**BIOREMEDIATION OF CRUDE OIL POLLUTED SOIL USING *Pseudomonas aeruginosa* AND *Aspergillus niger***

**ABSTRACT**

The effectiveness of *Pseudomonas aeruginosa*, *Aspergillus niger* and the consortium of these two bioremediants in remediating soils polluted with crude oil was studied. Soil samples were contaminated with different levels of crude oil (4%V/W of crude oil -0.2kg/5kg of soil 80,000kg/ha; 8%V/W of crude oil -0.4kg/5kg of soil = 160,000kg/ha; and 12%V/W of crude oil =0.6kg/5kg of soil 240.000kg/ha), the contaminated soils were inoculated with three different concentrations (50mls, 100mls and 150mls) of the bioremediants, these were left to stand for three months (90 days), and the total hydrocarbon content (THC), was measured at the start (day 0) and at the end (day 90) of the experiment. Results showed that the 4% V/W setups had the highest level of bioremediation when inoculated with a consortium of 150mls of *Pseudomonas aeruginosa* and 150mls of *Aspergillus niger*, reducing the THC considerably (from 7200 to 3600mg/kg).This demonstrates how bacteria and fungi work together to break down the contamination in soil polluted with crude oil. A decrease in THC of the contaminated soil inoculated with the two bioremediants both alone and in consortium (150mls each of *Pseudomonas aeruginosa* and *Aspergillus niger*, which is also their recommended application rate) was also recorded, indicating their strong potential in the breakdown of crude oil. The combined use of *Pseudomonas aeruginosa* and *Aspergillus niger* as bioremediants, rather than their individual usage, is recommended as a preferable method for remediating soil contaminated with crude oil.

**Keywords:** *Aspergillus niger*, Crude Oil, *Pseudomonas aeruginosa*, Bioremediation, Total Hydrocarbon Content

**INTRODUCTION**

Soil is the primary supporter of the terrestrial environment which are constantly interacting with one another to influence the evolution and final destination of inorganic and organic contaminants (Chen *et al*., 2019). However, soil pollution which is often characterized as the presence of a chemical agent or compound that is foreign and/or present in greater concentration in the soil environment and poses a risk to species that are not explicitly targeted has frequently reported (FAO & UNEP, 2021). According to Ebadi *et al*. (2018), soil pollution is a threat that is frequently difficult to see or analyze immediately, making it a concealed, and intentional or unintentional spills or leaks of solvents or petroleum products as a result of improper disposal, handling, or storage is one of the primary causes of soil pollution (Rodríguez, 2018). The primary reasons for concern about soil pollution include health risks, direct contact with contaminated soil, breathing contaminated vapor, or secondary contamination of water supplies inside and beneath the soil (Alori & Fawole, 2017, Alori *et al*., 2017).

Crude oil is a complex mixture of persistent and environmentally hazardous hydrocarbons and other chemical components, and one of the main contributors to soil pollution is crude oil spills (Ortega *et al*., 2018). Crude oil spills is an international environmental problem that endangers both the environment and human health by polluting the soil with petroleum hydrocarbons (Yang *et al*. 2017). Also, it significantly affects the number and activity of soil microorganisms as well as the chemical characteristics of the soil (Nwankwoala & Omofuophu 2019). Additionally, crude oil pollution has negative impacts including: clogged pore space, reduced soil aeration, water infiltration, and increased bulk density of soil that affects plant growth, reduced soil fertility, decreased agricultural productivity, and altered ecosystem aesthetic value (Brown & Tari, 2015). Moreover, because of their persistence and biological toxicity, carcinogenic and mutagenic crude oil chemicals have the potential to produce fatal alterations in genetic material even at low levels of pollution (Dhote *et al*., 2017; Fatimah *et al*., 2017).

One of the fundamental issues facing the oil industry today is how to increase the recovery of the significant portion of oil that remains unrecovered in both new and old depleted producing fields is (Ojewumi *et al*., 2017). The well-known bioremediation method is a fantastic spill cleanup method that uses microorganisms' natural capacity to decompose materials to break down and reduce environmental pollutants (Ojewumi *et al*., 2018). Of all the methods used to clean up the affected areas, in situ bioremediation with native microorganisms is by far the most popular (Ojewumi *et al*., 2017; Nuhu *et al*., 2021).

*Aspergillus niger* is widely used in biotransformations and waste management, and according Safiyanu *et al*. (2015), and Maxwell *et al*. (2023), the fungus is most frequently found in mesophilic settings, such as decomposing vegetation or soil and plants. Filamentous fungi provide useful enzymes that aid in the breakdown of hydrocarbons (Alori & Babalola, 2018), and their aggressive development, increased biomass output, and widespread hyphal proliferation in soil are the causes of this (Nuhu *et al*., 2021). Also, the possibility for biodegradation technology is provided by fungi (Maxwell et al., 2023).

*Pseudomonas aeruginosa* is a rod-shaped, gram-negative bacterium that is roughly 0.5–1.0 µm wide and 1–5 µm long, breaks down crude oil and has remarkable dietary diversity (Nuhu *et al*., 2021). In addition to degrading aromatic hydrocarbons, *Pseudomonas aeruginosa* is capable of breaking down toluene, which is the most basic form of methylbenzene, and it is present in soil, water, people, animals, plants, sewage, and hospitals (Maxwell *et al*., 2023). Temperature, oxygen, pH, and the amount of nitrogen and phosphorus present are all essential for microbial growth, and the kind of soil in which biodegradation takes place affects both the rate and degree of biodegradation (Alori & Babalola, 2018). Fungi and bacteria are the primary microorganisms that break down petroleum hydrocarbons (Nuhu *et al*., 2021), and using microorganisms to break down hydrocarbons has been the most eco-friendly way to clean up oil spills (Maxwell *et al*., 2023).

In recent years, as people have looked for more sustainable ways to clean up polluted surroundings, interest in the microbial biodegradation of pollutants and bioremediation, which uses microbial activity to degrade and detoxify environmental contaminants, has increased. The aim of the study is to assess the potentials of *Pseudomonas aeruginosa* and *Aspergillus niger* for bioremediation of crude oil polluted sandy loam ultisol.

**MATERIALS AND METHODS**

**Study Area**

Samples of soil were collected from an oil producing area (Emohua) in River State in South-South Nigeria. Emohua is located at latitude 4o 52’ 12” N \*, and longitude 6o 51’ 30" E. The climate is a humid tropical climate, with mean annual rainfall of 2138 mm minimum and maximum temperatures of 22 and 29o C respectively. It has high humidity. With respect to rainfall, two distinct seasons (rainy which lasts for eight months and dry which lasts for four months) prevail in both locations. Rainfall distribution in this location is bimodal and the soil is deep and well-drained.

**Soil**

The research site's soil originated from a geomorphic requin of coastal-plain sand. Generally, sedimentary rocks over which sandy loam/Typical paleudult developed.

**Sources of Crude Oil**

The crude oil (Bonny light), which is black in color, was procured from the Nigeria Petroleum Development Company (NPDC) Odidi flow-station, located in Warri. With the exception of the control, 1700 g of crude oil were applied to all of the experimental plots, resulting in an initial THC concentration of 9296.825 mg/kg.

**Research Design**

A 3 x 10 factorial screen house experiment was arranged in a completely randomized design/split plot design and carried out with three replications.

**Soil Sampling**

A composite soil samples was randomly collected under a grass/legume fallow from an oil producing area (Emohua in Rivers state) at a depth of 0-30cm. Sampling was done early in June. Undisturbed soil samples were collected into sack bags using shovels and maintaining a distance of about 1 m apart extending down to 15 cm depth. These samples were composited into one and stored in sack bags for the research work, i.e., contamination with crude oil and remediation with *Pseudomonas spp* and *Aspergillus spp*. Some of the samples of soil were taken to the laboratory for physicochemical analysis and to the screen house for the remediation study.

From the bulk soil sample, five kilograms of the air-dried soil (sandy-loam) were measured into each of the experimental pots/buckets. Then, these buckets were randomly punctured in various places to make needle-like holes, which allowed air to travel through but not soil particles. The pollutions were administered by spraying the oil onto the soil. The polluted pots were incubated for 3 months.

**Sampling Period**

Samples were subsequently collected and analyzed before contamination, two weeks after contamination, before inoculation, and then left to stand for three months after which samples were collected for analysis.

**Soil Sample Collection**

The chemical properties of the soil were assessed after being passed through a filter (2 mm) for preliminary investigations to ascertain their chemical properties. After tilling with a soil spatula, the soil samples for laboratory examination were taken from the various treatment pots in sterile sample bags at a depth of 0-15 cm. Four to ten random sites per pot were used to gather soil samples, which were then bulked to create a composite sample. For microbiological and chemical tests, small amounts (5 g) of the composite samples were taken and placed in sterile vials. All microbiological analysis was carried out in the Microbiology laboratory of Gifted Green Research while chemical analysis was carried out at Soil, Plant, Fertilizer and Water Laboratory, (NRCRI), Umudike in Abia State, Nigeria.

**Biodegradation Process**

5kg of soil was placed into each of the experimental pots. The experimental pot was polluted with different volumes of crude oil (4%v/w, 8% v/w and 12 % v/w (0.2kg/5kg soil w/w = 80,000kg/ha; 0.4kg/5kg soil w/w = 160,000kg/ha and 0.6kg/5kg of soil w/w = 240,00kg/ha) to the soil. They were allowed to stay for 14 days undisturbed. This was to allow crude oil to volatilize and sorb before being treatment with microorganisms; *Pseudomonas aeruginosa* and *Aspergillus niger* each and in consortium were added at three different concentrations 50, 100 and 150 mls accordingly. This was replicated 3 times for each of the experimental pots inoculated with the microorganism. Three (3) of the pots were designated as pristine crude oil polluted soil without treatments to serve as controls while other seven (7) pots received the different levels of treatments respectively. This gave a total of 30 treatments and 90 observations.

**Application of Micro-organisms and Crude Oil Treatment**

To evaluate the efficiency of bacterial and organic treatments, crude oil contamination at 4%v/w, 8%v/w and 12%v/w, (0.2kg/5kg soil w/w = 80,000kg/ha; 0.4kg/5kg soil w/w = 160,000kg/ha and 0.6kg/5kg of soil w/w = 240,00kg/ha in one furrow slice of soil) were selected as pollution levels, respectively. The soil was polluted with oil compounds at four levels including a contaminated soil sample without treatment which was used as a control. Two factors were evaluated: soil contamination at three levels and effect of the selected strains of microorganisms; one strain of *Pseudomonas aeruginosa* as well as one strain of *Aspergillus niger*.

**Data Analysis**

The soil samples were bulked for analysis and the levels of pollution became reps for statistical analysis. The effect of the strains of microorganisms (*Pseudomonas aeruginosa* and *Aspergillus* *niger*) on the contaminated soil was statistically analyzed by performing a one-way analysis of variance (ANOVA) on the soil data using the software IBM SPSS Statistics 22 and subjected to analysis of Variance at 95% of Significance. The means were compared using LSD at 95% confidence interval. Some chemical properties of the soil were also assessed. Also, correlations were run on the different treatments and chemical properties at different sampling periods to establish the degree of relationships among them. This was done using the software IBM SPSS Statistics 22.

**RESULTS**

**Initial Soil Chemical Properties**

The results of the initial chemical properties of the soil used are presented in Table 1. The initial (soil sample without any form of contamination or treatment) showed pH values of 5.7 in water and 4.8 in potassium chloride, values for calcium, magnesium, potassium and sodium are 1.6, 0.34, 0.31, and 1.64 respectively, total hydrocarbon content was 1200, among others. Immediately after contamination with crude oil, pH values in water increased to 6.9 in EMO C4, 7.2 in EMO C8, and 7.9 in EMO C12, pH values in potassium chloride increased to 6.2 in EMO C4, 6.8 in EMO C8, and 6.9 in EMO C12, total hydrocarbon content values increased to 7200 in EMO C4, 9800 in EMO C8, and 13200 in EMO C12, among others.

**Table 1: Values of the Chemical Properties of the Soil used for the Study**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **SOC** | **SOM** | **P** |  | **Ca** | **Mg** | **K** | **Na** | **EA** |  |  |  |  |
|  |  | **pH in** **KCI** | **(%)** |  |  | **ECEC** |  |  |  |  |  |  | **BS****(%)** | **AI3+****(cmol/kg** |
|  | **pH in H2O** |  |  |  | **(mg/kg)** |  |  |  **Cmol kg-1** | **THC****Mg/kg** | **TN****(%)** |  |  |
| Initial | 5.7 | 4.8 | 2.07 | 3.57 | 0.4 | 4.3 | 1.6 | 0.34 | 0.31 | 1.64 | 7.7 | 1200 | 0.2 | 78.7 | 0.58 |
| EMO C4 | 6.9 | 6.2 | 6.20 | 5.90 | 7.8 | 112.3 | 6.2 | 0.54 | 0.51 | 0.68 | 20.24 | 7200 | 0.32 | 96.6 | 0.18 |
| EMO C8 | 7.2 | 6.8 | 3.52 | 6.07 | 4.6 | 16.4 | 9.0 | 0.50 | 0.57 | 0.34 | 26.9 | 9800 | 0.34 | 98.7 | 0.08 |
| EMO C12 | 7.9 | 6.9 | 3.70 | 6.38 | 8.4 | 20.7 | 12.8 | 0.69 | 0.66 | 0.26 | 35.11 | 13200 | 0.38 | 99.3 | 0.08 |

**KEY:** SOM – Soil Organic Matter; SOC – Soil Organic Carbon; ECEC – Effective Cation Exchange Capacity

BS – Base Saturation; TN - Total Nitrogen; THC – Total Hydrocarbon

Initial – soil sample immediately after collection

EMO C4 – soil sample contaminated with 4% V/W of crude oil = 0.2kg/5kg of soil = 80,000kg/ha;

EMO C8 – soil sample contaminated with 8% V/W of crude oil = 0.4kg/5kg of soil = 160,000kg/ha

EMO C12 – soil sample contaminated with 12% V/W of crude oil = 0.6kg/5kg of soil = 240,000kg/ha

EMO C4, EMO C8 and EMO C12 are soil samples with the three different levels of contamination, 4%v/w, 8%v/w and 12%v/w.

**Soil Chemical Properties after Three Months**

At the end of the three months period of the study, selected chemical properties of the various levels of the contaminated soils treated with different levels of *Pseudomonas aeruginosa* are shown in Table 2. pH values in water ranged between 6.6 and 7.3 in controls and in various levels of treatment with *Pseudomonas aeruginosa* and showed no significant difference, pH values in potassium chloride ranged between 5.9 and 6.9 in controls and in various levels of treatment with *Pseudomonas aeruginosa* and showed no significant difference, least total hydrocarbon value of 4500 was recorded in the treatment PS C4 150, followed by 5000 PS C4 100, highest hydrocarbon content value of 12800 was recorded in control C12 ctrl, followed by 11000 in the treatment PS C12 50, among others.

**Table 2: Mean Values of the Chemical Properties of *Pseudomonas aeruginosa* Treated Soils after Three Months**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **SOC** | **SOM** | **P** |  | **Ca** | **Mg** | **K** | **Na** | **EA** |  |  |  |  |
|  |  | **pH in** **KCI** | **(%)** |  |  | **ECEC** |  |  |  |  |  |  | **BS****(%)** | **AI3+****(cmol/kg** |
|  | **pH in H2O** |  |  |  | **(mg/kg)** |  |  |  **Cmol kg-1** | **TN****(%)** | **THC****Mg/kg** |  |  |
| C4 ctrl | 6.7 | 5.9 | 3.15 | 5.43 | 25.8 | 10.6 | 5.2 | 0.50 | 0.47 | 0.74 | 17.51 | 0.20 | 7000 | 95.8 | 0.26 |
| C8 ctrl | 6.9 | 6.5 | 3.39 | 5.84 | 30.4 | 14.5 | 7.1 | 0.55 | 0.54 | 0.42 | 23.11 | 0.34 | 9700 | 98.2 | 0.12 |
| C12 ctrl | 7.3 | 6.9 | 3.47 | 5.98 | 35.8 | 19.3 | 10.4 | 0.65 | 0.60 | 0.34 | 31.29 | 0.38 | 12800 | 98.6 | 0.10 |
| PS C4 50 | 6.7 | 6.1 | 3.25 | 5.60 | 24.6 | 11.1 | 4.4 | 0.46 | 0.40 | 0.82 | 17.18 | 0.29 | 5200 | 95.2 | 0.28 |
| PS C8 50 | 6.9 | 6.4 | 3.42 | 5.90 | 26.4 | 13.4 | 5.6 | 0.51 | 0.48 | 0.64 | 20.63 | 0.30 | 8800 | 96.9 | 0.22 |
| PS C12 50 | 7.2 | 6.9 | 3.48 | 6.00 | 30.3 | 17.1 | 7.6 | 0.62 | 0.58 | 0.48 | 26.36 | 0.31 | 11000 | 98.2 | 0.14 |
| PS C4 100 | 6.7 | 6.0 | 3.32 | 5.72 | 24.0 | 11.3 | 4.0 | 0.46 | 0.45 | 0.81 | 17.02 | 0.30 | 5000 | 95.2 | 0.26 |
| PS C8 100 | 6.9 | 6.3 | 3.43 | 5.91 | 27.0 | 13.6 | 6.2 | 0.52 | 0.49 | 0.62 | 21.42 | 0.30 | 7600 | 97.1 | 0.20 |
| PS C12 100 | 7.3 | 6.8 | 3.49 | 6.02 | 30.8 | 17.5 | 8.0 | 0.60 | 0.57 | 0.48 | 27.15 | 0.31 | 9800 | 98.2 | 0.16 |
| PS C4 150 | 6.6 | 5.9 | 3.32 | 5.72 | 24.0 | 11.2 | 4.2 | 0.47 | 0.45 | 0.80 | 17.12 | 0.29 | 4500 | 95.3 | 0.24 |
| PS C8 150 | 6.9 | 6.3 | 3.45 | 5.95 | 27.4 | 13.7 | 6.2 | 0.52 | 0.50 | 0.62 | 21.53 | 0.30 | 7000 | 97.1 | 0.18 |
| PS C12 150 | 7.3 | 6.9 | 3.53 | 6.09 | 30.5 | 17.6 | 8.4 | 0.61 | 0.59 | 0.46 | 27.45 | 0.31 | 9200 | 98.3 | 0.12 |

**KEY:** SOM – Soil Organic Matter; SOC – Soil Organic Carbon; ECEC – Effective Cation Exchange Capacity

BS – Base Saturation; TN - Total Nitrogen; THC – Total Hydrocarbon

C4 ctrl, C8 ctrl, C12 ctrl – soil contaminated with crude oil at the three levels without any microorganisms

PS C4 50; PS C8 50; PS C12 50 – soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 50mls of *Pseudomonas aeruginosa* each.

PS C4 100; PS C8 100; PS C12 100 - soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 100mls of *Pseudomonas aeruginosa* each.

PS C4 150; PS C8 150; PS C12 150 - soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 150mls of *Pseudomonas aeruginosa* each.

**Chemical Properties of Crude Oil Contaminated Soil Treated with *Aspergillus niger* after Three Months**

The results for some selected chemical properties of the crude oil contaminated soil treated with *Aspergillus niger* at the end of the three months are shown in Table 3. pH values in water ranged between 6.6 and 7.3 in controls and in various levels of treatment with *Aspergillus niger* and showed no significant difference, pH values in potassium chloride ranged between 5.7 and 6.9 in controls and in various levels of treatment with *Aspergillus niger* and showed no significant difference, least total hydrocarbon value of 4700 was recorded in the treatment AS C4 150, followed by 5200 AS C4 100, highest hydrocarbon content value of 12800 was recorded in control C12 ctrl, followed by 11400 in the treatment AS C12 50, among others.

**Table 3: Mean Values of the Chemical Properties of the *Aspergillus niger* Soil after Three Months**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **SOC** | **SOM** | **P** |  | **Ca** | **Mg** | **K** | **Na** | **EA** |  |  |  |  |
|  |  | **pH in** **KCI** | **(%)** |  |  | **ECEC** |  |  |  |  | **TN****(%)** | **THC****Mg/kg** | **BS****(%)** | **AI3+****(cmol/kg** |
|  | **pH in H2O** |  |  |  | **(mg/kg)** |  |  |  **Cmol kg-1** |  |  |  |  |
| C4 ctrl | 6.7 | 5.9 | 3.15 | 5.43 | 25.8 | 10.6 | 5.2 | 0.50 | 0.47 | 0.74 | 17.51 | 0.20 | 7000 | 95.8 | 0.26 |
| C8 ctrl | 6.9 | 6.5 | 3.39 | 5.84 | 30.4 | 14.5 | 7.1 | 0.55 | 0.54 | 0.42 | 23.11 | 0.34 | 9700 | 98.2 | 0.12 |
| C12 ctrl | 7.3 | 6.9 | 3.47 | 5.98 | 35.8 | 19.3 | 10.4 | 0.65 | 0.60 | 0.34 | 31.29 | 0.38 | 12800 | 98.6 | 0.10 |
| AS C4 50 | 6.6 | 5.7 | 3.23 | 5.57 | 24.0 | 10.8 | 4.4 | 0.45 | 0.39 | 0.88 | 16.93 | 0.27 | 5600 | 94.8 | 0.30 |
| AS C8 50 | 6.8 | 6.1 | 3.35 | 5.78 | 26.1 | 13.0 | 5.4 | 0.50 | 0.48 | 0.66 | 20.04 | 0.29 | 9400 | 96.7 | 0.24 |
| AS C12 50 | 7.1 | 6.6 | 3.40 | 5.86 | 30.1 | 16.9 | 7.2 | 0.60 | 0.57 | 0.52 | 25.79 | 0.30 | 11400 | 97.98 | 0.16 |
| AS C4 100 | 6.6 | 6.0 | 3.27 | 5.64 | 24.1 | 11.1 | 4.1 | 0.46 | 0.44 | 0.86 | 16.96 | 0.27 | 5200 | 94.93 | 0.28 |
| AS C8 100 | 6.8 | 6.2 | 3.38 | 5.83 | 26.3 | 13.1 | 6.0 | 0.51 | 0.47 | 0.64 | 20.72 | 0.28 | 8000 | 96.1 | 0.16 |
| AS C12 100 | 7.2 | 6.8 | 3.43 | 5.91 | 30.4 | 17.0 | 7.7 | 0.59 | 0.56 | 0.48 | 26.33 | 0.30 | 10200 | 98.18 | 0.12 |
| AS C4 150 | 6.7 | 6.0 | 3.24 | 5.59 | 24.2 | 10.6 | 4.1 | 0.46 | 0.44 | 0.86 | 16.46 | 0.27 | 4700 | 94.78 | 0.26 |
| AS C8 150 | 6.9 | 6.4 | 3.40 | 5.86 | 26.8 | 13.4 | 6.1 | 0.51 | 0.48 | 0.64 | 21.13 | 0.28 | 7200 | 96.97 | 0.18 |
| AS C12 150 | 7.3 | 6.9 | 3.45 | 5.97 | 30.0 | 17.4 | 8.2 | 0.58 | 0.56 | 0.48 | 27.22 | 0.31 | 9600 | 98.24 | 0.14 |

**KEY:** SOM – Soil Organic Matter; SOC – Soil Organic Carbon; ECEC – Effective Cation Exchange Capacity

BS – Base Saturation; TN - Total Nitrogen; THC – Total Hydrocarbon

AS C4 50; AS C8 50; AS C12 50 – soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 50mls of *Aspergillus niger*each.

AS C4 100; AS C8 100; AS C12 100 - soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 100mls of *Aspergillus niger*each.

AS C4 150; AS C8 150; AS C12 150 - soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 150mls of *Aspergillus niger*each.

**Chemical Properties of the Crude Oil Contaminated Soil Treated with the Combination of *Pseudomonas aeruginosa* and *Aspergillus niger* after Three Months**

The result at the end of the three months for some chemical properties of the crude oil contaminated soil treated with the combination of *Pseudomonas aeruginosa* and *Aspergillus niger* at varying levels are shown in Table 4. The least pH in water value (6.6) was recorded in PSAS C4 150, while the highest value (7.4) was recorded in PSAS C12 100 and PSAS C12 150, the least pH in potassium chloride value (6.0) was recorded in PSAS C4 150, while the highest value (6.9) was recorded in PSAS C12 100 and PSAS C12 150, the least total hydrocarbon content value of 3600 was recorded in PSAS C4 150, followed by 4000 recorded in PSAS C4 100, among others.

**Table 4.: Mean Values of the Chemical Properties of the *Pseudomonas aeruginosa* and *Aspergillus niger* Combination Treatment Soil after Three Months**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **SOC** | **SOM** | **P** | **Ca** | **Mg** | **K** | **Na** | **EA** | **ECEC** |  |  |  |  |
|  |  | **pH in** **KCI** | **(%)** |  |  |  |  |  |  |  |  |  | **BS****(%)** | **AI3+****(cmol/kg** |
|  | **pH in H2O** |  |  |  | **(mg/kg)** |  |  |  **Cmol kg-1** | **TN****(%)** | **THC****Mg/kg** |  |  |
| PSAS C4 50 | 6.7 | 6.1 | 3.35 | 5.77 | 24.4 | 11.4 | 4.5 | 0.46 | 0.40 | 0.78 | 17.55 | 0.28 | 4600 | 95.55 | 0.26 |
| PSAS C8 50 | 6.9 | 6.5 | 3.50 | 6.03 | 26.6 | 13.6 | 5.7 | 0.52 | 0.48 | 0.64 | 20.94 | 0.29 | 7500 | 96.94 | 0.16 |
| PSAS C12 50 | 7.3 | 6.8 | 3.62 | 6.24 | 30.5 | 17.6 | 7.6 | 0.61 | 0.58 | 0.44 | 26.82 | 0.32 | 9600 | 98.36 | 0.12 |
| PSAS C4 100 | 6.7 | 6.1 | 3.39 | 5.84 | 24.2 | 11.4 | 4.3 | 0.47 | 0.40 | 0.76 | 17.33 | 0.28 | 4000 | 95.61 | 0.24 |
| PSAS C8 100 | 6.8 | 6.3 | 3.46 | 5.97 | 26.8 | 13.8 | 6.3 | 0.52 | 0.49 | 0.62 | 21.73 | 0.29 | 6400 | 97.15 | 0.16 |
| PSAS C12 100 | 7.4 | 6.9 | 3.52 | 6.07 | 30.9 | 18.0 | 8.1 | 0.61 | 0.58 | 0.44 | 27.73 | 0.32 | 8600 | 8.41 | 0.12 |
| PSAS C4 150 | 6.6 | 6.0 | 3.34 | 5.76 | 24.4 | 11.6 | 4.3 | 0.47 | 0.40 | 0.76 | 17.54 | 0.28 | 3600 | 95.67 | 0.28 |
| PSAS C8 150 | 7.1 | 6.8 | 3.47 | 5.98 | 27.3 | 13.8 | 6.4 | 0.52 | 0.50 | 0.62 | 21.83 | 0.30 | 6200 | 97.16 | 0.22 |
| PSAS C12 150 | 7.4 | 6.9 | 3.58 | 6.17 | 30.8 | 18.0 | 8.6 | 0.61 | 0.59 | 0.42 | 28.22 | 0.32 | 7800 | 98.51 | 0.16 |

**KEY:** SOM – Soil Organic Matter; SOC – Soil Organic Carbon; ECEC – Effective Cation Exchange Capacity

BS – Base Saturation; TN - Total Nitrogen; THC – Total Hydrocarbon

PSAS C4 50; PS C8 50; PS C12 50 – soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 50mls of *Pseudomonas aeruginosa* and *Aspergillus niger*each.

PSAS C4 100; PS C8 100; PS C12 100 - soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 100mls of *Pseudomonas aeruginosa* and *Aspergillus niger*each.

PSAS C4 150; PS C8 150; PS C12 150 - soil contaminated with 4% V/W, 8% V/W, and 12% V/W of crude oil and inoculated with 150mls of *Pseudomonas aeruginosa* and *Aspergillus niger*each.

**DISCUSSION**

The removal of dangerous chemicals from soil, groundwater, sediment, and surface water through remediation offers a chance to lessen pollution and, consequently, pollution-related fatalities (Landrigan *et al*., 2018). Also, the co-application of plants and bacteria is regarded as a successful method for cleaning up soil that has been contaminated by crude oil, while the host plant provides a place for growth and colonization, the bacteria supply nutrients, and the bacteria breakdown organic pollutants at multiple locations throughout the host plant (Fatima *et al*. 2017). Using microorganisms to reduce the bioavailability of pollutants (particularly organic contaminants) allows microbial remediation, which makes them less hazardous to the ecosystem, simple to understand (Sylvester & Emike. 2017). These microorganisms have the ability to metabolize or break down-contaminants by utilizing them as food, and in order to bioremediate soil pollution, particular competent microbe strains have been frequently exploited (Atha *et al*., 2016; Chao et al 2017). Due to the fact that most remediation techniques are site-specific, Hussain *et al*. (2018) assert that it is crucial to apply appropriate bioremediation technology.

In this study, the initial soil sample without any form of contamination or treatment shows the soil of the study area to be acidic with pH of 4.8 (less than 5.5). Immediately after contamination with crude oil, pH, calcium, and magnesium values slightly increased while total hydrocarbon content and aluminum values did not increase significantly. The THC of the soil has a value of 1200mg/kg which was far above the 50mg/kg set by DPR for agricultural soils. This value changed remarkably after introducing the crude oil into the soil as seen in EMO C4, EMO C8 and EMO C12 (Table 1). These findings are similar to those reported by Ojewumi (2018) and Maxwell *et al*. (2023) for initial soil chemical properties before contamination and subsequent remediation.

At the end of the three months of the study, selected chemical properties of the various levels of the contaminated soils treated with different levels of *Pseudomonas aeruginosa* showed that the pH of the soil increased from acidic to moderately acidic ranges between 6.1-6.9 (greater than 5.5) (Table 2). Crude oil contaminated soils have been known to have high pH content, and this has been attributed to the presence and metabolic activities of the elements present in crude oil such as Hydrocarbon. The organic matter content of the soil decreased compared to their levels immediately after application of crude oil as seen in EMO C4, C8 and C12 and ranged between 5.90-6.0%. The available phosphorus decreased, ranging between 27-38mg/kg. The ECEC of the soils also decreased ranging between 31.29 20.63cmol/kg. The total Nitrogen of soils also reduced as seen in their values ranging between 0.293 0.309%. Similar decrease was also seen in base saturation and Aluminum levels of the soil. Total hydrocarbon content was significantly lower in most of the treatments compared with the control (Table 2).

Also, the results for some selected chemical properties of the crude oil contaminated soil treated with *Aspergillus niger* at the end of the three months showed that the pH of the soil increased from acidic to moderately acidic with ranges between 5.7 and 6.6 (greater than 5.5). The organic matter content of the soil, the available phosphorus, the total Nitrogen, base saturation, Aluminum levels, and total hydrocarbon contents decreased compared to their levels immediately after application of crude oil (Table 3). Furthermore, the result at the end of the three months for some chemical properties of the crude oil contaminated soil treated with the combination of *Pseudomonas aeruginosa* and *Aspergillus niger* at varying levels showed that the pH of the soil increased from acidic to moderately acidic with ranges between 6.1-6.8 (greater than 5.5), with the approach to neutral seen in the PSASC4 50 soil. The organic matter content of the soil, the available phosphorus, the total Nitrogen, base saturation, Aluminum levels, and total hydrocarbon content decreased compared to their levels immediately after application of crude oil (Table 4).

While we have reported variations in pH for control and different treatment levels, pH was not used to determine the bioremediation potential of *Pseudomonas aeruginosa* and *Aspergillus niger* because Ojewumi *et al*. (2018) had earlier reported that the soil pH during the bioremediation period is not a very reliable indicator of the level of bioremediation. In this study, total hydrocarbon content decreased compared to their levels immediately after application of crude oil and was used to determine the level of bioremediation by *Pseudomonas aeruginosa* and *Aspergillus niger*. This approach was also adopted by Ojewumi *et al*. (2018) and Maxwell *et al*. (2023). Furthermore, the decrease in total hydrocarbon content and other soil chemical properties reported in this study is supported by the findings of Adebiyi *et al*. (2015), Osalodion & Ita (2021), and Maxwell *et al*. (2023) who showed a decrease in total hydrocarbon content and the different soil chemical parameters in their study. While this study did not report any significant difference in the bioremediation potential of *Pseudomonas aeruginosa* and *Aspergillus niger*, Ojewumi *et al*. (2018) reported that raw crude polluted soil is better remedied by *Pseudomonas aeruginosa* (bacteria) and *Aspergillus niger* (fungi) alone and separately than by treated crude polluted soil, while Maxwell *et al*. (2023) posited that *Pseudomonas aeruginosa* outperformed *Aspergillus niger*. While our study used these microorganisms to remediate crude oil polluted soil, the study of Maxwell *et al*. (2023) used these organisms in the remediation of polluted water. This could explain the variation in the bioremediation potential of the microorganisms.

**CONCLUSION**

The consortium performance of *Pseudomonas aeruginosa* and *Aspergillus niger* on the soil that had been contaminated by crude oil as amendments demonstrates the potential and usefulness of these microbial strain as a cost-effective bioremediation method. The samples of polluted soil amended with both the *Pseudomonas aeruginosa* and *Aspergillus niger* exhibit a better level of restoration than the samples of polluted soil amended with only one of the two organisms. It was determined that utilizing both *Pseudomonas aeruginosa* and *Aspergillus niger* together is a more effective bioremediant than using any one of them separately based on the results obtained. Therefore, a consortium of *Pseudomonas aeruginosa* (bacteria) and *Aspergillus niger* (fungi) is recommended for effective bioremediation.

**COMPETING INTERESTS DISCLAIMER**:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

1. Adebiyi, F.M., Oluyemi, E.A. & Akande, A.A. (2015). Measurements of Physicochemical, Elemental, and Total Petroleum Hydrocarbon Contents as Pollution Indicators of Soils in Petroleum Products Retailing Station Surrounding Areas. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects*, 37, 299-308.
<https://doi.org/10.1080/15567036.2011.585385>
2. Alori. E.T. & Babalola, O.O. (2018). Microbial inoculant for improving crop quality and human health. *Frontiers in Microbiolology*, 9:22131-12
3. Alori, E.T. & Fawole, O.B. (2017). Microbial inoculants-assisted phytoremediation for sustainable soil management. (Eds.) Ansari AA, Gill R. Gill SS, Lanza GR. Newman L, editors. Phytoremediation: Management of environmental contaminants, vol. 5: 3-17.
4. Alori, E.T., Dare, M. O. & Babalola, O.O. (2017). Microbial inoculants for soil quality and plant health. In: Lichtfouse E, editor. *Sustainable agriculture review*, 22:281-308.
5. Athar, H.U., Ambreen S., Javed. M. Hina, M., Rasul, S., Zafar, Z.U., Manzoor, H. Ogbaga, C.C., Afzal, M. & Al-Qurainy, F. (2016). Influence of sub-lethal crude oil concentration on growth, water relations and photosynthetic capacity of maize (*Zea mays* L.) plants. *Environmental Science and Pollution Research*, 23, 1-12
6. Brown, I. & Tari, E. (2015). Evaluation of the effect of petroleum exploration and production activities in the social environment in Eboyi land, Nigeria. *International Journal of Scientific and Technology Research*, 4 (4): 273-282.
7. Chao, C., Qiu, L., Liu, C.& Yu, J. (2017). Elect of digerent enrichment strategies on microbial community structure in petroleum-contaminated marine sediment in Dalian, China. Marine Pollution Bulletin., 117, 274.
8. Chen, X.D., Dunfield, K.E., Fraser, T.D., Wakelin, S.A., Richardson, A.E. & Condron, L.M. (2019). Soil biodiversity and biogeochemical function in managed ecosystems. *Soil Research*, 58:1-20.
9. Dhote, M., Kumar, A., Jajoo, A. & Juwarkar, A. (2017). Assessment of hydrocarbon degradation potentials in plant-microbe interaction system with oil sludge contamination: A sustainable solution. *International Journal of Phytoremediation*, 19, 1085-1092.
10. Ebadi. A., Khoshkholgh Sima, N.A., Olamaee, M., Hashemi, M., & Ghorbani, N.R. (2018). Remediation of saline soils contaminated with crude oil using the halophyte Salicornia persica in conjunction with hydrocarbon-degrading bacteria. *Journal of Environmental Management*, 219.260-268.
11. FAO & UNEP. (2021). Environmental, health and socio-economic impacts of soil. Global assessment of soil pollution: Report. 4
12. Fatima, K., Imran A., Naveed, M. & Afzal, M. (2017). Plant-bacteria synergism: An innovative approach for the remediation of crude oil-contaminated soils. *Soil Environment*, 36, 93-113.
13. Hussain, I., Puschenreiter, M., Gerhard, S., Schöftner, P. & Yousaf, S. (2018). Rhizoremediation of petroleum hydrocarbon-contaminated soils: Improvement opportunities and field applications. Environ Exp Bot. 147: 202-219. *Journal of Environmental Management*, 71, 95-122.
14. Landrigan, P.J., Fuller, R., Acosta, N.J.R., Adeyi, O., Arnold, R., Basu, N.N., Baldé, A.B., Bertollini, R., BoseO'Reilly, S., Boufford, J.I., Breysse, P.N., Chiles, T., Mahidol, C., CollSeck A.M., Cropper, M.L., Fobil, J., Fuster, V., Greenstone, M., Haines, A. & Zhong, M., (2018). The Lancet Commission on pollution and health. The Lancet Commissions, 391(10119), 462-512.
15. Maxwell, O.I., Onyebuchukwu, M.G., Chinonso, O.E., Nnaemeka, I.C. & Afamefuna, E.K. (2023). Kinetics of Bioremediation of Oil Contaminated Water Dispersed by Environment-Friendly Bacteria (*Pseudomonas aeruginosa*) and Fungi (*Aspergillus niger*). *Advances in Chemical Engineering and Science*, 13, 19-35. doi: [10.4236/aces.2023.131003](https://doi.org/10.4236/aces.2023.131003).
16. Nuhu, M.M., Rene, E.R. & Ishaq, A. (2021) Remediation of Crude Oil Spill Sites in Nigeria: Problems, Technologies, and Future Prospects. *Environmental Quality Management*, 31, 165-175. <https://doi.org/10.1002/tqem.21793>
17. Nwankwoala, H., & Omofuophu, E. (2019). Modeling and assessment of human health risks from exposure to hydrocarbon contaminated soils in parts of Bonny, Rivers State, Nigeria. *Journal of Environmental Earth Science*, 2(3):1-7.
18. Ojewumi, M.E., Anenih, E.A., Taiwo, O.S., Adekeye, B.T., Awolu, O.O. & Ojewumi, E.O. (2018). A Bioremediation Study of Raw and Treated Crude Petroleum Oil Polluted Soil with *Aspergillus niger* and *Pseudomonas aeruginosa*. *Journal of Ecological Engineering*, 19. 226–235.
19. Ojewumi M.E., Emetere M.E., Babatunde D.E. & Okeniyi, J.O. (2017). In Situ Bioremediation of Crude Petroleum Oil Polluted Soil Using Mathematical Experimentation. International Journal of
20. Ortega, M. F., Guerrero D.E.. Garcia-Martinez M.J., Bolonio D., Llamas J.F. & Canoira L. (2018). Optimization of Land Farming Amendments Based on Soil Texture and Crude Oil. Concentration. *Water, Air & Soil Pollution*, 229:229-34.
21. Osalodion, E.U. & Ita, E.U. (2021). Effects of Time on pH, Total Bacteria Counts, and Total Hydrocarbon Contents in the Bioremediation of Crude Oil Contaminated Soil Using Indigenous Bio-Stimulants. *Tanzania Journal of Science*, 47, 344-353.
22. Rodriguez-Eugenio, N., McLaughlin, M. & Pennock, D. (2018). Soil pollution: A hidden reality. Rome FAO (2018), 142
23. Safiyanu, I., Sani, I. & Rita, S.M. (2015). Review on Bioremediation of Oil Spills Using Microbial Approach. Volume 3.
24. Sylvester, U. &, Emik,e O. (2017). Induced degradation of crude oil polluted soil by microbial augmentation. *Journal of Engineering Studies and Research*, 23, 4.
25. Yang, Z.H., Lien, P.J., Huang, W.S., Surampalli, R.Y. & Kao, C.M. (2017). Development of the risk assessment and management strategies for TPH contaminated sites using TPH fraction methods. *Journal of Hazardous and Toxic Radioactive Waste*, 21 (1):3-10.