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LABORATORY INVESTIGATION OF WATER-BASED BIO-STIMULANT FOR BIOREMEDIATION OF OIL-CONTAMINATED SOIL

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Abstract: **This study presents a laboratory investigation of a water-based bio-stimulant formulated from cow dung, goat dung, and sawdust for the bioremediation of oil-contaminated soil. The bio-stimulant was prepared as organic slurries and applied to crude oil-polluted soil under controlled laboratory conditions to evaluate its effectiveness in enhancing soil recovery. Physio-chemical parameters such as moisture content, chloride concentration, total hardness, total organic carbon, and organic matter were analysed before and after treatment to assess remediation performance. The results showed that bio-stimulant application improved soil properties by increasing organic matter and moisture content while reducing chloride and hardness levels compared to untreated samples. The pH analysis confirmed that the formulations maintained near-neutral conditions conducive to microbial activity. Growth observation using corn seeds further demonstrated successful germination and early root development in bio-stimulant-treated soils, indicating reduced toxicity and improved nutrient availability. The study concludes that water-based bio-stimulants derived from locally available organic wastes can serve as an effective, low-cost, and sustainable approach for the remediation of oil-contaminated soils, supporting both environmental restoration and resource recovery in affected regions.**

Keywords: **Investigation, Water-Based, Bio-stimulant, Bioremediation and Oil-Contaminated Soil**

I. INTRODUCTION

Studies show that organic-based bio-stimulants not only accelerate degradation but also improve soil porosity, pH, and microbial diversity [17] Bioremediation continues to emerge as a sustainable response to the challenges posed by oil-contaminated soils. Numerous studies have examined the role of bio-stimulants, particularly compost and organic waste materials, in enhancing microbial activity and facilitating the breakdown of petroleum hydrocarbons. [17]

In a more targeted study, [8] focused on benzopyrene, one of the most persistent polycyclic aromatic hydrocarbons (PAHs) in contaminated soils. Using an effective microbial fermented (EMF) solution, they achieved complete degradation of the pollutant under optimized conditions. Beyond removing hydrocarbons, the bio-stimulant enhanced enzyme activity and boosted the expression of genes responsible for degradation. This highlights the potential of microbial formulations to influence deeper soil biochemical processes, not just surface remediation.

[16] tested cow dung as a bio-stimulant for crude oil degradation in Delta State. Their results indicated a significant increase in hydrocarbon-degrading bacteria and a steady reduction in TPH over a six-week period. The microbial response was stronger in soils already rich in organic matter, confirming the role of soil condition in determining bioremediation success. This provides further support for using animal waste as a cost-effective, nutrient-rich amendment that stimulates native microbial populations.

[6] addressed the issue of acidified wetland conditions, common in the Niger Delta, by combining food waste digestate and the non-toxic surfactant Tween 80. Their 49-day mesocosm experiment recorded over 90% hydrocarbon degradation, even in highly acidic soils. This highlights the adaptability of bio-stimulant-based remediation strategies under challenging environmental conditions.

[7] studied compost amendments in diesel-contaminated soils and discovered that, besides supporting hydrocarbon degradation, compost reduced methane emissions by enhancing microbial gene expression related to both carbon cycling and pollutant breakdown. This dual function of remediation and greenhouse gas mitigation positions bio-stimulants as multifunctional tools for sustainable environmental management.

[1] employed cassava peels and poultry droppings as nutrient sources for remediation. Their research demonstrated effective TPH degradation alongside improvements in nitrogen and phosphorus content, suggesting that bio-stimulants can restore both soil quality and fertility.

[16] compared chemical surfactants and organic bio-stimulants such as spent tea leaves and goat manure. While chemical agents produced faster short-term degradation, bio-stimulants provided better long-term soil health and microbial stability. This underscores the trade-off between rapid remediation and ecological balance, favoring natural solutions for sustainable recovery.

[2] explored the use of brewery wastewater and poultry manure on urban-polluted soils. Their findings revealed enhanced microbial diversity, organic carbon restoration, and better soil texture, supporting the broader view that bio-stimulants can be adapted for various contamination scenarios.

Water-based systems also enhance microbial respiration by maintaining aeration and regulating moisture levels. This is especially beneficial in arid or sandy soils with poor water retention. Studies such as those by [17] and [14] found that compost extracts in liquid form sustained microbial populations more effectively than dry compost. These aqueous systems create biologically active zones where microbes thrive and degradation proceeds more consistently. They also allow continuous leaching of inhibitory compounds, further aiding soil recovery.

Compared with solid compost bio-stimulants, which often release nutrients slowly and distribute unevenly, liquid bio-stimulants can be sprayed, poured, or injected at different depths for better coverage. However, they are more sensitive to microbial die-off if not used promptly or preserved properly [7]. Balancing accessibility with microbial stability remains one of the main challenges in their formulation.

Recent innovations have introduced plant-based gels, aloe vera extracts, and low-viscosity slurries to maintain microbial viability and reduce evaporation. Experimental trials in Nigeria and Southeast Asia have shown that even simple liquid compost systems can achieve over 80% hydrocarbon reduction within two months [2]. These results provide scientific and economic justification for adopting water-based systems in low-resource regions.

Another growing area of interest is the use of biosurfactants in water-based systems. Natural compounds such as saponins and rhamnolipids can increase hydrocarbon solubility and improve microbial access to pollutants. [11] demonstrated that combining digestate with the biosurfactant Tween 80 significantly improved total petroleum hydrocarbon degradation in wetland soils. Such findings suggest that biosurfactants can complement water-based bio-stimulants, enhancing performance without introducing synthetic chemicals.

Field experiments have also proven that water-based bio-stimulants can be applied using low-cost tools like watering cans, backpack sprayers, or small irrigation systems. [16] reported that manure-based aqueous solutions applied to backyard oil spill sites in Delta State achieved over 70% TPH removal within six weeks. These examples confirm the real-world applicability of water-based systems and their ability to bridge the gap between research and local practice.

Importantly, this approach aligns with the principles of green chemistry and sustainable development. By using water as a carrier and organic waste as feedstock, water-based bio-stimulants promote circular economy practices, converting agricultural by-products into remediation resources. This addresses pollution, waste management, and local economic challenges simultaneously. When implemented in oil-producing regions such as the Niger Delta, the method supports environmental recovery while empowering communities with accessible, low-cost technology.

Soil aeration remains essential for maintaining aerobic degradation pathways. In compacted or clay-rich soils, limited oxygen diffusion forces microbes into slower anaerobic processes, delaying hydrocarbon breakdown and sometimes producing more toxic intermediates. In contrast, sandy and loamy soils with better aeration allow for faster microbial metabolism and cleaner degradation [16].

Surfactants play a pivotal role in enhancing hydrocarbon remediation by reducing surface and interfacial tension between water and oil phases. Their main function is to increase the solubility and mobility of hydrophobic contaminants such as alkanes and polycyclic aromatic hydrocarbons (PAHs), thereby making these pollutants more bioavailable to degrading microbes. While synthetic surfactants such as SDS and Tween 80 have been widely used for this purpose, concerns about their environmental persistence and toxicity have led to growing interest in naturally derived biosurfactants [10]

Biosurfactants are amphiphilic compounds produced by bacteria, yeasts, and fungi during hydrocarbon metabolism. Common examples include rhamnolipids from Pseudomonas aeruginosa, chlorolipids from Candida bombicola, and plant-derived saponins. These biomolecules reduce surface tension as effectively as synthetic agents but are more biodegradable and less toxic. Their environmental compatibility makes them particularly valuable for large-scale clean-ups in sensitive areas such as wetlands, agricultural soils, and groundwater recharge zones [12].

However, the production of biosurfactants within the soil environment is often limited by nutrient availability and microbial physiology. Some remediation protocols use direct addition of purified biosurfactants, while others stimulate in situ production through the addition of nutrient-rich organic materials. The co-application of poultry manure and biosurfactant-producing bacteria, for instance, has achieved hydrocarbon reductions of more than 50 percent within six weeks in pilot studies [3].

[6] explored how soil pH and structure affect treatment outcomes using food-waste digestate and Tween 80 surfactant. In acidic wetland soils, the liquid digestate achieved nearly 90 percent degradation within 50 days, outperforming both synthetic surfactants and solid composts. However, performance declined sharply in clay soils with poor drainage. This underscores the need to adapt bio-stimulant formulations to specific soil types.

Beyond technical performance, the success of any remediation strategy depends greatly on its social and economic viability. Bio-stimulant-based bioremediation, particularly when derived from agricultural or organic waste, presents a practical pathway for communities to address soil pollution using locally available resources. This approach democratizes environmental management, enabling resource-constrained regions to implement clean-up prohibitive cost of imported chemical agents [5].

The participatory nature of community-based remediation is critical to its sustainability. Involving local residents, especially youth and women, in compost production, site preparation, and monitoring promotes ownership and resilience. Field experiences in Bayelsa and Rivers State show that when farmers are trained to produce and apply water-based bio-stimulants, they gain both practical skills and a deeper sense of environmental responsibility [2]

Bio-stimulant-enhanced remediation is also closely aligned with SDG 13 (Climate Action), particularly in oil-producing and climate-vulnerable regions such as the Niger Delta. Oil contamination disrupts soil carbon balance and increases greenhouse gas emissions, especially when treated with combustion-based or chemical-intensive methods. In contrast, bio-stimulant-assisted bioremediation is a low-emission, regenerative process that supports carbon sequestration and improves soil fertility [6]; [7] This dual impact promotes both local resilience and global climate mitigation efforts.

Ex-situ bioremediation techniques have emerged as effective tools for addressing oil-contaminated soils, especially in regions where in-situ methods are limited by soil type, depth, or pollutant concentration. These approaches involve excavating contaminated soil and treating it under controlled conditions using bioreactors that optimize temperature, pH, aeration, and nutrient balance [4]; [9]. The controlled environment accelerates microbial activity and enhances hydrocarbon degradation compared to conventional landfarming or composting methods.

Slurry-phase bioreactors are among the most common designs for treating oil-contaminated soils. In these systems, soil is mixed with water and nutrients to form a uniform slurry that is continuously aerated and agitated. This promotes rapid microbial degradation by increasing hydrocarbon bioavailability in the aqueous phase. Studies have reported degradation efficiencies exceeding 90% within six to ten weeks under optimal conditions [3]. Despite their effectiveness, the energy and infrastructure demand of these systems remain a challenge in low-resource settings.

Recent advances include Two-Phase Partitioning Bioreactors (TPPBs), which incorporate a non-aqueous phase such as biodegradable polymers or silicone oil to sequester hydrophobic pollutants like polycyclic aromatic hydrocarbons (PAHs). These reactors maintain low aqueous concentrations, reducing microbial toxicity and promoting sustained degradation. As microbes metabolize the soluble fraction, more pollutants are released from the non-aqueous phase, creating a continuous degradation cycle [18]. This approach enhances microbial stability and efficiency, making it suitable for highly polluted soils.

Immobilized cell bioreactors represent another major innovation. In these systems, microbes are attached to carriers such as alginate beads, biochar, or coconut husks, which prevent microbial washout and allow prolonged retention time. This design is effective for treating high-strength petroleum waste and enables reuse across multiple treatment cycles, reducing long-term costs [15]. Immobilized systems also perform well in variable conditions, making them adaptable to fluctuating pollutant loads.

The development of modular and portable bioreactors is helping bridge this gap. Compact units powered by solar energy and equipped with basic aeration and filtration systems are now being deployed for small-scale or rural applications. These units often rely on inexpensive bio-stimulants such as poultry litter or spent mushroom substrate to maintain microbial viability [7]. They offer practical solutions for communities with limited infrastructure and expand the accessibility of advanced remediation technologies.

The aim of this research is to investigate the effectiveness of a water-based bio-stimulant for the bioremediation of oil-contaminated soil under controlled laboratory conditions. And the objective is to formulate and characterize water-based bio-stimulant for the treatment of oil-contaminated soil, to determine and evaluate the physio-chemical properties of the soil before and after contamination and treatment using selected parameters and to also assess the growth response of seeds as an indicator of soil recovery after treatment.

II. MATERIALS AND METHOD

The materials used for this research include:

i. Soil sample collected from Rivers State University.
ii. Condemned crude oil obtained locally.
iii. Organic wastes: cow dung, goat dung, and sawdust.
iv. Distilled water for solution preparation.
v. Corn seeds for growth observation.
vi. Rubber containers for experimental setup.
vii. Glassware such as beakers, measuring cylinders, and funnels.
viii. Weighing balance for mass determination.
ix. Burette, pipette, and retort stand for titration procedures.
x. Filter paper for separation processes.
xi. Wash bottle for dispensing distilled water.
xii. Oven for moisture analysis.

2.2 Methods

2.2.1 Soil Sample Collection and Preparation

The polluted soil sample used for this study was collected from Niger Delta Reservoir. Sampling was done from the surface layer (0–15 cm depth) to represent the natural condition of the soil most affected by contamination. Obvious debris such as roots and stones were removed manually. The

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polluted soil was used in its collected state without sieving, in order to reflect field conditions as closely as possible. Although laboratory studies often involve sieving to obtain uniform grain size, this step was omitted in the present work due to time constraints. The soil was, however, adequately homogenized by mixing before being apportioned into the experimental containers as shown on Figure 1.

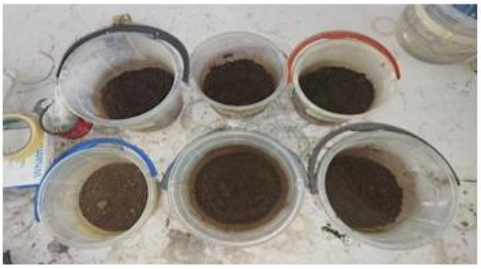

Figure 1: This figure presents the initial setup of the experiment showing the labelled containers

2.2.2Bio-stimulant Preparation and Characterization

Bio-stimulants were prepared from three organic waste materials: cow dung, goat dung, and sawdust. Each material was weighed to 100 g and mixed separately with 100 mL of distilled water in clean containers to form slurries. For the combined treatment, equal portions of 33.3 g of cow dung, 33.3 g of goat dung, and 33.4 g of sawdust were measured, giving a total of 100 g, and mixed with 100 mL of distilled water. All mixtures were thoroughly stirred using a stirrer to ensure uniform consistency and were left to stand for 24 hours to allow partial microbial activation before application to the contaminated soil.

To characterize the prepared bio-stimulants, pH determination was carried out using standardized pH test strips. Each slurry sample was gently stirred to ensure homogeneity. A pH strip was immersed into the slurry for approximately 30 seconds, after which the resulting colour change was compared with the manufacturer's pH colour chart to record the corresponding pH range. This procedure was repeated for all bio-stimulant types to evaluate their acidity or alkalinity, which serves as an indicator of microbial suitability and compatibility with the target soil.

2.2.3 Experimental Setup

Six rubber containers were used to model different treatment conditions. Each container was filled with 1 kg of soil. The containers were labelled as follows:

i. Soil only (control)
ii. Soil + Oil
iii. Soil + Oil + Cow dung
iv. Soil + Oil + Goat dung
v. Soil + Oil + Sawdust
vi. Soil + Oil + Cow dung + Goat dung + Sawdust

Crude oil contamination was introduced by adding 100 mL of crude oil to each container, except the control (S). The contaminated soils were thoroughly mixed to ensure uniform distribution of oil and left for 24 hours to allow proper infiltration. After this period, the prepared bio-stimulant slurries were added to the respective containers according to their labels. The mixtures were then monitored for changes in soil properties and plant growth response over time.

2.2.4Polluted Soil Analysis

Soil analysis was carried out to assess the physicochemical properties of the samples before and after contamination, as well as after the application of bio-stimulants. These analyses provided a basis for comparing the effectiveness of different treatments in enhancing hydrocarbon degradation and soil recovery. The following parameters were determined: moisture content, chloride concentration, hardness, total organic carbon (TOC), and organic matter.

2.2.4.1 Moisture Content

The oven-drying method was used to determine the moisture content of the soil sample. The procedure was as follows:

A crucible was weighed using a digital balance, and the weight was recorded. Exactly 5 g of polluted soil sample was added to the plain crucible, and the combined weight was recorded. The polluted soil sample with the crucible was placed in an oven set at 50 °C for 24 hours. After drying, the sample was removed and allowed to cool in a desiccator to avoid moisture absorption from the atmosphere. The sample was reweighed, and the moisture content was calculated as the difference between the initial and final soil weights relative to the dry weight of the soil.

2.2.4.2 Chloride Test

The chloride content of the soil extract was determined using the argentometric titration method. The procedure was as follows:

Five grams (5 g) of the polluted soil sample was weighed and placed into a closed-cap container. Fifty millilitres (50 mL) of distilled water were added, and the mixture was agitated thoroughly to extract soluble salts. The suspension was filtered using filter paper to obtain a clear filtrate. Five millilitres (5 mL) of the filtrate were measured into a clean conical flask. Two drops of potassium chromate indicator were added, producing a yellow solution. The solution was titrated against a standard silver nitrate ($AgNO_3$) solution until the colour changed from yellow to brick red, indicating the end point.

The chloride concentration was calculated using the formula:

$$\text{Chloride}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{V x N x 35.45 x 1000}}{\text{Volume of aliquot (mL)}} \qquad (1)$$

Where:

V = volume of $AgNO_3$ used (mL)
N = normality of $AgNO_3$ solution
35.45 = molar mass of chloride ion (g/mol)
Volume of aliquot = 5 mL

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2.2.4.3 Hardness Test

The total hardness of the soil extract was determined using the EDTA complexometric titration method. The procedure was as follows:

Five grams (5 g) of the polluted soil sample was weighed and placed into a beaker. Fifty millilitres (50 mL) of distilled water were added, and the mixture was thoroughly agitated in a closed container to extract soluble minerals. The suspension was filtered to obtain a clear filtrate and five millilitres (5 mL) of the filtrate were measured into a clean conical flask then two drops of ammonium buffer solution were added to maintain pH 10. Two drops of Eriochrome Black C indicator were then added, turning the solution violet. The solution was titrated against a standard EDTA solution until the colour changed from violet to pure blue, indicating the end point.

The total hardness of the sample was calculated using the formula:

$$\text{Hardness } \left(\frac{\text{mg}}{\text{L}} \text{as CaCO}\right) = \frac{\text{V x N x 50,000}}{\text{Volume of aliquot (mL)}} \qquad (2)$$

Where:

V = volume of EDTA used (mL)
N = normality of EDTA solution
Volume of aliquot = 5 mL
50,000 = equivalent weight factor for $CaCO_3$

2.2.4.4 Total Organic Carbon (TOC)

The total organic carbon (TOC) of the soil was determined using the Walkley–Black wet oxidation method. The procedure was as follows:

Two grams (2 g) of air-dried soil sample was weighed into a clean conical flask. Five millilitres (5 mL) of 1 N potassium dichromate ($K_2Cr_2O_7$) solution were added to the flask then ten millilitres (10 mL) of concentrated sulfuric acid (H_2SO_4) were carefully introduced, and the flask was swirled gently to ensure proper mixing. The mixture was left to stand for 30 minutes to allow oxidation of organic carbon. After cooling, the solution was diluted with distilled water to a final volume of 100 mL, three to four drops of ferroin indicator were added. The mixture was titrated with standard Ferrous Ammonium sulphate (FAS) solution until the colour changed from blue-green to reddish-brown, which indicated the end point.

A reagent blank was also prepared (without soil) and titrated in the same way.

The percentage organic carbon was calculated using the relation:

$$\text{Organic Carbon (OC)} = \frac{(V_b - V_s) \text{ x N x } 0.003 \text{ x } 100}{W} \qquad (3)$$

Where:

V_b = volume of FAS used for blank (mL)
Vs = volume of FAS used for sample (mL)
N = normality of FAS solution
W = weight of soil sample (g)
0.003 = milliequivalent weight of carbon in grams

The percentage organic matter was then obtained by multiplying the organic carbon by the Van Bemmelen factor (1.724):

$$\text{Organic Matter (OM)} = \%\text{OC X } 1.724 \qquad (4)$$

2.2.6 Data Handling and Analysis

The data generated from the various soil analyses (moisture content, chloride, hardness, total organic carbon, and organic matter) will be recorded and processed using standard analytical formulas. Results will be presented in tables and graphs to enable clear comparison between the control soil (uncontaminated), oil-contaminated soil, and the bio-stimulant-amended soils. The extent of remediation will be assessed by evaluating the differences in soil parameters across treatments. Statistical comparisons may also be made where applicable to highlight the significance of observed changes.

III. RESULT AND DISCUSSION

The graphical representation of the pH distribution across the bio-stimulant samples is shown in Figure 2. The figure illustrates the comparative pH values of the individual slurries and the combined formulation, highlighting the equilibrium achieved when all components were blended. The balanced pH observed in the final mixture validates the success of the formulation process and its readiness for soil application.

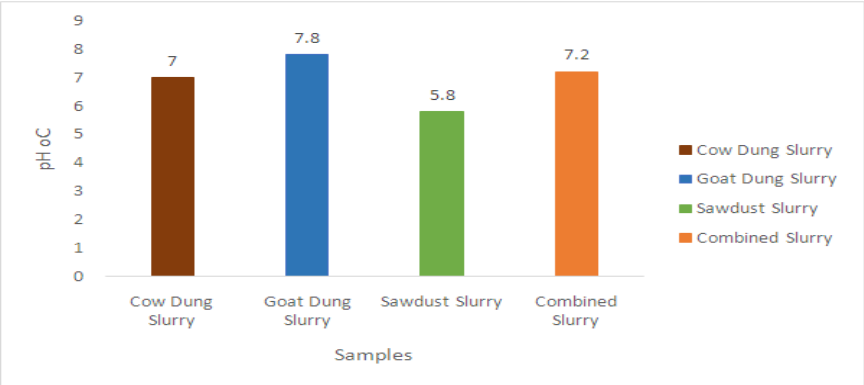

Figure 2: pH Values of Water-Based Bio-stimulants

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The results indicate that the slurries exhibited slightly acidic to neutral pH values, ranging between 5.0 and 8.5. Specifically, cow dung slurry recorded a near-neutral pH, goat dung slurry showed an alkaline tendency, and sawdust slurry exhibited mild acidity. The blended slurry maintained a balanced pH range favourable for microbial activity and nutrient bioavailability. These results confirm that the bio-stimulant were well-buffered and suitable for the subsequent remediation experiments.

3.2 Physio-chemical Properties of Soil Before and After Treatment

3.2.1 Moisture Content

The moisture content of the soil samples for Day 0 and Day 7 is presented in Figure 3

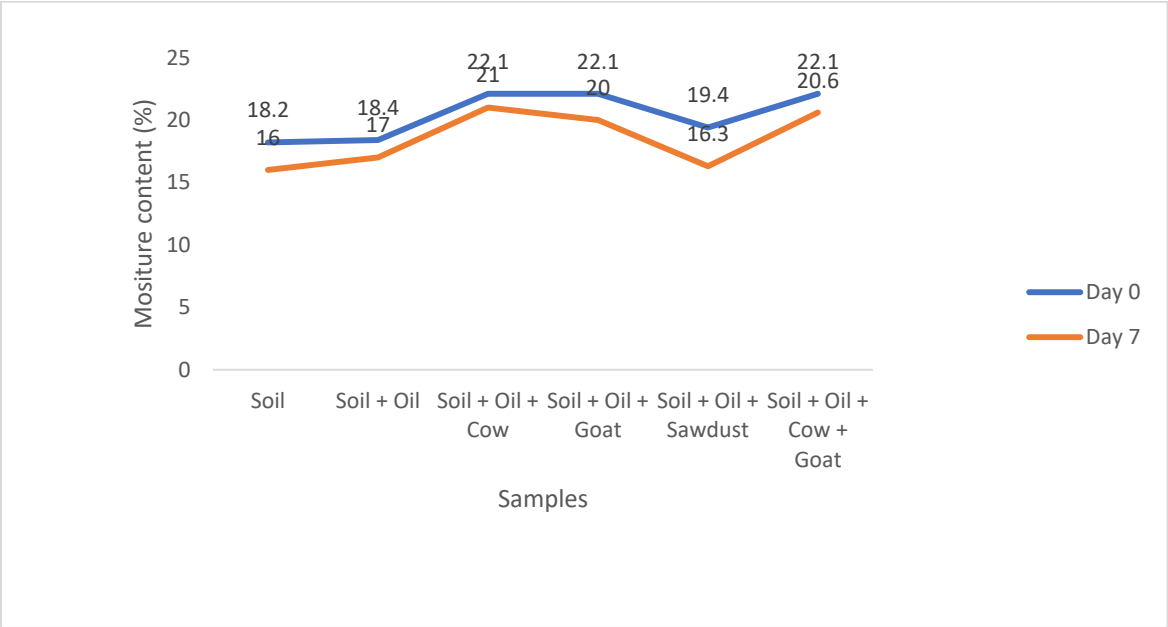

Figure 3: Moisture content of soil samples on Day 0 and Day 7

On Day 0, initial moisture contents were relatively high across all treatments, indicating the soil's natural water retention before significant microbial activity began. The control sample (Soil only) showed moderate moisture, while the oil-contaminated sample (Soil + Oil) retained slightly less, suggesting that crude oil interfered with normal water absorption and capillarity. Among the bio-stimulant-amended samples, Soil + Oil + Cow Dung and Soil + Oil + Goat Dung recorded higher moisture retention, likely due to the organic matter present in the amendments.

By Day 7, a general reduction in moisture content was observed across all samples, which may be attributed to ongoing microbial degradation and the gradual evaporation of soil water. The Soil + Oil + Cow Dung sample maintained the highest residual moisture, followed by Soil + Oil + Goat Dung and the mixed amendment (Soil + Oil + Cow Dung + Goat Dung + Sawdust). This trend suggests that organic amendments improved the soil's water-holding capacity and maintained a favourable moisture environment for microbial activity and hydrocarbon degradation.

Comparatively, the Soil + Oil treatment showed the sharpest moisture decline between Day 0 and Day 7, reinforcing that crude oil contamination tends to reduce soil porosity and inhibit water retention. Sawdust, although organic, retained less moisture than dung-based treatments—likely due to its fibrous and carbon-rich composition that decomposes more slowly, providing fewer hydrophilic sites for water absorption. The progressive decline in moisture content over time supports the bioremediation process, as moderate drying enhances aeration and oxygen diffusion, stimulating aerobic microbial degradation. Overall, the presence of organic bio-stimulants moderated water loss and created more stable micro-environments for sustained microbial action.

3.2.2 Chloride Ion Concentration

The chloride concentration of the soil samples at Day 0 and Day 7 is presented in Figure 4

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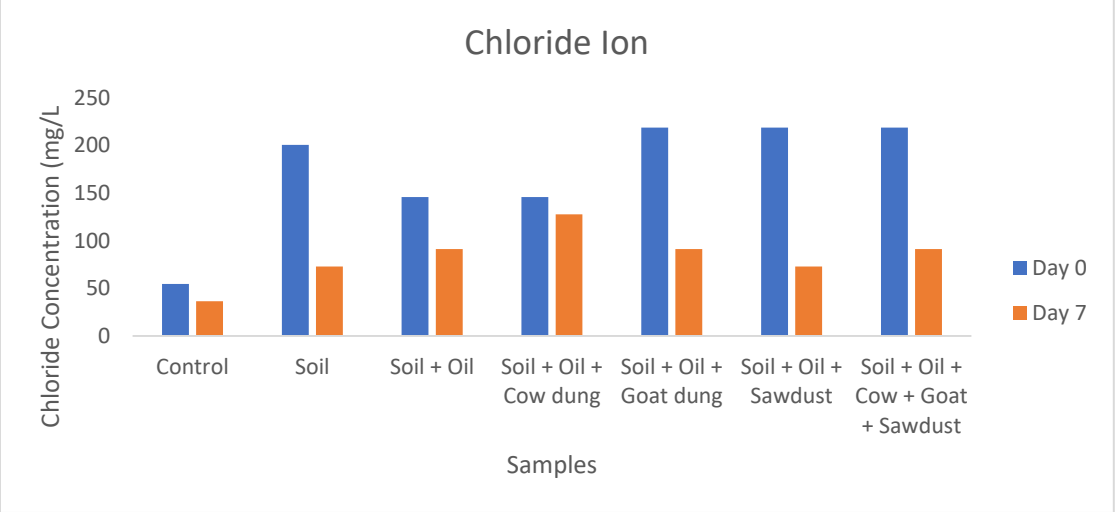

Figure 4: Chloride concentration of soil samples on Day 0 and Day 7.

On Day 0, chloride concentration varied across treatments. The highest values were observed in Soil + Oil + Goat (218.66 mg/L), Soil + Oil + Sawdust (218.66 mg/L), and Soil + Oil + Cow + Goat + Sawdust (218.66 mg/L). Moderate values were recorded in Soil (200.43 mg/L), while the lowest concentrations occurred in Soil + Oil (145.77 mg/L) and Soil + Oil + Cow (145.77 mg/L).

By Day 7, chloride concentrations generally decreased across all samples. Soil + Oil + Cow recorded the highest value (127.55 mg/L), followed by Soil + Oil (91.11 mg/L), Soil + Oil + Goat (91.11 mg/L), and Soil + Oil + Cow + Goat (91.11 mg/L). The lowest concentrations were seen in Soil (72.89 mg/L) and Soil + Oil + Sawdust (72.89 mg/L).

The observed reduction in chloride concentration between Day 0 and Day 7 suggests that bio-stimulant amendments enhanced microbial activity and nutrient cycling, thereby facilitating degradation of hydrocarbons and modification of soil ionic balance. Cow dung amendment maintained relatively higher chloride levels, possibly due to its richer nutrient profile, which sustains microbial communities and slows rapid chloride depletion. In contrast, sawdust-amended soils showed greater reduction, reflecting its lower nutrient availability and slower decomposition rate.

When compared to the control (Soil only), which decreased from 200.43 mg/L at Day 0 to 72.89 mg/L at Day 7, oil contamination alone (Soil + Oil) resulted had slower chloride reduction, with values reducing from 145.77 mg/L to 91.11 mg/L. This indicates that crude oil contamination disrupts natural ionic balance, while bio-stimulant amendments improve soil conditions and support remediation processes.

3.2.3 Total Hardness Determination

The total hardness of the soil samples was determined on Day 0 and Day 7. The graphical summary of the results is shown in Figure 5

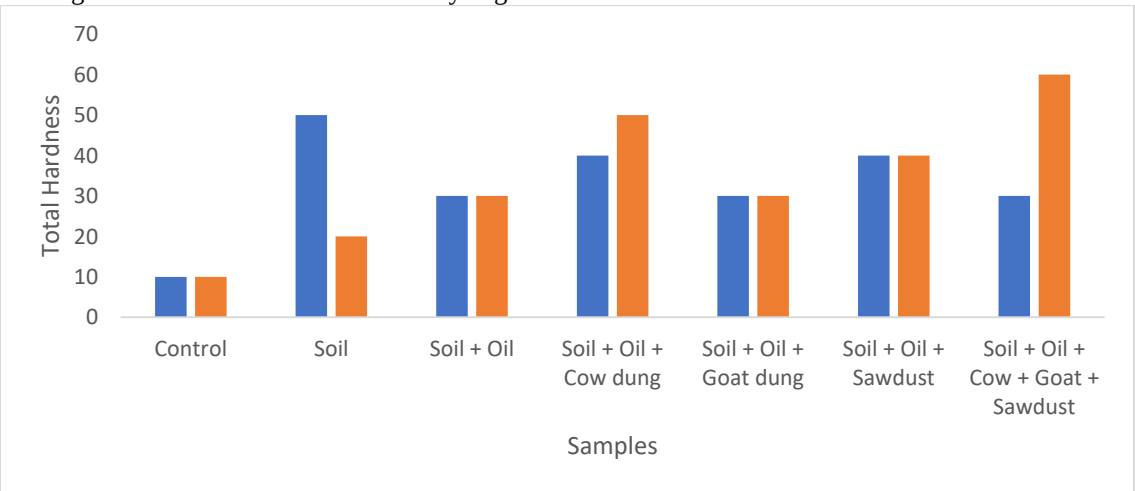

Figure 5: Total hardness of soil samples on Day 0 and Day 7

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On Day 0, total hardness varied across treatments. The Soil sample recorded 50.05 mg/L, while the Soil + Oil sample had 30.03 mg/L. Among the amended samples, Soil + Oil + Cow showed the highest hardness (40.04 mg/L), followed by Soil + Oil + Sawdust (40.04 mg/L), while Soil + Oil + Goat and Soil + Oil + Cow + Goat + Sawdust had 30.03 mg/L each.

By Day 7, hardness levels increased in most samples. Soil + Oil + Cow had the highest value (50.05 mg/L), followed by Soil + Oil + Sawdust (40.04 mg/L), Soil + Oil + Goat (30.03 mg/L), and Soil + Oil (30.03 mg/L). The Soil (control) showed a lower hardness of 20.02 mg/L, while the combined amendment (Soil + Oil + Cow + Goat) recorded the highest overall value of 60.06 mg/L.

The increase in hardness observed between Day 0 and Day 7 in the amended soils indicates the release of mineral ions during the degradation process stimulated by bio-stimulants. Cow dung amendment consistently produced higher hardness values compared to goat dung and sawdust, possibly due to its richer mineral composition and higher microbial activity. The control soil, however, showed a significant reduction, reflecting natural depletion of soluble salts in the absence of contamination or amendments.

When compared with the Soil + Oil sample, which maintained relatively low hardness levels (30.03 mg/L on both days), the bio-stimulant-amended soils demonstrated improved ionic exchange and mineral cycling. This suggests that the application of organic amendments not only supports remediation but also enhances soil fertility by releasing essential minerals during hydrocarbon degradation.

3.2.4 Total Organic Carbon (TOC) and Organic Matter (OM) Results

The Total Organic Carbon (TOC) and Organic Matter (%OM) results for the soil samples at Day 0 and Day 7 are presented in Figures 6a and 6b

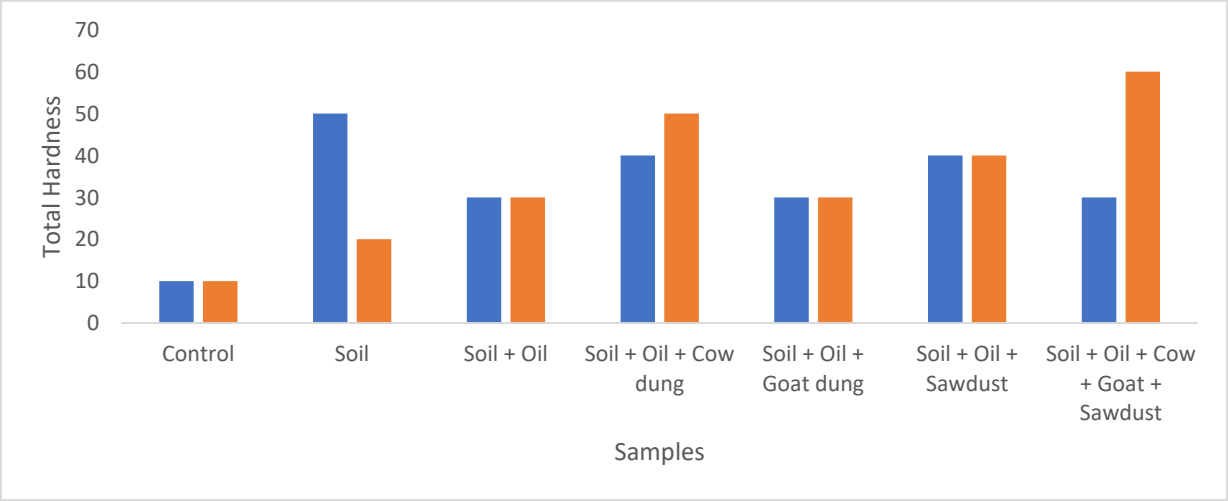

Figure 6a: **Percentage Total Organic Carbon (%OC) of Soil Samples on Day 0 and Day 7**

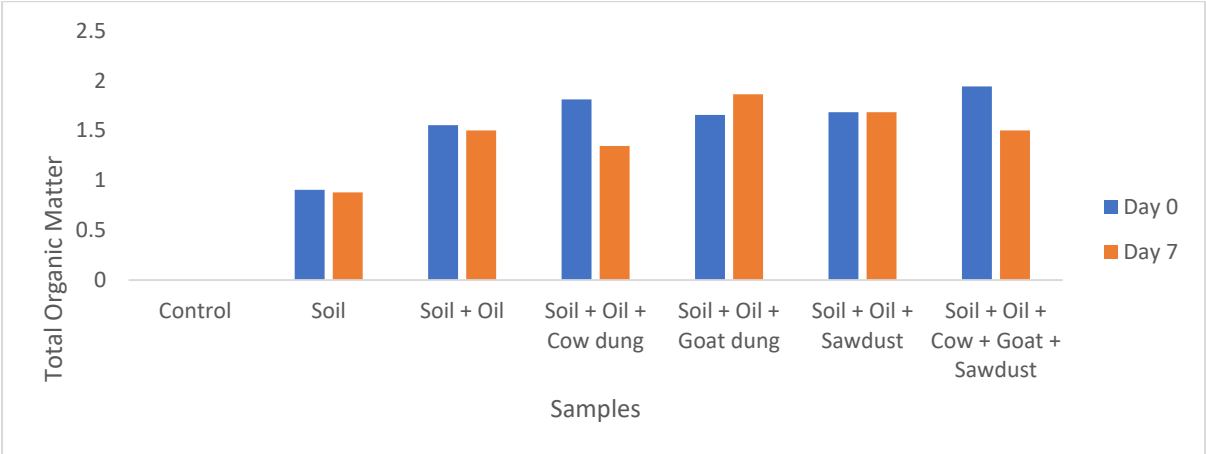

Figure 6b: Percentage Organic Matter (%OM) of Soil Samples on Day 0 and Day 7

On Day 0, the TOC levels were generally high across all treatments, reflecting the heavy organic load introduced by crude oil contamination and the freshly applied amendments. The control soil recorded 0.525% OC and 0.905% OM, while

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the oil-contaminated soil showed an increase to 0.900% OC and 1.552% OM, indicating that the crude oil added significant carbon content. Among the bio-stimulant treatments, Soil + Oil + Cow showed 1.050% OC and 1.811% OM, while Soil + Oil + Goat and Soil + Oil + Sawdust recorded 0.960% OC / 1.655% OM and 0.975% OC / 1.682% OM, respectively. The combined treatment (Soil + Oil + Cow + Goat + Sawdust) produced the highest initial carbon load with 1.125% OC and 1.940% OM, indicating nutrient enrichment from the organic mixtures.

By Day 7, the TOC and OM values generally reduced across most treatments, showing that active microbial degradation had begun. The control sample slightly decreased to 0.510% OC and 0.879% OM, while the oil-contaminated soil reduced to 0.870% OC and 1.500% OM, signifying slow natural degradation. The Soil + Oil + Cow treatment dropped to 0.780% OC and 1.344% OM, suggesting moderate hydrocarbon breakdown due to enhanced microbial activity. Interestingly, the Soil + Oil + Goat treatment increased marginally to 1.080% OC and 1.862% OM, which may indicate ongoing decomposition and microbial biomass build-up. The sawdust treatment remained relatively stable (0.975% OC, 1.682% OM), implying a slower but sustained carbon mineralization process. The combined treatment (Soil + Oil + Cow + Goat + Sawdust) reduced to 0.870% OC and 1.500% OM, demonstrating balanced bio-stimulant synergy that supported consistent degradation.

Overall, the observed reduction in TOC and OM between Day 0 and Day 7 confirms that the bio-stimulants significantly enhanced hydrocarbon degradation. The organic amendments provided essential nutrients that supported microbial growth, which in turn converted complex hydrocarbons into simpler carbon compounds. The results affirm that cow dung, goat dung, and sawdust, both individually and in combination, can serve as effective, eco-friendly water-based bio-stimulants for the remediation of oil

3.2.5 Evaluation of Bio-stimulant Effectiveness in Soil Remediation

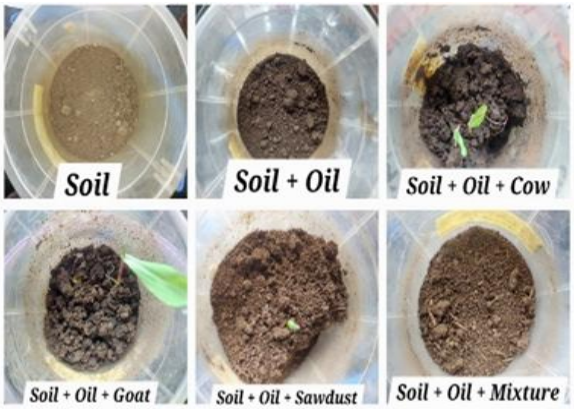

Figure 7a: **Bio-stimulant Effectiveness in Soil Remediation**

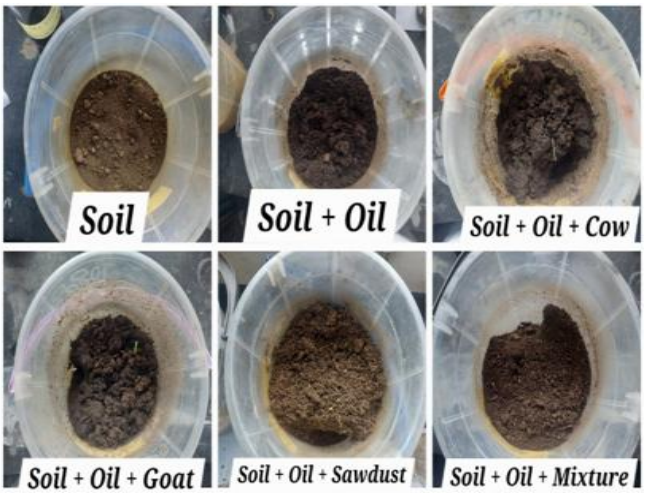

Figure 7b: Bio-stimulant Effectiveness in Soil Remediation

The effectiveness of the formulated water-based bio-stimulants was evaluated through growth observation tests using maize (corn) seeds as bioindicators. The test aimed to assess soil recovery, nutrient availability, and toxicity reduction following bio-stimulant application on crude oil–contaminated soil. Six treatments were observed over a 14-day period, including the control soil, contaminated soil, and soils treated with individual and combined bio-stimulants.

By the seventh day, no germination was observed in the control (Soil only) and the crude oil–contaminated soil (Soil + Oil). The absence of sprouting in the control could be attributed to low moisture content and limited nutrient availability, while the lack of germination in the oil-polluted sample reflected the inhibitory effects of hydrocarbons on oxygen diffusion and enzyme activation essential for seed development.

In contrast, the bio-stimulant-amended soils exhibited significant improvements in seed emergence and early growth. Treatments with cow dung, goat dung, and sawdust-based bio-stimulants (Soil + Oil + Cow Dung, Soil + Oil + Goat Dung, and Soil + Oil + Sawdust) all showed visible sprouting by Day 7. This suggests that the bio-stimulants effectively improved soil aeration, microbial activity, and nutrient release, thereby mitigating the toxic influence of hydrocarbons and supporting seed germination. The observed results also imply that the water-based formulations enhanced bioavailability of nitrogen and phosphorus—critical for enzymatic and metabolic functions during early plant growth.

The combined bio-stimulant treatment (Soil + Oil + Cow Dung + Goat Dung + Sawdust) showed delayed germination, with root emergence recorded on Day 14. However, the seedlings failed to survive beyond this stage. This outcome may be linked to insufficient moisture retention or limited microbial stability within the blend, leading to suboptimal nutrient supply for sustained growth. Nonetheless, initial germination in this treatment indicates partial soil recovery, demonstrating that water-based bio-stimulants hold promise

for restoring contaminated soils when optimized for dosage and moisture balance.

Overall, the growth observation confirmed that the application of water-based bio-stimulants substantially enhanced soil fertility and biological activity in oil-contaminated soils. The individual bio-stimulants outperformed the untreated controls, showing potential for restoring soil productivity in degraded environments. The findings further validate that organic water-based formulations are effective, affordable, and environmentally friendly alternatives for small-scale bioremediation initiatives.

IV. CONCLUSION

After the analysis the following conclusions were drawn;

i. The cow dung, goat dung, and sawdust slurries exhibited near-neutral to slightly alkaline pH values, indicating conditions conducive for microbial activity essential in hydrocarbon degradation.
ii. The combined slurry provided a balanced C:N ratio, suggesting that the blend could support sustained biodegradation while maintaining soil pH stability.
iii. The Treated samples showed increased organic matter and moisture content, while parameters such as chloride and hardness were moderated toward values typical of healthy soils.
iv. The best performance was observed in soils amended individually with cow dung and goat dung slurries, reflecting their strong nutrient profiles and microbial enhancement potential.

Nomenclature and Abbreviations

- MC = Moisture Content
- Cl^- = Chloride ion concentration
- TOC = Total Organic Carbon
- OM = Organic Matter

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