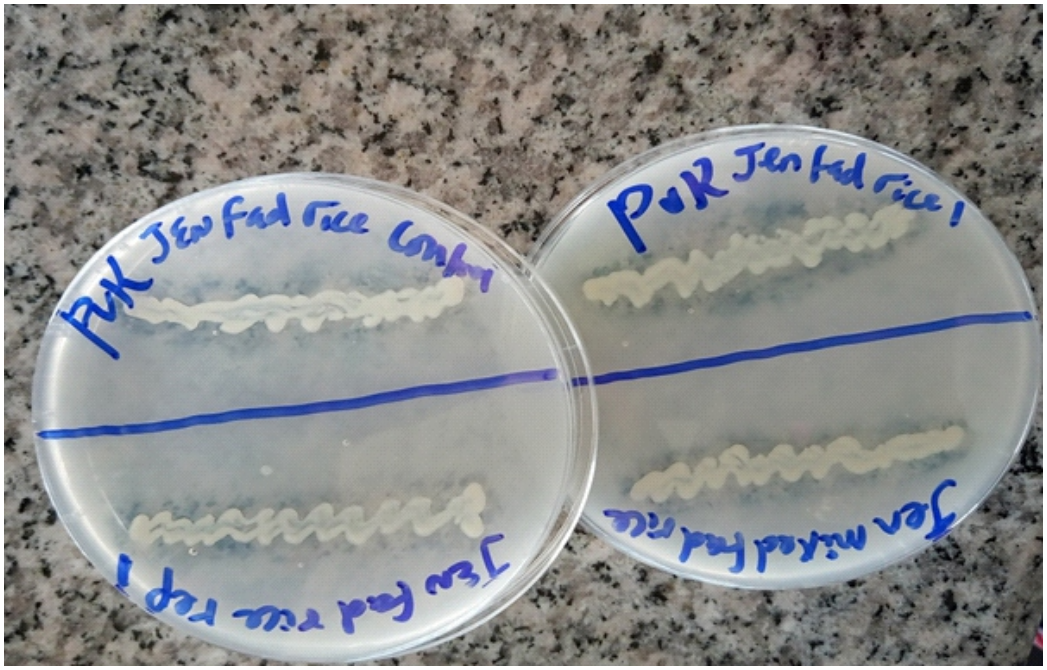
The inhibitory concentrations of heavy metals, and phosphate solubilizing, nitrogen fixing and toxicant sequestration ability of the bacterial isolates are shown in Table 2. All the Bacterial isolates were tolerant to Chromium, lead and cadmium at the various concentrations tested. However, some of the isolates (*E. cloaca, B.paranthracis, B. subtilis. B. thuringiensis* and *V. fluvialis)* at the concentration of 1.0 nM and B. *anthracis*, B. *amyloliquefaciens, B. nitratireducens, B. nitratireducens* and *E. hormaechei* at 4.0 nM were non-tolerant to nickel. All the isolates showed potential for phosphatase catalysis, as they solubilized phosphatase and were also nitrogen fixing and toxicant sequestration bacteria. Plate 1(A) and (B) shows the phosphate solubilizing potential of microorganisms with clear halo zone.

**Table 2**: The inhibitory concentrations of heavy metals and phosphate solubilizing, nitrogen fixing and toxicant sequestration ability of the bacterial isolates.

| | Heavy metal tolerance | | | | | Growth promoting factors | | |
|---|---|---|---|---|---|---|---|---|
| **IDENTIFIED BACTERIA** | **Chromium** (1.0mM) | **Lead** (16.0mM) | **Cadmium** (1.6mM) | **Nickel** (1.0mM) | **Nickel** (4.0mM) | **Phosphate solubilizing** | **Nitrogen fixing** | **Toxicant sequestration** |
| *Bacillus anthracis* | + | + | + | + | - | + | + | + |
| *Enterobacter cloacae* | + | + | + | _ | + | + | + | + |
| *Bacillus paranthracis* | + | + | + | - | + | + | + | + |
| *Bacillus amyloliquefaciens* | + | + | + | + | - | + | + | + |
| *Bacillus subtilis* | + | + | + | - | + | + | + | + |
| *Bacillus nitratireducens* | + | + | + | + | - | + | + | + |
| *Bacillus cereus* | + | + | + | + | - | + | + | + |
| *Bacillus thuringiensis* | + | + | + | _ | + | + | + | + |
| *Vagococcus fluvialis* | + | + | + | _ | + | + | + | + |
| *Enterobacter hormaechei* | + | + | + | + | - | + | + | + |

+: Positive -: Negative

A



B

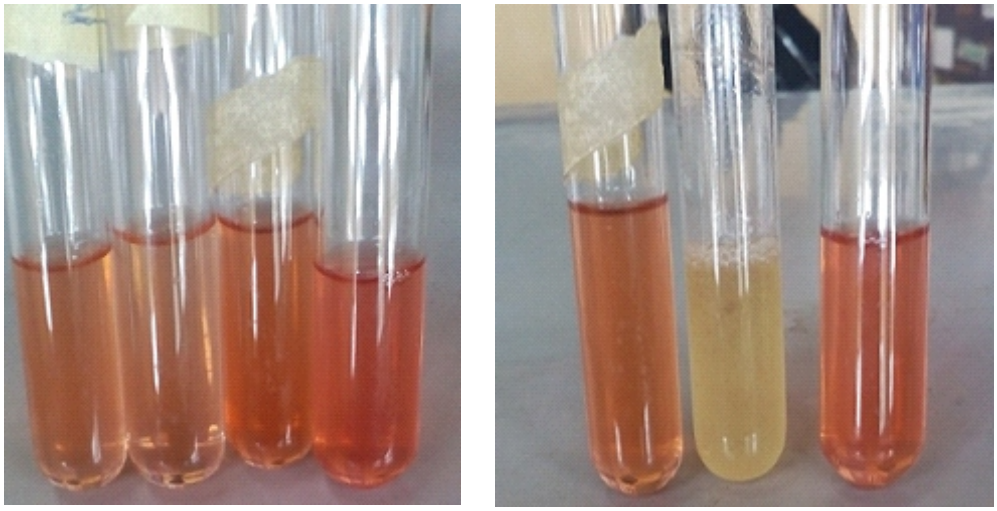
Plate 1: Phosphate solubilizing bacteria with clear halo zone
(Plate 1 A and B showing the phosphate solubilizing potential of bacteria with clear halo zone).

Table 3 shows the affinity of the bacterial isolates to produce siderophore and indole acetic acid, and ability to degrade hydrocarbon. The isolates showed the ability to turn the blue color of siderophore to yellow/orange showing their affinity for iron sequestration. The bacteria also displayed affinity for siderophore except *Bacillus paranthracis*. Meanwhile the lowest affinity was observed in *Bacillus anthracis* and *Enterobacter cloacae*. Similarly, the isolates showed ability to produce indole acetic acid by the change in color of the salkowiski's reagent to pink. All the isolates were able to degrade hydrocarbon (crude oil and phenanthrene) except *B. subtilis, B. nitratireducens* and V. *fluvialis* that were non-tolerant to crude oil. *Bacillus amyloliquefaciens* displayed very strong ability to degrade crude oil. Plate 2 shows indole acetic acid production by the bacterial isolates while plate 3A depicts isolates that showed strong siderophore production by complete turning of the dark blue CAS indicator color to orange and Plate 3 B showing no siderophore production by the isolates by retention of the CAS blue color.

**Table 3**: The affinity of the bacterial isolates to produce siderophore and indole acetic acid and their ability to degrade hydrocarbon.

| | Growth factors | | Hydrocarbon | |
|---|---|---|---|---|
| **IDENTIFIED BACTERIA** | **Siderophore** | **Indole Acetic Acid** | **Crude oil** | **Phenanthrene** |
| *Bacillus anthracis* | + | + | ++ | + |
| *Enterobacter cloacae* | + | ++ | ++ | + |
| *Bacillus paranthracis* | - | + | + | ++ |
| *Bacillus amyloliquefaciens* | ++ | + | +++ | + |
| *Bacillus subtilis* | ++ | + | - | + |
| *Bacillus nitratireducens* | +++ | + | - | ++ |
| *Bacillus cereus* | +++ | + | ++ | ++ |
| *Bacillus thuringiensis* | +++ | ++ | ++ | + |
| *Vagococcus fluvialis* | +++ | + | - | ++ |
| *Enterobacter hormaechei* | +++ | +++ | + | + |

+ Affinity; ++ High affinity; +++ Very High affinity; - No affinity



A B

Plate 2: Indole acetic acid production by the bacterial isolates.
A: Positive for indole production B: The control (middle) against the change in color.
Color change from yellow (control) to pink color indicates IAA (Indole acetic acid) production ability

A B

Plate 3: Siderophore production by the bacterial isolates
A: Isolates on plates that showed strong siderophore production by complete turning of the dark blue CAS indicator color to orange. B-Left: Isolates on plates that showed no siderophore production by retaining the CAS blue color.
B-Right: Isolates on plates that showed siderophore production by gradually turning the blue CAS indicator yellow.

## DISCUSSION

Enhancing soil fertility is one of the most popular methods for raising agricultural yield through the use of economical, environmentally friendly technologies like biofertilizer, particularly when maintaining high levels of available nitrogen (N) and phosphorus (P), the two most scarce nutrients in the soil, and also eliminating hazardous substances that still pose a risk and find their way into the soil. Most researches on biofertilizer have dwelled more on the awareness and in-depth studying of either phosphate solubilization or $N_2$ fixation. However, when toxicants like heavy metal, hydrocarbons and pesticides also pollute

the soil, the productive capacity of the soil is compromised. Samples in this study were analyzed for heavy metals and to know their ability to degrade toxicants for environmental bioremediation and for eventual use for the production of biofertilizer for sustainable agriculture. Due to the growing global population, there is a need to attain food security and boost crop productivity, which has resulted in the widespread use of chemical fertilizers and pesticides in agricultural soils (Sudhakaran *et al*., 2018; Kayode *et al*., 2021). However, the most important environmental contaminant has primarily been identified as trace elements or heavy metals (Emenike *et al*., 2020).

Heavy metals have their way of getting into the soil; hence, the need to analyze soil, especially agricultural soil for heavy metals. Some metals that are tagged heavy metals are actually micronutrients as their presence in soil does not pose any problem either to the soil or the public health. Metals such as iron, manganese, copper, zinc, when found in soil samples are usually considered non-toxic but metals such as cadmium, chromium, nickel, lead, arsenic when detected raise concern because of their deleterious effects.

In this study, both cultural and molecular tools were used in isolating and identifying organisms that have the capacity to solubilize phosphate, fix nitrogen while also degrading environmental pollutants like pesticides, heavy metals and hydrocarbons to serve as biofertilizer for improved and increased crop yield and also bioremediating pollutants. The organisms involved in all of these are highly varied in the soil and they play important roles. This study, puts to test, isolates for heavy metal resistance, phosphate solubilization, nitrogen fixation and sequestration ability of toxicants (Table 2). This study tested for the tolerance of isolates on heavy metals; contamination of soil by harmful metals like chromium (Cr), lead (Pb), cadmium (Cd), and nickel (Ni) presents a serious risk to ecosystem health, agricultural output, human health, and soil fertility and water quality. Lead is ranked number two among hazardous wastes on the Agency for Toxic Substance and Disease Registry (ATSDR) list, followed by cadmium at number seven and nickel at number fifty-seven, all of which are deemed to pose a substantial risk to human health (Glick, 2015).

The bacteria showed highest tolerance to Cr at 1.0mM, Pb at 16.0mM, Cd at 1.6mM and to Ni at 1.0mM and 4.0mM which is in line with the work of Oyetibo *et al*., (2010) that bacteria exhibited tolerance to high concentrations of $Cd^{2+}$, $Co^{2+}$, $Ni^{2+}$, and $Cr^{6}$. This study examined the requirements for viable bacteria to perform in the presence of target heavy metals (HMs) and other contaminants such as hydrocarbons and pesticides in order for active bacteria-based bioremediation to be a dependable method of sequestering toxicants. All the isolates subjected to heavy metal test showed resistance to it and also exhibited strong ability to fix nitrogen and also sequester toxicants (Table 2).

The isolation of the solubilizing ability of bacteria was done on plates with Pikovskaya's agar; Solubility of insoluble P by microbes was reported by Pikovskaya (1948). Bacteria of the genus *Bacillus* are among the most efficient phosphate producers (Muleta *et al*., 2013; Prakash and Arora, 2019). Additionally, Bacillus has an advantage over other bacteria due to their endosporeforming ability, which allows them to resist environmental changes that lead to unsafe conditions, such as biofungicides or bioinoculants (Qiao *et al*., 2014). Phosphate solubilizing ability was exhibited by the formation of clear halo zone on plates (Plate 1). On PVK agar, the clearing zone has grown, which may indicate a rise in acid secretion and change from insoluble phosphate into soluble form. Accordingly, the capacity of these microbes to lower the pH of their surroundings by releasing protons or organic acids may be the cause of the phosphate solubilization potential seen in all of the species (Hariprasad and Niranjana, 2009; Charana and Yoon, 2013).

Since P stimulates both growth and phosphate solubilizing bacteria (PSB, which may ensue), it is not required to enhance overall development as is often done after it is understood that it may. Instead, testing should be conducted further to establish a direct contribution to nutrition. from other sources), and the capacity to fix P does not always equate to the capacity to advance plant growth (Bashan *et al*., 2013; Collavino *et al*., 2010)

The majority of studies on phosphate solubilizing microorganisms (PSMs) have demonstrated that, in addition to producing gluconic acid, these microbes also generate indole-3-acetic acid (IAA), 1 aminocyclopropane-1-carboxylate (ACC) deaminase, and siderophores.

In this study, the molecular tools identified organisms (Table 1): *Bacillus anthracis., Enterobacter cloacae., Bacillus paranthracis., Bacillus amyloliquefaciens., Bacillus subtilis., Bacillus nitratireducens., Bacillus cereus., Bacillus thuringiensis., Vagococcus fluvialis and Enterobacter hormaechei.*

The bacteria identified in this study were analyzed for the enzyme indoleacetic acid (IAA) (Figure 1) and all showed a positive effect on IAA production; IAA is a plant enzyme that has a variety of physiological roles and is one of the most significant plant growth regulators generated by PSM (Oves *et al*., 2013).

In this study, the production of IAA was demonstrated in the presence of tryptophan. The amount of IAA produced by bacterial species may vary depending on the part of the biosynthetic pathway, the location of the cells, and the presence and sequence of enzymes that convert free IAA to the coalesced form. Siderophores, which are many iron receptors, are one of the biocontrol methods used to inhibit the growth of phytopathogens. By turning the blue CAS agar colour to yellow (Plate 2), the organisms showed affinity for siderophore except for *Bacillus paranthracis* that showed no affinity. Improvement of plant growth can also occur directly and against pathogens (biocontrol) through the synthesis of antibiotics or secondary metabolites using systemic resistance (SR) (van Loon *et al*., 1998, 2007).

## CONCLUSION AND RECOMMENDATIONS

Organisms identified in this research showed a great deal of nitrogen fixing ability, heavy metal tolerance and hydrocarbon degrading abilities that showed great prospect to serve as both biofertilizer and bioremediator of environmental pollutants.

Enhancing soil fertility is among the most popular methods for raising agricultural output. It is still difficult to keep the two most limiting nutrients, phosphorus (P) and nitrogen (N) in soil at high levels. Most research on organic fertilizers has focused on recognizing and ameliorating the effects of the deficiency ofnitrogen availability. However, it is known that any nodule formation process is limited by the presence of phosphorus. Therefore, it is important to develop research on phosphate solubilizers and nitrogen fixers as biofertilizers, as well as microbes that can help solve the problem of pesticides, hydrocarbons and heavy metals contamination while also acting as biofertilizer.

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## CONFLICT OF INTEREST

There is no conflict of interest among authors

## AUTHORS' CONTRIBUTIONS

T.M, Momoh-Salami: Investigation, analysis, writing. N.M, Akinyemi: Investigation. G.A, Ajiboye: Data collection, analysis. G.O, Oyetibo: Conceptualization, methodology, investigation, proof reading. L.A, Adams: Methodology, Data, reading. L.A, Ogunkanmi: Methodology, data collation. M.O, Ilori: Conceptualization, Funding, Methodology, Data acquisition, reading.

**REFERENCES**

Ajai, O. 2010. Balancing of interests in environmental law in Nigeria. Environ Law Africa 379

Bashan, Y., Kamnev, A.A. and de Bashan, L.E. 2013. A proposal for isolating and testing phosphate solubilizing bacteria that enhance plant growth. *Biology and Fertility of Soils* 49:1–2.

Cabaj, A. and Kosakowska, A. 2007. Iron-dependent growth of and siderophore production by two heterotrophic bacteria isolated from brackish water of the southern Baltic Sea. *Microbiology Research*

Charana, W.B. and Yoon, M.H. 2013. Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. *African Journal of Microbiology Research* 3:266-275.

Clark, D.P., Dunlap, P.V., Madigan, M.T. and Martinko, J.M. 2009. Brock. Biology of Microorganisms. San Francisco: Pearson. p 485

Collavino, M.M., Sansberro, P.A., Mroginski, L.A. and Aguilar, O.M. 2010. Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. *Biology and Fertility Soils* 46:727–738

Compant, S., Clément, C. and Sessitsch, A. 2010. Plant growth promoting bacteria in the rhizo and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* 42:669–678

Emenike P.C., Neris J.B., Tenebe I.T., Nnaji C.C. and Jarvis P. 2020. Estimation of Some Trace Metal Pollutants in River Atuwara southwestern Nigeria and spatio-temporal human health risks assessment. *Chemosphere* (1)239: 124770.

Eze, C.N., Odoh, C.K., Eze, E.A., Enemuor, S.C., Orjiakor, I.P. and Okobo, U.J. 2018. Chromium (III) and its effects on soil microbial activities and phytoremediation potentials of Arachis hypogea and *Vigna unguiculata*. *African Journal of Biotechnology* 17(38):1207-1214.

Glick, B.R 2015. Resource acquisition. In: Beneficial plant-bacterial interactions. *Springer*, New York, pp 29–63

Hariprasad, P. and Niranjana, S.R. 2009. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. *Plant Soil* 316:13-24.

Ite, A.E., Ibok, U.J., Ite, M.U. and Petters, S.W. 2013. Petroleum exploration and production: past and present environmental issues in the Nigeria's Niger Delta. *American Journal of Environmental Protection* 1(7):8–90

Kayode, O.T., Aizebeokhai, A.P. and Odukoya, A.M. 2021. Sustainable Use of Chemical in agricultural Soils and Implications for Precision Agriculture. *Journal of Environmental Treatment Techniques* 9(2):361-367.

Kumar, A., Chaturvedi, A.K., Yadav, K., Arunkumar, K.P., Malyan, S.K., Raja, P., Kumar, R., Khan, S.A., Yadav, K.K., Rana, K.L., Kour, D., Yadav, N. and Yadav, A.N. 2019. Fungal Phytoremediation of Heavy Metal-Contaminated Resources: Current Scenario and Future Prospects. In: Yadav, A.N., Singh, S., Mishra, S. and Gupta, A. (eds) Recent advancement in white biotechnology through Fungi: Volume 3: Perspective for Sustainable Environments. Springer International Publishing, Cham, pp 437–461.

Muleta, D., Assefa, F., Borjesson, E. and Granhall, U. 2013. Phosphate solubilizing rhizobacteria associated with *coffea arabica* L. in natural coffee forests of southwestern Ethiopia. *Journal of the Saudi Society of Agricultural Sciences* 12(1):73-84

Odoh, C.K., Akpi, U.K. and Anya, F. 2017. Environmental impact of mineral exploration in Nigeria and their phytoremediation strategies for sustainable ecosystem. *Global Journal of Science Frontier Research* 17(3):19–27.

Odoh, C.K., Eze, C.N., Akpi, U.K. and Unah, V.U. 2019a. Plant growth promoting rhizobacteria (PGPR): a novel agent for sustainable food production. *American Journal of Agricultural and Biological Sciences* 14(35): 54.

Odoh, C.K., Zabbey, N., Sam, K. and Eze, C.N. 2019b. Status, progress and challenges of phytoremediation—An African scenario. *Journal of Environmental Management* 237:365 378.

Oves, M., Khan, M.S. and Zaidi, A. 2013. Chromium reducing and plant growth promoting novel strain *Pseudomonas aeruginosa* OSG41 enhance chickpea growth in chromium amended soils. *European Journal of Soil Biology* 56:72–83

Oyetibo, G.O., Ilori, M.O., Obayori, O.S. and Amund. 2015, O.O. Metal biouptake by actively growing cells of metal-tolerant bacterial strains. *Environmental Monitoring and Assessment* 187: 525

Oyetibo, G.O., Ilori, M.O., Adebusoye, S.A., Obayori, O.S and Amund, O.O. 2010. Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. *Environmental Monitoring and Assessment* 168:305–314

Patten, C.L and Glick, B.R. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology* 42:207–220

Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiology* 17:362–370.

Prakash, J. and Arora, N.K. 2019. Phosphate solubilizing *Bacillus spp*. enhances growth, phosphorus uptake and oil yield of *mentha arvensis. Biotechnology* 9(4): 126

Qiao, J.Q., Wu, H.J., Rong Huo, R., Gao, X.W and Borriss, R. 2014. Stimulation of plant growth and biocontrol by *Bacillus amyloliquefaciens* subsp. plantarum FZB42 engineered for improved action. *Chemical and Biological Technologies for Agriculture* 1:12

Sam, K., Coulon, F. and Prpich, G. 2017. A multi-attribute methodology for the prioritization of oil contaminated sites in the Niger Delta. *Science of the Total Environment* 579:1323–1332.

Shin, S.H., Lim, Y., Lee, S.E., Yang, N.W. and Rhee, J.H. 2001. CAS agar diffusion assay for the measurement of siderophores in biological fluids. *Journal of Microbiological Methods* 44(1):89-95.

Sohrt, J., Lang, F. and Weiler, M. 2017. Quantifying components of the phosphorus cycle in temperate forests. *Wiley Interdisciplinary Reviews* 4: e1243.

Sudhakaran M., Ramamoorthy D., Savitha V., Balamurugan S. 2018. Assessment of Trace Elements and Its Influence on Physicochemical and Biological Properties in Coastal Agroecosystem Soil, Puducherry Region. *Geology, Ecology and Landscapes* 2(3): 169-176

Takada, H.Y and Matsumoto, N. 2005. Skim milk drastically improves the efficacy of DNA extraction from andisol, a volcanic ash soil. *Japan Agricultural Research Quarterly*. DOI: 10.6090/jarq.39.247

van Loon, L.C. 1998. Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology*. 119:243-254.

van Loon, L.C., Bakker, P. A. H. M. and Pieterse, C. M. J. 2007. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* 36: 453-483

Walker, D.J., Clemente, R., Roig, A. and Bernal, M.P. 2003. The effect of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environmental Pollution* 22:303–312

Zabbey, N., Sam, K. and Onyebuchi, A.T. 2017. Remediation of contaminated lands in the Niger Delta, Nigeria: prospects and challenges. *Science Total Environment* 586:952–965.