



GREEN REMEDIATION AND ENHANCED METAL ACCUMULATION USING *Moringa oleifera* LEAF EXTRACTS AND HYDROCARBON-DEGRADING MICROORGANISMS IN CRUDE OIL-IMPACTED SOILS OF THE NIGER DELTA, NIGERIA

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ABSTRACT

This emerging technology called “Phytoremediation” which uses plants to remove pollutants from the environment is preferred over traditional methods, because it offers site restoration, partial decontamination, and maintenance of biological activity and physical structure whilst being potentially cheap, visually unobtrusive and with a possibility of bio-recovery of metals. Because of these advantages, phytoremediation is considered as a 'green', sustainable pollution removal process. The present study was designed to evaluate Phytoremediation potential of *Moringa oleifera* leaf extract on soil contaminated with Spent Engine Oil. Top soil (0-15cm depth) was randomly collected and 5kg each of the composite samples were transferred into fifty (50) plastic buckets with drainage holes at the base, plugged with cotton wool to retain the soil. The Plastic buckets were arranged into five treatment groups with Ten (10) replicates each. The polythene bags were arranged in a completely randomized block design. Spent Engine oil obtained from mechanic shops in Bedwell Calabar, Cross River State, was applied as the pollutant. The Spent engine oil was introduced at different concentration levels (0.3, 0.5, 0.7, and 1.0 l/5kg) into the treatment groups apart from the control (0.0 l/5kg). The results across the various treatment levels were subjected to statistical analysis, using Analysis of variance (ANOVA) and the results showed that physicochemical parameters (soil pH and organic carbon), Heavy Metals present, Total Petroleum Hydrocarbon and Microbial Counts were significantly higher ($P < 0.05$) in treatment groups than the control. The result also showed a significant reduction ($P < 0.05$) of the Total petroleum hydrocarbon after treatment.

KEYWORDS: *Moringa oleifera*, phytoremediation, soil pollution, crude oil spillage

INTRODUCTION

Phytoremediation is a broad term that has been in use since 1991 to describe the use of plants to reduce the volume, mobility, or toxicity of contaminants in soil, groundwater, or other contaminated media (Abou-Shanab et al., 2007).

Petroleum in its natural state is referred to as crude oil. Crude oil is mainly either black or green but it can be light yellow. It varies considerably in density and is described as heavy, average or light (Agboun et al., 2016). Petroleum is at present Nigeria's and indeed the world's most important derived energy source. Soil contamination is caused by the presence

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of xenobiotic (human-made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals, or improper disposal of waste. The most common chemicals involved are petroleum hydrocarbons (such as naphthalene and benzoic, solvents, pesticides, lead and other heavy metals.

Contamination is correlated with the degree of industrialization and intensity of chemical usage. Controlled and uncontrolled disposal of waste, accidental and process oil spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants into non-contaminated sites as dust or leachate and contribute towards contamination of our ecosystem (Ekpo et al., 2021). Concern over soil contamination by crude oil or hydrocarbon products in general, is gathering momentum after a similar feeling has been around for a while on oil spills, which enjoy more media coverage because of the often-spectacular visual effects images conveyed to people. The primary aims of any remediation are reduction of actual or potential environmental threat and reduction of potential risks so that unacceptable risks are reduced to acceptable levels (Ogbonna et al., 1991). Consequently, the need for remediation will depend on the degree of actual or potential environmental threat or the level of risk (Baker et al., 1994). Remediation of a contaminated site is achieved by one or more of the following objectives: Modification of the contaminants to a less toxic form, Removal or destruction of the contaminants and Isolation of the contaminant from the target by interrupting the pathway of exposure (Osuji et al., 2004). Environmental quality is measured by quantitative data and/or by performance of environmental media such as air, soil and water.

However, these media have capacity to cleanse or regenerate themselves biologically or mechanically. The impacts of urbanization or industrialization continuously limit or stress their natural cleansing potential. This in turn has led to the decline in the quality of air, soil and water in areas where industrial development activities are prevalent (Ekpo et al., 2021). A wide variety of chemicals have been detected in soil, water, and air (Ekpo et al., 2021). Heavy metals pose a critical concern to human health and the environment due to their common occurrence as contaminants, low solubility in biota, and the classification of several heavy metals as carcinogenic and mutagenic (Ekpo et al., 2021).

Bioremediation processes may be directed towards accomplishing; Complete oxidation of organic contaminants (termed mineralization), Biotransformation of organic chemicals into smaller less toxic metabolites, or Reduction of highly electrophilic halo- and nitro-groups by transferring electrons from an electron donor (typically a sugar or fatty acid) to the contaminant, resulting in a less toxic compound. With

increasing numbers of successfully demonstrated cleanups, biological remediation alone or in combination with other methods, has gained an established place as a soil restoration technology. Various strains of microorganisms are found in most soils and are able to utilize hydrocarbons present as a food source, enzymes released by the microorganisms break down the microorganism to substances that can easily be consumed by these microbes.

However without some help these processes proceed slowly, this process may be so slow that it may take decades before the spills are completely remediated as a result of that our concern here is to speed up this bioremediation process and this is achieved by the provision of a suitable ambient conditions for this natural bioremediation process to take place. We may not

necessarily have to introduce non-resident micro-organism to the soil because the crude oil polluted environment already have microbes there that feed on the crude, all we need to do is to give these microbes a better conditioning so that they can reproduce more and increase their population so as to quickly feed on the crude. Some microorganisms are present in the contaminated site, but for effective remediation, growth of microorganism should be stimulated. Bio-stimulation is the process of adding nutrient, electron acceptor and oxygen to stimulate existing bacteria involve in bioremediation. This is the process of optimizing the environmental conditions of the remediation site. Additives are usually added to the subsurface through injection wells. Subsurface characteristics such as ground water velocity, hydraulic conductivity of the subsurface, and lithology of the subsurface are important in developing a bio-stimulation system. The indigenous microorganism present in the soil is responsible for degradation of the pollutant, but bio-stimulation can be improved by bio-augmentation (Salt et al., 1998). Bio-augmentation is the addition of a group of indigenous microbial strains or genetically engineered microbes to treat the contaminated soil. It is effective where native microorganisms are not identified in the soil or do not have the metabolic capability to perform the remediation process. Most of bioremediation method aims in enhancing oxygen supply to contaminated sites assuming that the principal mechanism of hydrocarbon removal is aerobic respiration. But addition of urea and ammonia-based fertilizers sometimes used for oil-spill bioremediation can potentially exert an oxygen demand due to biological ammonia oxidation. On some sites, mass transfer of oxygen may not be sufficient to replenish oxygen consumed by microbial metabolism, though penetration of oil into deeper sediment layers is also likely to be reduced in fine sediments. Under such conditions anaerobic hydrocarbon degradation may be of relevance (Udo and Fayemi, 1995).

In the study by Agboun et al. (2016), moringa leaf was used to investigate its usefulness in carrying out bioremediation program effectively with less inhibiting effect on the microbial activities. Components that may influence the reaction mechanisms were monitored upon the influence of the toxic nature or characteristics of the crude oil contamination. The research demonstrates the advantageous functions of moringa leaf on bioremediation of contaminated soil environment. During microbial degradation of petroleum hydrocarbons, the n-alkane chain length is

one of the most important factors because shorter-chain petroleum hydrocarbons are generally degraded more rapidly than longer-chain hydrocarbons. *Moringa oleifera* is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 or 12 m in height. It has a spreading, open crown of drooping, fragile branches, feathery foliage of tripinnate leaves, and thick, corky, whitish bark. The leaves are bipinnate or more commonly tripinnate, up to 45 cm long, and are alternate and spirally arranged on the twigs.



Figure 1: Leaf of *Moringa oleifera*

The environmental problems resulting from oil pollution/contamination have been a major research issue in Nigeria over the years owing to its effect on agriculture the nation's economy, and society at large. Spillage of crude oil on soils hinders plant growth and productivity. The retarded plant growth caused by oil spillage usually results from insufficient aeration caused by air displacement from the pore spaces between the soil particles by the crude oil. Insufficient aeration also causes root stress, which consequently reduces plant growth. Soils contaminated with petroleum crude oil are of no benefit to the agriculturist and other uses of land due to nutrient depletion.

There is therefore the need to redeem the soil for use in agriculture. However, moringa leaf can be applied to the soil as a remediating factor. This is because moringa leaf does not only enrich the soil it also ameliorates the crude oil pollution effect on the soil (Agboun et al., 2016).

MATERIALS AND METHODS

2.1 Study Site

The study was carried out in the Environmental Laboratory of the Department of Genetics and Biotechnology, University of Calabar, Nigeria.

2.2 Sample Collection:

Topsoil (0-15 cm depth) was collected randomly from the University of Calabar farm to create a composite sample representing the study area. Five kilograms (5 kg) from each composite sample were transferred into fifty (50) polythene bags, each equipped with drainage holes at the base. To ensure soil retention, the bags were lined with cotton wool. The subsequent procedures involved the application of two distinct treatments: spent engine oil as the pollutant and a leaf extract of *Moringa oleifera* as a potential remediation agent. The spent engine oil used in this study was sourced from a local mechanic shop in Bedwell. Different concentrations of spent

engine oil (0.3, 0.5, 0.7, and 1.0 liters per 5 kg of soil) were introduced into treatment groups, with the exception of the control group which received no oil (0.0 liter per 5 kg). The oil was carefully mixed with the soil within each polythene bag. Subsequently, the set-up was allowed to stand for a period of one week to facilitate acclimatization between the soil and the oil.

To explore the potential remediation effects of *Moringa oleifera*, a leaf extract was prepared and applied to the contaminated soil after the acclimatization period. Fresh and clean leaves of *Moringa oleifera* were collected from a reliable source. These leaves were thoroughly washed to eliminate any external impurities. Subsequently, the leaves were dried in a shaded area to prevent degradation of their bioactive compounds. Once dried, the *Moringa oleifera* leaves were ground into a fine powder using a suitable grinding apparatus or a mortar and pestle. This process ensured efficient extraction of bioactive components from the leaves.

A ratio of 100 grams of *Moringa oleifera* leaf powder was mixed with 500 milliliters of distilled water. The mixture was stirred rigorously to ensure the even dispersion of the leaf powder within the water. The leaf extract mixture was allowed to stand for a specified period, such as 24 hours. During this time, the bioactive compounds were gradually extracted from the *Moringa oleifera* leaves into the water.

Following the one-week acclimatization of the soil-oil mixture, the prepared *Moringa oleifera* leaf extract was uniformly applied to the soil within each polythene bag. Careful mixing was carried out to ensure thorough distribution of the leaf extract throughout the soil. Throughout the experimental duration, moisture content adjustments were performed by applying water on a weekly basis, maintaining the moisture level at 60% of the soil's moisture holding capacity. This comprehensive approach aimed to evaluate the effectiveness of the

Moringa oleifera leaf extract in mitigating the negative impact of the introduced spent engine oil on the soil, while also maintaining rigorous scientific standards for reproducibility and transparency.

2.3 Laboratory Analysis:

In the pursuit of comprehensive insights into the effects of contamination and potential remediation, a series of analyses were conducted on the soil samples collected from the polythene bags. These analyses encompassed microbial assessments, determination of heavy metal concentrations, and evaluation of physicochemical properties. The detailed methodologies employed for the determination of heavy metals and physicochemical properties are elaborated below:

2.3.1 Determination of Heavy Metals:

Soil samples were collected from each polythene bag prior to contamination with spent engine oil, as well as thirty (30) days after contamination. The collected soil samples were air-dried and finely ground to achieve homogeneity and facilitate accurate measurements. A subsample of each dried and ground soil sample was subjected to digestion using appropriate acidic reagents, ensuring the conversion of metals into soluble forms suitable for analysis. The digested samples were then subjected to an extraction procedure, using a suitable solvent to obtain the metal ions in solution.

The concentrations of specific heavy metals, such as lead (Pb), cadmium (Cd), mercury (Hg), and others of interest, were quantified using established analytical techniques. These techniques could include atomic absorption spectroscopy (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), or other suitable methods.

2.3.2 Physicochemical Properties Determination:

The assessment of various physicochemical properties aimed to capture important characteristics of the soil affected by spent engine oil contamination. The moisture content of soil samples was determined by weighing a known amount of soil before and after drying in an oven at a specified temperature (e.g., 105°C). Moisture content was expressed as a percentage of the initial weight. Bulk density was determined by dividing the mass of the dried soil by its volume. Soil cores were collected and weighed, and their volume were determined through a known volume of water displacement.

The pH of the soil samples was measured using a soil-to-water ratio of 1:2.5 (w/v). A pH meter calibrated with standard buffer solutions (pH 4, 7, and 10) was used for accurate pH determination. Organic carbon content was determined using the Walkley-Black method (Walkley and Black, 1934) and nitrogen content was determined using the Kjeldahl method (Kjeldahl, 1883).

The available phosphorus content in the soil was determined using the Olsen phosphorus method. Ten grams of air-dried and ground soil sample were mixed with 20 ml of Olsen's extractant solution (0.5 M sodium bicarbonate) and shaken for 30 minutes.

After centrifugation, the supernatant was analyzed for phosphorus content using the molybdenum blue method, with absorbance measured using a spectrophotometer. Exchangeable cations (potassium, sodium, calcium, and magnesium) were extracted using either 1 M ammonium acetate or Mehlich-3 solution.

After shaking and centrifugation, the supernatant was analyzed using flame photometry for potassium and atomic absorption spectrophotometry for sodium, calcium, and magnesium. Concentrations of exchangeable cations were calculated based on standard curves obtained from known cation standards. The specific details of the analytical procedures, including reagent concentrations, instrument settings, and calibration procedures, were recorded to ensure reproducibility and accuracy.

Cation Exchange Capacity (CEC) and Electrical Conductivity (EC): The CEC of the soil and its electrical conductivity were determined using suitable methods, contributing to a holistic understanding of the soil's ion exchange and salinity characteristics.

2.4 Bacteria counts and isolation

2.4.1 Enumeration of total heterotrophic bacteria

The total heterotrophic bacterial (THB) count was determined using the spread plate method on nutrient agar (NA) according to the American Public Health Association (APHA) standard method (APHA, 2005). Soil suspensions were prepared by 10-fold serial dilutions with one gram of soil and 0.1 ml of 10^{-6} and 10^{-7} dilution were spread on the plates in triplicates. The colony forming units (CFU) of the bacteria were counted after incubation at 28°C for 18 hours.

2.4.2 Enumeration and isolation of crude oil degrading bacteria

Crude oil utilizing bacteria in the soil samples were enumerated by the viable count method using the surface spreading technique. The mineral salts medium was solidified by the addition of 1.5% agar. Soil suspensions were prepared by 10-fold serial dilutions with 1g of soil and 0.1 ml of 10^{-4} and 10^{-6} dilution was spread on the plates in triplicates. After inoculation of the agar plates with the sample, a sterile filter paper (Whatman No.1), saturated with crude oil, was aseptically placed onto the inside of the lid (cover) of the Petri dishes. The filter paper saturated with crude oil served as a sole carbon and energy source for growth of the organisms on the surface through vapour phase transfer. The plates were then incubated in an inverted position at room temperature for 7 days, after which the average counts from triplicate plates were counted and recorded.

2.4.3 Enumeration of total heterotrophic fungi

In this method the total number of fungi present in the soil was enumerated using surface spreading techniques. Serial dilution of the soil samples were prepared from 10^{-1} to 10^{-7} and 0.1 ml of 10^{-7} dilution was plated out onto Sabouraud dextrose agar (SDA) plates supplemented with 15 mg penicillin/ml and 100 mg/ml of streptomycin to inhibit bacteria growth. The plates were also prepared in triplicate as for

bacteria and were all incubated at 370 °C for 72 hours before the colonies were counted and expressed as colony forming units per gram of the soil sample (CFUg⁻¹).

2.4.4 Enumeration and isolation of crude oil utilizing fungi

This was done using the surface spreading technique. The same procedure as described in the enumeration of crude oil degrading bacteria was used. But in this case, 0.1 ml of 10⁻⁶ dilution was plated onto mineral salt agar medium containing 15 mcgml⁻¹ of penicillin and 100 mcgml⁻¹ of streptomycin to inhibit bacterial growth. After inoculation of the agar plates with the samples, a sterile filter paper (Whatman No. 1), saturated with crude oil was also aseptically placed onto the inside cover of the Petri dishes to serve as a source of carbon and energy for the growth of microorganisms through vapour pressure phase transfer. All plates were prepared in triplicates, inverted and incubated at 280 °C for 7 days before the colonies were counted and expressed as colony forming units per gram of the soil sample (CFUg⁻¹). The isolates were sub-cultured onto freshly prepared Sabouraud dextrose agar (SDA) plates. Isolated colonies were further purified by subculturing and identified macroscopically and microscopically using the wet mount method (cotton-blue in lactophenol). Organisms were identified using the scheme of JSC (Mbagwu, 1992).

RESULTS

3.1 Physicochemical parameters of Soil Samples before and after Treatment:

The physicochemical parameters of the soil samples before and after treatment applications are presented in Table 1.

3.1.1 Soil moisture content:

The results indicated that the moisture content of the soils from the five treatment levels were found statistically significant ($P < 0.05$), (Table 1). The control with zero amounts (0L/5kg), had the highest mean soil moisture content (9.01 ± 0.10). Treatment 4 (1L/5kg), had the least mean Soil Moisture content (7.99 ± 0.10) after treatment.

3.1.2 Bulk density:

The bulk density of soils from the different treatment groups had no significant difference ($P > 0.05$) except for treatment 1 which was not different from the control group. The control with zero amounts (0L/5kg), had the lowest mean bulk density after treatment (1.20 ± 0.07). Treatment 4 (1L/5kg), had the highest mean bulk density (1.55 ± 0.08). There were significant differences in the soil bulk density across the various treatment levels (Table 1).

3.1.4 Soil pH:

There were variations in soil pH levels from the soil samples analyzed. The control with zero amounts (0L/5kg), had the lowest mean soil pH (5.00 ± 0.11). Treatment 4 (1L/5kg), had the highest soil pH (6.90 ± 0.10). The result also showed that there was an increase in the soil pH level with an increase in the level of the contaminant.

3.1.4 Organic carbon contents of the soils:

Organic carbon levels of the soils varied significantly ($P < 0.05$) across the various treatment levels. The control with zero amounts (0L/5kg), had the lowest mean soil organic carbon after treatment (2.50 ± 0.21). This was followed by Treatment 1 (0.3L/5kg) (3.98 ± 0.12), Treatment 2 (0.5L/5kg) (4.52 ± 0.11), Treatment 3 (0.7L/5kg) (4.81 ± 0.14). Treatment 4 (1L/5kg), had the highest mean organic carbon content (5.13 ± 0.12) (Table 1). However, there were significant differences in the soil organic carbon content across the various treatment levels (Table 1). From the result, it was observed that there was an increase in soil organic carbon content with a corresponding increase in the concentration of the pollutant (Spent engine oil).

3.1.5 Nitrogen content of the soil (g/kg):

Analysis of the samples showed that there was no significant difference ($P > 0.05$). The control with zero amount pollutant (0L/5kg), had the lowest mean soil nitrogen content after treatment (0.12 ± 0.01). This was followed by Treatment 1 (0.3L/5kg) (0.17 ± 0.01), Treatment 2 (0.5L/5kg) (0.20 ± 0.04), Treatment 3 (0.7L/5kg) (0.26 ± 0.11). Treatment 4 (1L/5kg), had the highest mean organic carbon content (0.29 ± 0.10).

3.1.6 Available phosphorus:

The levels of available phosphorus from the soil analyzed were significantly different ($P < 0.05$). The control with zero amount pollutant (0L/5kg), had the highest mean soil available phosphorus after treatment (9.41 ± 0.12). This was followed by Treatment 1 (0.3L/5kg) (5.86 ± 0.22), Treatment 2 (0.5L/5kg) (5.55 ± 0.21), and Treatment 3 (0.7L/5kg) (4.50 ± 0.18). Treatment 4 (1L/5kg), had the lowest mean available phosphorous (2.29 ± 0.20).

3.1.7 Exchangeable potassium:

Potassium is a vital constituent of all plant and animal tissues. The results indicated that there were no significant differences in potassium concentration from the various treatment levels ($P > 0.05$) except for control. The control with zero amount pollutant (0L/5kg), had the highest mean soil available potassium after treatment (0.19 ± 0.01). This was followed by Treatment 1 (0.3L/5kg) (0.16 ± 0.24), Treatment 2 (0.5L/5kg) (0.12 ± 0.02), and Treatment 3 (0.7L/5kg) (0.10 ± 0.01). Treatment 4 (1L/5kg), had the lowest mean exchangeable potassium (0.09 ± 0.01).

3.1.8 Exchangeable sodium:

Sodium levels were significantly different ($P < 0.05$) in all the treatment levels. The control with zero amount pollutant (0L/5kg), had the highest mean exchangeable sodium (0.14 ± 0.01). This was followed by Treatment 1 (0.3L/5kg) (0.16 ± 0.04), Treatment 2 (0.5L/5kg) (0.19 ± 0.06), and Treatment 3 (0.7/5kg) (0.19 ± 0.06). Treatment 4 (1L/5kg), had the lowest mean exchangeable sodium (0.20 ± 0.06).

3.1.9 Exchangeable calcium:

The result indicated that calcium contents were significantly different in the various treatment levels of the sample analyzed ($P < 0.05$) (Table 1). The control with zero amount pollutant (0L/5kg), had the

highest mean exchangeable calcium (1.50 ± 0.14). This was followed by Treatment 1 (0.3L/5kg) (0.92 ± 0.13), Treatment 2 (0.5L/5kg) (0.81 ± 0.09), and Treatment 3 (0.7/5kg) (0.73 ± 0.11). Treatment 4 (1L/5kg), had the lowest mean exchangeable calcium (0.61 ± 0.12).

3.10 Exchangeable magnesium:

The results indicate that there were significant differences in magnesium content of the soils ($P < 0.05$) (Table 1). The control with zero amount pollutant (0L/5kg), had the highest mean exchangeable magnesium (0.14 ± 0.02). This was followed by Treatment 1 (0.3L/5kg) (0.10 ± 0.02), Treatment 2 (0.5L/5kg) (0.09 ± 0.12), and Treatment 3 (0.7/5kg) (0.07 ± 0.02). Treatment four (1L/5kg), had the lowest mean exchangeable calcium (0.06 ± 0.01).

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF POLLUTED SOILS BEFORE AND AFTER MORINGA APPLICATION

POLLUTED SOIL BEFORE MORINGA APPLICATION					
Measured Parameter/Unit	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
Soil Moisture Content (%)	9.00 ^a ± 0.10	8.86 ^a ± 0.08	8.60 ^b ± 0.05	8.61 ^b ± 0.05	8.58 ^b ± 0.06
Bulk Density (mg/dm ³)	1.20 ^d ± 0.07	1.39 ^d ± 0.05	1.52 ^d ± 0.10	1.60 ^b ± 0.09	1.78 ^b ± 0.10
pH (H ₂ O)	5.02 ^c ± 0.11	5.73 ⁿ ± 0.09	5.80 ^a ± 0.11	5.90 ^b ± 0.11	6.98 ^a ± 0.09
Org.C (g/kg)	2.50 ^e ± 0.21	4.00 ^f ± 0.13	4.62 ^{ab} ± 0.11	4.81 ^{ad} ± 0.22	5.02 ^{db} ± 0.10
Org.M (g/kg)	1.72 ^a ± 0.06	2.44 ^a ± 0.09	2.48 ^{ba} ± 0.08	3.00 ^{ab} ± 0.06	3.42 ^{aa} ± 0.06
N (g/kg)	0.12 ^b ± 0.01	0.18 ^b ± 0.03	0.21 ^a ± 0.04	0.25 ^a ± 0.02	0.30 ^b ± 0.001
P (g/kg)	9.40 ^b ± 0.12	5.90 ^{an} ± 0.22	5.60 ^a ± 0.21	4.80 ^a ± 0.19	3.00 ^{ab} ± 0.20
Ca(cmol/kg)	0.42 ^{ab} ± 0.06	0.40 ^e ± 0.08	0.37 ^{ff} ± 0.04	0.34 ^b ± 0.09	0.26 ^{ab} ± 0.09
Mg(cmol/kg)	0.14 ^{cb} ± 0.03	0.11 ^d ± 0.01	0.09 ^{ab} ± 0.01	0.08 ^{ab} ± 0.01	0.06 ^{ae} ± 0.01
K(cmol/kg)	0.19 ^{aa} ± 0.23	0.16 ^e ± 0.02	0.13 ^{ba} ± 0.02	0.11 ^f ± 0.01	0.09 ^a ± 0.01
Na(cmol/kg)	0.14 ^{ba} ± 0.06	0.16 ^c ± 0.04	0.17 ^{ad} ± 0.09	0.19 ^e ± 0.06	0.21 ^a ± 0.6
CEC(cmol/kg)	7.20 ^a ± 0.07	3.85 ^d ± 0.09	5.56 ^a ± 0.12	3.45 ^h ± 0.11	3.24 ^a ± 0.12
EC (µS/m)	1.50 ^e ± 0.14	0.92 ^a ± 0.13	0.81 ^a ± 0.09	0.73 ^k ± 0.11	0.61 ^d ± 0.12
POLLUTED SOIL AFTER MORINGA APPLICATION					
Measured Parameter/Unit	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
Soil Moisture Content (%)	9.01 ^a ± 0.10	8.84 ^a ± 0.08	8.52 ^b ± 0.21	8.23 ^b ± 0.06	7.99 ^c ± 0.10
Bulk Density (mg/dm ³)	1.20 ^b ± 0.07	1.30 ^b ± 0.05	1.46 ^d ± 0.08	1.50 ^d ± 0.06	1.55 ^d ± 0.08
pH (H ₂ O)	5.00 ^a ± 0.11	5.70 ^b ± 0.10	5.76 ^c ± 0.09	5.85 ^a ± 0.11	6.90 ^a ± 0.10
Org.C (g/kg)	2.50 ^{ab} ± 0.21	3.98 ^{bc} ± 0.12	4.52 ^{bc} ± 0.11	4.81 ^{bc} ± 0.14	5.13 ^{bc} ± 0.12
Org.M (g/kg)	1.72 ^c ± 0.06	2.46 ^a ± 0.09	2.80 ^a ± 0.08	2.92 ^a ± 0.06	3.00 ^a ± 0.07
N (g/kg)	0.12 ^d ± 0.01	0.17 ^e ± 0.01	0.20 ^e ± 0.04	0.26 ^e ± 0.11	0.29 ^e ± 0.10
P (g/kg)	9.41 ^f ± 0.12	5.86 ^f ± 0.22	5.55 ^f ± 0.21	4.50 ^e ± 0.18	2.29 ^a ± 0.20
Ca(cmol/kg)	0.42 ^a ± 0.06	0.40 ^e ± 0.08	0.36 ^d ± 0.04	0.34 ^d ± 0.09	0.24 ^d ± 0.09
Mg(cmol/kg)	0.14 ^b ± 0.02	0.10 ^a ± 0.02	0.09 ^a ± 0.12	0.07 ^a ± 0.02	0.06 ^a ± 0.01
K(cmol/kg)	0.19 ^c ± 0.01	0.16 ^h ± 0.24	0.12 ^h ± 0.02	0.10 ^h ± 0.01	0.09 ^h ± 0.01
Na(cmol/kg)	0.14 ^e ± 0.01	0.16 ^a ± 0.04	0.19 ^a ± 0.06	0.20 ^a ± 0.06	0.22 ^a ± 0.02
CEC(cmol/kg)	7.20 ^{ab} ± 0.07	3.85 ^{ab} ± 0.09	5.56 ^{ab} ± 0.12	3.45 ^{ab} ± 0.11	3.24 ^{ab} ± 0.12
EC (µS/m)	1.50 ^e ± 0.14	0.92 ^e ± 0.13	0.81 ^{ab} ± 0.09	0.73 ^e ± 0.11	0.61 ^h ± 0.12

*Means with different superscripts along each horizontal array indicate significant difference (p<0.05).

3.2 Heavy Metals concentration in Polluted Soils Before and after Treatment Applications

The results of heavy metals concentration in the polluted soils before and after treatment applications are shown in Table 2. The results show that the control with no amount of pollutant (0L/5kg), had the least amount of heavy metals while the soil with the highest concentration of pollutant (Spent engine oil), had the highest amount of heavy metals after

treatment. Though the general results show that, after treatment with *Moringa oleifera* leaf extract, there was a slight decrease/reduction in the amount of heavy metals present across the various treatment groups. The results showed that there were significant differences ($P > 0.05$) in the amount of heavy metal across the various pollutants levels after treatment was applied.

TABLE 2: Heavy Metals Concentration in polluted Soils Before and after Treatment

POLLUTED SOIL BEFORE TREATMENT APPLICATION					
Measured Parameter/Unit	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
Fe (mg/L)	0.32 ^a ± 0.10	0.38 ^a ± 0.08	0.45 ^b ± 0.05	0.50 ^b ± 0.05	0.60 ^b ± 0.06
Cr (mg/L)	0.57 ^c ± 0.03	0.61 ⁿ ± 0.09	0.77 ^a ± 0.11	0.96 ^b ± 0.11	1.00 ^a ± 0.09
Mn (mg/L)	0.05 ^d ± 0.01	0.08 ^d ± 0.01	0.09 ^d ± 0.10	1.05 ^b ± 0.09	1.24 ^b ± 0.10
Cu (mg/L)	0.91 ^e ± 0.20	1.20 ^f ± 0.13	1.30 ^{ab} ± 0.11	2.81 ^{ad} ± 0.22	4.02 ^{db} ± 0.10
Zn(mg/L)	0.06 ^a ± 0.01	0.08 ^a ± 0.09	0.10 ^{ba} ± 0.08	0.20 ± 0.06	0.50 ^{aa} ± 0.06
Pb (mg/L)	0.02 ^b ± 0.01	0.18 ^b ± 0.03	0.23 ^a ± 0.04	0.25 ^a ± 0.02	0.30 ^b ± 0.001
As (mg/L)	0.26 ^b ± 0.08	0.30 ^{an} ± 0.22	0.35 ^a ± 0.21	0.40 ^a ± 0.19	0.60 ^{ab} ± 0.20
Li (mg/L)	0.56 ^{ab} ± 0.06	0.62 ^e ± 0.08	0.69 ^{ff} ± 0.04	0.75 ^b ± 0.09	0.80 ^{ab} ± 0.09
Al (mg/L)	0.84 ^{cb} ± 0.03	0.90 ^d ± 0.01	1.23 ^{ab} ± 0.01	1.50 ^{ab} ± 0.01	1.70 ^{ae} ± 0.01
Hg (mg/L)	0.03 ^{aa} ± 0.23	0.05 ^e ± 0.02	0.09 ^{ba} ± 0.02	0.60 ^f ± 0.01	0.70 ^a ± 0.01
Cd (mg/L)	0.04 ^{ba} ± 0.06	0.07 ^c ± 0.04	0.09 ^{ad} ± 0.09	0.19 ^e ± 0.06	0.25 ^a ± 0.06
Ni (mg/L)	0.31 ^a ± 0.07	0.38 ^a ± 0.09	0.43 ^a ± 0.12	0.70 ^h ± 0.11	0.90 ^a ± 0.12

POLLUTED SOIL AFTER TREATMENT APPLICATION					
Measured Parameter/Unit	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
Fe (mg/L)	0.32 ^a ± 0.10	0.40 ^a ± 0.08	0.42 ^a ± 0.08	0.46 ^a ± 0.08	0.57 ^a ± 0.08
Cr (mg/L)	0.57 ^c ± 0.03	0.59 ^c ± 0.04	0.70 ⁿ ± 0.09	0.87 ⁿ ± 0.09	0.90 ⁿ ± 0.09
Mn (mg/L)	0.05 ^d ± 0.01	0.08 ^d ± 0.01	0.09 ^d ± 0.01	1.00 ^d ± 0.01	1.20 ^d ± 0.01
Cu (mg/L)	0.91 ^e ± 0.20	1.19 ^f ± 0.12	1.30 ^f ± 0.13	2.50 ^f ± 0.13	3.50 ^f ± 0.13
Zn(mg/L)	0.06 ^a ± 0.01	0.07 ^a ± 0.01	0.09 ^a ± 0.09	0.20 ^a ± 0.06	0.50 ^a ± 0.06
Pb (mg/L)	0.02 ^b ± 0.01	0.17 ^b ± 0.05	0.20 ^b ± 0.03	0.23 ^b ± 0.03	0.28 ^b ± 0.01
As (mg/L)	0.26 ^b ± 0.08	0.29 ^{an} ± 0.22	0.33 ^{an} ± 0.22	0.42 ^{an} ± 0.22	0.58 ^{an} ± 0.22
Li (mg/L)	0.56 ^{ab} ± 0.06	0.60 ^e ± 0.08	0.65 ^e ± 0.08	0.70 ^e ± 0.08	0.78 ^e ± 0.08
Al (mg/L)	0.84 ^{cb} ± 0.03	0.87 ^d ± 0.01	1.20 ^d ± 0.01	1.30 ^d ± 0.01	1.65 ^d ± 0.01
Hg (mg/L)	0.03 ^{aa} ± 0.23	0.07 ^e ± 0.02	0.09 ^e ± 0.02	0.67 ^e ± 0.02	0.70 ^e ± 0.02
Cd (mg/L)	0.04 ^{ba} ± 0.06	0.08 ^c ± 0.04	0.08 ^c ± 0.04	0.25 ^c ± 0.04	0.28 ^c ± 0.06
Ni (mg/L)	0.31 ^a ± 0.07	0.37 ± 0.09 ^a	0.40 ^b ± 0.09	0.66 ± 0.09 ^c	0.72 ± 0.09 ^c

*Means with different superscripts along each horizontal array indicate significant difference ($p < 0.05$).

3.2 Microbial population in the Polluted Soils before and after Treatment

The results in Table 3 show the microbial population of hydrocarbon utilizing bacteria and fungi present in the polluted soil before and after treatment. It was observed that there was a significant ($P < 0.05$) increase in the microbial population, after being treated with the *Moringa oleifera* leaf extract. The

result also reveals that there was a decrease in microbial population with increased concentration of the pollutant (spent engine oil). The Control (0L/5kg), had the least amount of heavy metals. The result also showed that there was an increase in the amount of heavy metal, with an increase in concentration of the pollutant

TABLE 3: Microbial Population of the polluted Soil before and After Treatment

POLLUTED SOIL BEFORE TREATMENT					
Measured Parameter/Unit	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
THB (10^7 CFU/g)	6.02 ^a ± 2.04	5.42 ^b ± 2.05	5.32 ^b ± 2.01	5.00 ^c ± 1.98	4.60 ^c ± 1.72
CUB (10^6 CFU/g)	2.01 ^e ± 0.81	1.89 ^d ± 0.79	1.80 ^d ± 0.62	1.62 ^c ± 0.52	1.52 ^f ± 0.46
THF (10^7 CFU/g)	2.96 ⁿ ± 1.81	2.87 ⁿ ± 1.76	2.70 ^p ± 1.61	2.40 ^k ± 1.42	2 ^{ab} .00 ± 1.03
CUF (10^6 CFU/g)	1.50 ^a ± 0.6	1.30 ^a ± 0.60	1.25 ^c ± 0.58	1.18 ^d ± 0.52	1.00 ^d ± 0.46
POLLUTED SOIL AFTER TREATMENT					
Measured Parameter/Unit	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
THB (10^7 CFU/g)	6.50 ^a ± 2.06	5.98 ^b ± 2.05	5.46 ^b ± 2.01	5.00 ^k ± 1.98	4.60 ^k ± 1.72
CUB (10^6 CFU/g)	2.01 ^j ± 0.81	1.96 ^g ± 0.81	1.82 ^h ± 0.62	1.68 ⁿ ± 0.49	1.63 ^m ± 0.46
THF (10^7 CFU/g)	2.97 ^d ± 1.80	2.93 ^d ± 1.78	2.70 ^e ± 1.61	2.44 ^f ± 1.47	2.30 ^h ± 1.05
CUF (10^6 CFU/g)	1.58 ^a ± 0.68	1.33 ^b ± 0.61	1.25 ^c ± 0.58	1.20 ^d ± 0.52	1.43 ^e ± 0.46

Means with the same superscript along each horizontal array indicate no significant difference ($p > 0.05$).

THB= Total heterotrophic bacteria, CUB= Crude oil utilizing bacteria, THF= Total heterotrophic fungi, CUF= crude oil utilizing bacteria, CFU/g= coliform forming unit per gram.

3.4

Total Petroleum Hydrocarbon:

The Potentials of *Moringa oleifera* in the degradation of total petroleum hydrocarbon (TPH) in spent engine oil polluted soil is presented in Table 4. The result shows that there were significant reductions ($p < 0.05$) in the TPH of the soils samples polluted with spent engine oil at different concentration and then treated with *Moringa oleifera* leaf extract. The result showed that the control (0L/5kg) had the least total hydrocarbon before and after treatment. However, the highest petroleum hydrocarbon was recorded in treatment 4 (1L/5kg). The result showed that total petroleum hydrocarbon increased with increased concentration of the pollutant. However, after treatment with *Moringa oleifera* the result showed a

significant degradation in the total petroleum hydrocarbon across the various pollution levels.

Table 4 also presents the levels of Total Petroleum Hydrocarbon (TPH) in soil samples before and after treatment, across different durations. The data show variations in TPH concentrations among treatment groups and over time. Prior to treatment, TPH levels generally increased with higher pollutant concentrations, peaking at Day 28. Following treatment, TPH concentrations exhibited a decreasing trend, with some fluctuations, but without significant differences ($p > 0.05$) among treatment groups. The results underscore the potential effectiveness of the treatments in reducing TPH content, though further analysis is needed to assess treatment impacts more comprehensively.

TABLE 4: Total Petroleum Hydrocarbon Content in Polluted Soil before and after Treatment

POLLUTED SOIL BEFORE TREATMENT					
Duration (Days)	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
Day 7	43 ^a ± 2.50	642 ^b ± 5.08	680 ^b ± 7.01	720 ^c ± 10.7	780 ^c ± 8.73
Day 14	42 ^e ± 2.36	641 ^d ± 6.79	678 ^d ± 6.62	718 ^c ± 9.56	782 ^f ± 8.80
Day 21	43 ⁿ ± 2.50	650 ⁿ ± 8.76	690 ^p ± 8.61	730 ^k ± 10.20	795 ^{ab} ± 8.76
Day 28	42.7 ^a ± 2.40	662 ^a ± 0.60	700 ^c ± 8.92	745 ^d ± 10.56	800 ^d ± 9.53

POLLUTED SOIL AFTER TREATMENT					
Duration (Days)	Control (0L/5kg)	Trt1 (0.3L/5kg)	Trt2 (0.5L/5kg)	Trt3 (0.7L/5kg)	Trt4 (1L/5kg)
Day 7	42 ^a ± 2.06	628 ^b ± 4.98	670 ^b ± 2.01	710 ^k ± 1.98	720 ^k ± 1.72
Day 14	41.56 ^j ± 2.12	615 ^g ± 0.81	665 ^h ± 0.62	690 ⁿ ± 0.49	705 ^m ± 0.46
Day 21	40.00 ^d ± 2.07	619 ^d ± 1.78	650 ^e ± 1.61	680 ^r ± 1.47	690 ^h ± 1.05
Day 28	39.23 ^a ± 2.04	612 ^b ± 0.61	620 ^c ± 0.58	650 ^d ± 0.52	670 ^e ± 0.46

Data were expressed in mean and standard error ($X \pm S.E$) in triplicate. Mean followed with the same superscript along each horizontal array indicate no significant difference ($p > 0.05$).

4.0 DISCUSSION:

The study seeks to evaluate the phytoremediation potential of *Moringa Oleifera* leaf extracts, when used to treat soil polluted with spent engine oil. From the study it was observed that there were significant changes in the physicochemical parameters of the polluted soils before and after treatment application. Heavy metals, Microbial population of Hydrocarbon utilizing microbes as well as Total petroleum hydrocarbon of the polluted soils before and after treatment applications were also significant.

The microbial population of total heterotrophic and crude oil utilizing bacteria and fungi in the soil before pollution with spent engine oil showed that the mean heterotrophic counts were relatively low in the pristine control soil. The following bacteria species were identified in the soil: *Bacillus* spp, *Micrococcus* spp, *E. coli*, *Proteus* spp and the gram positive bacteria were found to be the dominant bacteria species. The fungi species identified in the soil includes: *Rhizopus* spp, *Mucor* spp and *Aspergillus* spp. The pollution of the soil with spent engine oil shows that the total average counts of crude oil utilizing bacteria and fungi were generally higher in polluted soil than in the natural soil. The following bacteria species were identified in the soil: *Bacillus* spp, *Micrococcus* spp, *Acinetobacter* spp and *Pseudomonas* spp. (Table 5) .

The fungi species also identified include: *Mucor* spp, *Aspergillus* spp and, *Penicillium* spp. (Ani, 2006) also observed that the presence of petroleum might cause an increase in microbial population in the soil.

However, it was observed from the study that, the amount of hydrocarbon utilizing microbes, increased after treatment was made. Also, the amount of total petroleum hydrocarbon also decreased across the various group when treatment was applied. This study conforms with Agboun et al. (2016) who reported potentials of using *Moringa oleifera* seeds in the bioremediation of soil contaminated by crude oil. From the results of this study, it can be concluded that *Moringa Oleifera* leaf extract possesses the ability to enhance the biodegradation of crude oil in soil. *Moringa Oleifera* leaf extract can serve as good materials for reclaiming/rehabilitating crude oil polluted soil. Their use in reclaiming oil polluted soil could also solve the problem of solid waste disposal in the environment.

Table 5: cultural, cell morphology and biochemical characterization and identification of hydrocarbon utilizing bacteria isolates

Sample Code	Isolate Number	Cultural Characterization of Test Bacteria Isolates	Cell Morphology of Bacteria Isolates	Gram's Reaction	Motility Test	Indole Test	MR-VP Test		Citrate Test	Catalase Test	Oxidase Test	Coagulase Test	Urease Test		C ₆ H ₁₂ O ₆				Probable Bacteria Isolates	
							Methyl Red	Voges-Proskauer					Lactose		Sucrose	Glucos	Gas prod	H ₂ S Prod		
SA1	1	Pale yellow, glistening, raised and smooth colony	Slender bacilli rod	-	+	-	-	-	+	+	+	-	+	-	-	-	-	+	-	AchromobactorSpp
SB1	1	Large, dry, irregular, flat and milky pigmented	Bacilli rod	+	+	-	-	+	+	+	-	-	-	-	-	-	+	-	+	Anthrax Bacillus
	2.	Smooth, greenish, round, flat colony	Curved rod	-	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	Pseudomonas acrograms
SB1	1.	Bright yellow, convex, circular, raised, moist	Cocci in pairs	+	-	-	+	-	+	+	+	-	-	-	-	-	-	+	-	MicrococasLuteus
	2	Smooth, round, moist flat, greenish pigmented	Curved rod	-	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	Pseudomonas acrograms
SA2	1.	Pale yellow, raised, glistening and smooth	Bacilli rod	-	+	-	-	-	+	+	+	-	+	-	-	-	-	+	-	Achromobactorspp
SB2	1.	Large, dry, irregular, flat and milky pigmented	Bacilli rod	+	+	-	-	+	+	+	-	-	-	-	-	-	+	-	+	Anthrax Bacillus

SC2	1	Gray pigmented, moist, raised moist circular	Slender rod	-	-	+	+	-	-	+	+	-	-	+	+	-	-	Flavo bacterium
SA3	1	Pale yellow, glistening, raised and smooth	Slender bacilli rod	-	+	-	-	-	+	+	+	-	+	-	-	-	+	AchromobactorSpp
SB3	1	Greenish pigmented, smooth, round, moist	Curved rod	-	+	-	+	-	+	+	+	-	-	-	-	-	-	Pseudomonas acroginosa
	2	Bright yellow, convex, circular, raised	Cocci in pairs	+	-	-	+	-	+	+	+	-	-	-	-	-	+	MicrococasLuteus
SC3	1	Bright yellow, convex, circular, raised colony	Cocci in pairs	+	-	-	+	-	+	+	+	-	-	-	-	-	+	MicrococasLuteus

SA1 – SC3= Sample codes

CONCLUSION:

The study investigated the potential of phytoremediation using "green plants" such as *Moringa Oleifera* leaf extract to restore environmental equilibrium in contaminated areas. The emerging and cost-effective phytoremediation technology harnesses the remarkable metabolic capabilities of plants to sequester various elements and compounds from the environment. The applicability of phytoremediation spans a wide spectrum of contaminants, ranging from heavy metals and radio nuclides to organic compounds such as chlorinated solvents, polycyclic aromatic hydrocarbons, pesticides, explosives, and surfactants. While promising, the technology is still in its early developmental stages, with limited full-scale implementations.

The study identified mechanic workshops as sites prone to heavy metal contamination due to automotive repair and maintenance activities. The potential of plants to absorb heavy metals during their growth adds value to their role in phytoremediation efforts. One significant challenge to advancing phytoremediation involves public concerns about genetic modification. While genetic engineering could enhance the efficacy of phytoremediation, apprehensions related to biodiversity loss and the introduction of foreign genes into the human food chain must be carefully addressed. In the context of the study's findings, phytoremediation holds considerable promise as a sustainable method to mitigate environmental stressors. Continued research and responsible development are essential to unlocking the full potential of phytoremediation and addressing existing challenges. As the global community seeks innovative solutions for environmental restoration, the application of phytoremediation offers a pathway towards a greener and healthier future.

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