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Physicochemical and Microbial Alterations in Crude Oil-Contaminated Soil Samples

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Abstract
Environmental pollution is becoming an issue of concern due to its negative impact on the living and non-living components of the environment. Crude oil and its products are the major sources of environmental pollution that renders negative impact on the soil mineral components and microorganism. This study was thus carried out to determine the effect of crude oil on soil physicochemical properties, bacterial and fungal population. The experiment lasted for 42 days and the analysis were carried out at days 0, 14, 28 and 42 using standard protocols. Results obtained show significant changes ($P \leq 0.05$) in hydrocarbons and other physicochemical parameters such as pH, nitrate, electrical conductivity, sodium ion, calcium ion, phosphate and magnesium ion, when the control was compared with crude oil contaminated soil. Also, soil bacterial and fungal count decreased significantly ($P \leq 0.05$) with increasing concentrations of crude oil indicating its toxicity to soil microbial consortium. However, results also showed that hydrocarbon-degrading microorganisms strived at higher concentrations but insignificantly ($P \leq 0.05$) in comparison with the control. Owing to the result obtained in this study, crude oil exploration should be properly managed to prevent spillages that negatively impact on the environment.

Keywords: Crude oil, Oil spillage, Soil, Physio chemistry, Bacteria, Fungi

INTRODUCTION
Globally, the oil sector is known for its negative and positive impacts on the environmental quality and the socioeconomic aspect of human existence (Elum *et al.*, 2016). While its products are major sources of fuel for domestic and industrial activities which positively impacts on job creation, it constitutes the main source of revenue generation for developing countries like Nigeria (Demir, 2012; Elum *et al.*, 2016). Despite these benefits, the oil sector has impacted the total quality of the environment negatively in a good number of ways during exploration, production processes and distribution. Some of these routes include; release of wastewater discharge, gas flaring and oil spillage (Olajire, 2014). Oil spillage, which is associated with crude oil exploration and exploitation largely, poses environmental risk factor due to its toxic hydrocarbon component and other impurities that are harmful to the biota (Edema, 2012).

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Crude oil is a naturally occurring complex mixture that consists of hydrocarbon (90%) and non-hydrocarbon (10%) components such as heavy metals like; lead, mercury and vanadium, trace elements such as nitrogen, sulphur and salts (Alinnor and Nwachukwu, 2013; Yasin *et al.*, 2013). Hydrocarbon components are divided into four categories including saturates, aromatics, asphaltenes and resins (Ite and Semple, 2012). The non-hydrocarbons components are mainly obtained from the earth as impurities such as mud, salts and water that enter the whole crude during its exploration and transportation process (Alsadi, 2018; Wang *et al.*, 1999). The elemental composition of petroleum is much less variable than that of coal: 83-87% carbon, 11-16% hydrogen, 0-4% oxygen plus nitrogen, and 0-4% sulfur (Alinnor and Nwachukwu, 2013, Yasin *et al.*, 2013). Alkanes are saturated hydrocarbons, ranging from pentane to pentadecane, they are the main constituents in gasoline or petrol. Alkanes above C_{17} present in crude oil causes increased viscosity, high cloud and pour points (Alinnor and Nwachukwu, 2013; Yasin *et al.*, 2013).

The alkyl derivatives of the most common aromatics in crude oil include Benzene xylene and toluene. High molecular weight aromatics of crude oil are more toxic than low molecular weight aromatics; however, unsaturated hydrocarbons with a low boiling point like Benzene, Xylene and Toluene have been reported as the very toxic crude oil component (Edema *et al.*, 2008; Edema, 2012). Aromatic hydrocarbons such as toluene, benzene, ethylene, xylene, especially polyaromatic hydrocarbons (PAHs) like benzo-A-pyrene are one of the most toxic components of whole crude (Kuppusamy *et al.*, 2020). The toxicity posed by the hydrocarbon and non-hydrocarbon components of crude oil has necessitated the consistent investigation of the detrimental effect of crude oil to the living and non-living environmental components that may result from oil spillages.

Again, oil spill is majorly caused by accidental leakages during exploration and transportation, leakages from old pipes, terrorism, theft and vandalization (Adishi and Olugbenga, 2017; Agbonifo, 2016; Albert *et al.*, 2019). These occurrences have resulted to the deterioration and damages of the different ecological compartments of the environment, mostly in the Niger Delta region area of Nigeria, where oil spill scenarios have been frequently reported (Akpan, 2022; Mongabay, 2021 and Nriagu *et al.*, 2016). Oil spills have resulted in the deterioration of the terrestrial and aquatic environment (Nnaji and Egwu, 2020). In the terrestrial environment, alterations in the soil physicochemical parameters and trace elements such as sodium, potassium, electrical conductivity, chloride and calcium ions, phosphorus and pH have being researched (Adeyemo and Alui, 2021; Devantha *et al.*, 2019; Nnaji and Egwu, 2020; Wang *et al.*, 2013).

Soil contamination directly impacts the plants cultivated on contaminated soil in the following ways; inhibition of germination, inhibition of nutrient and water uptake by root causing deficiency in some plant parts (Achuba and Iserhienrhien 2018; Correa *et al.*, 2022; FiriAppah *et al.*, 2014; Olubodun and Eriyamremu, 2013). Severe toxic effects have been traced to the aromatic components of crude oil, due to their persistence and hydrophobic nature in the environment (Honda and Suzuki, 2020; Patel *et al,* 2020), however, soil microorganisms play an important role in mitigating, oil pollution in the environment. Polluted sites are usually left without remediation in developing countries that are oil producing like Nigeria (Xu *et al*., 2018), and so they depend mainly on bioremediation by natural attenuation and this process is dependent on the degradative ability of soil bacteria and fungi (Xu *et al.*, 2018; Sui *et al*., 2021) .

A good number of bacterial and fungal species have been seen to degrade petroleum hydrocarbons in the soil. Bacteria of genera; *Alcaligens, Enterobacter, pseudomonas,* *Flavobacterium, Bacillus* and *Alcanivorax* (Anno *et al*., 2021). Fungi of the following genera, *Aspergillius, Fusarium, Mucur, Penicillium, trichoderma* and *Cryptococus* have been predominantly observed to breakdown hydrocarbons (Anno *et al.,* 2021). However, in very severe cases of crude oil- contamination, crude oil toxicity is imposed on both hydrocarbon degraders and other microbial non-hydrocarbon degraders, exposing the soil to greater risk. However, bioremediation by augmentation and genetic modification could serve as very good options to salvage the polluted soil (Anno *et al.,* 2021).

This study presents novelty in its comprehensive investigation of the impact of crude oil contamination on soil physicochemical properties, bacterial, and fungal populations. It provides a detailed understanding of the changes induced by crude oil in the soil environment over a 42-day period. The study highlights the toxic effects of crude oil on soil microbial populations and emphasizes the importance of preventing oil spillages to safeguard agricultural lands. It contributes to the understanding of soil degradation due to crude oil contamination and provides valuable insights for developing effective strategies for soil remediation and environmental management, addressing the global concern of environmental pollution caused by crude oil and its products.

MATERIALS AND METHODS

Sample collection and study location

The present study was conducted in the general laboratory at the Department of Biochemistry, Faculty of Life sciences, University of Benin, Benin City, Nigeria. The crude oil (10 L, bonny light) used was obtained from Warri refinery and petrochemical company in Delta state, Nigeria. The soil (sandy loam) sample (20 Kg) for this study was collected using sterilized hand trowel from a quiet region of the university, with minimal vehicular traffic. This was done to ensure that the soil samples were not contaminated with hydrocarbon compounds that might interfere with the result of the research study.

Treatment of the soil

The collected soil samples were sieved (2 mm) and air dried for 48 h, then 500 g was measured into sterilized polythene bags mixed with crude oil for the treated samples and no treatment for the control samples. The concentrations of whole crude for treatment were 2, 5, 10 and 20% v/w whole crude, gotten after a range finding test was done. Treatment was done on a daily basis by adding 2 mL crude oil to mimic the state of chronic soil pollution for 42 days. One (1) gram of soil was collected in sterile containers each for soil physicochemical properties, bacterial and fungal analyses at day 0, 14, 28 and 42.

Physicochemical analyses

Soil physicochemical parameters including total hydrocarbon content (THC), pH, nitrate, electrical conductivity, phosphate, sodium, calcium, potassium and magnesium ion were all analyzed on day 0, 14, 28 and 42. All parameter were analyzed according to the AOAC standards for soil physiochemistry (AOAC, 1984).

Bacterial analysis from crude oil-contaminated soil

Bacterial analysis was undertaken according to the procedure of Bergey's manual of determinative bacteriology (Brown, 1939). First of all nursery bags, glassware, and media were maintained in sterile conditions; distilled water and growth media were autoclaved at 121 ^{o}C for 15 min. Soil sample (1 g) was suspended in 10 mL distilled water to make a concentrate, serial dilution was done using 10^2 to 10^{10}, while dilution concentrations; 10^6, 10^8 and 10^{10} were eventually used to estimate heterogeneous bacteria in triplicates, using pour plate method. Pure isolates from representative bacterial community were maintained at 4 ^{0}C on nutrient agar, after incubation was carried out for seven (7) days at ambient temperature. Microscopic viewing, cultural and biochemical characteristics were used to quantify and identify the bacterial isolates

Enumeration, isolation and identification of fungi
One (1) gram of control and crude oil-contaminated sample was then diluted in 9 mL of sterilized water and serial dilution in ten folds from 1:10 to 1:10,000. A portion, 0.1 mL of the 10^{-4} dilution was plated on potato dextrose agar (PDA), after sterilization, amended with streptomycin (helps inhibit of bacteria) each sample in triplicates using the pour plate method. The plates were incubated at 27 ^{0}C for 72 h, after which viable fungi in the control and oil-contaminated soil were calculated with the number of colonies formed. The inoculum volume and dilution factors were expressed in colony forming unit per gram. Fungal specimens were prepared and identified through morphological and taxonomical characteristics under a light microscope (Barnett and Hunter, 1998).

Data analysis
The data collected in the course of this research were presented as mean ± standard error of mean (SEM). A one-way analysis of variance (ANOVA) was done to test for statistical significance while post hoc text (tukey) and dunnet were used to compare significant difference between control and percentage treatments within and between groups. Significant difference was set at $p \leq 0.05$ and the symbol * was used to denote significant changes in all graphical presentations.

RESULTS AND DISCUSSION
Crude oil contamination has undoubtedly remained a matter of great concern due to its negative effect on both terrestrial and aquatic environment. Total hydrocarbon content (THC) levels (Figure 1A) and phosphate ion concentration (Figure 1D) were seen to increase significantly ($p \leq 0.05$), while soil pH (Figure 1B), electrical conductivity (Figure 2A), nitrate (Figure 1C), sodium (Figure 2B), potassium (Figure 2C), calcium (Figure 2D), magnesium ion (Figure 2E) decreased significantly, with concentration and time, as reported in a recent research (Nnaji and Egwu, 2020). This implies that crude oil caused an increase in THC of the soil, due to the rich aliphatic and aromatic hydrocarbon content of crude oil (Nnaji and Egwu, 2020).

Nnaji and Egwu (2020) also reported that soil pH, nitrate, sodium, calcium, magnesium ion decreased significantly when crude oil contaminated soil was compared with control (Nnaji and Egwu, 2020). Devantha *et al*. (2019) also reported similar results for pH, EC and potassium ion as there were alterations in these soil properties due to crude oil contamination. Uquetan *et al.* (2017) also reported an increase in total petroleum hydrocarbons and a significant decrease in Na, Ca, and K ions due to crude oil contamination. Ebulue (2022) also conducted a research to ascertain the effect of crude oil on soil physicochemical and reported alterations such as significant increase in total petroleum hydrocarbon and decreases that were significant for Na, K, Ca and Mg ions which again, were in conformity to the results from this study. This suggests that crude oil contamination might have caused changes in the soil properties that could negatively impact on the soil productivity.

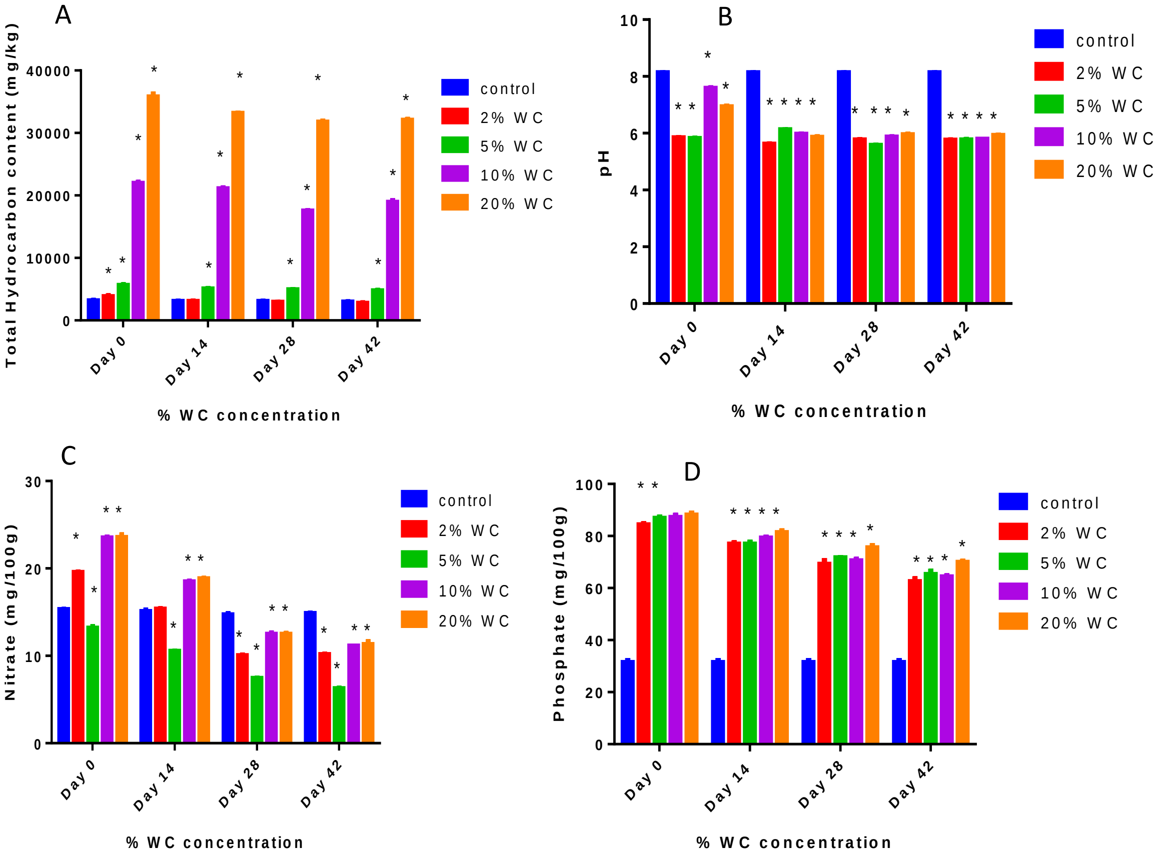

Figure 1: Changes in the physicochemical and microbial properties of crude oil-contaminated soil at varying concentrations for day 0, 14, 28 and 42. (A). Total hydrocarbon content. (B). pH level. (C). nitrate ion (D). phosphate ion concentration. Results are presented as mean ± SEM of three determinations and * indicates significant difference at $p \leq 0.05$

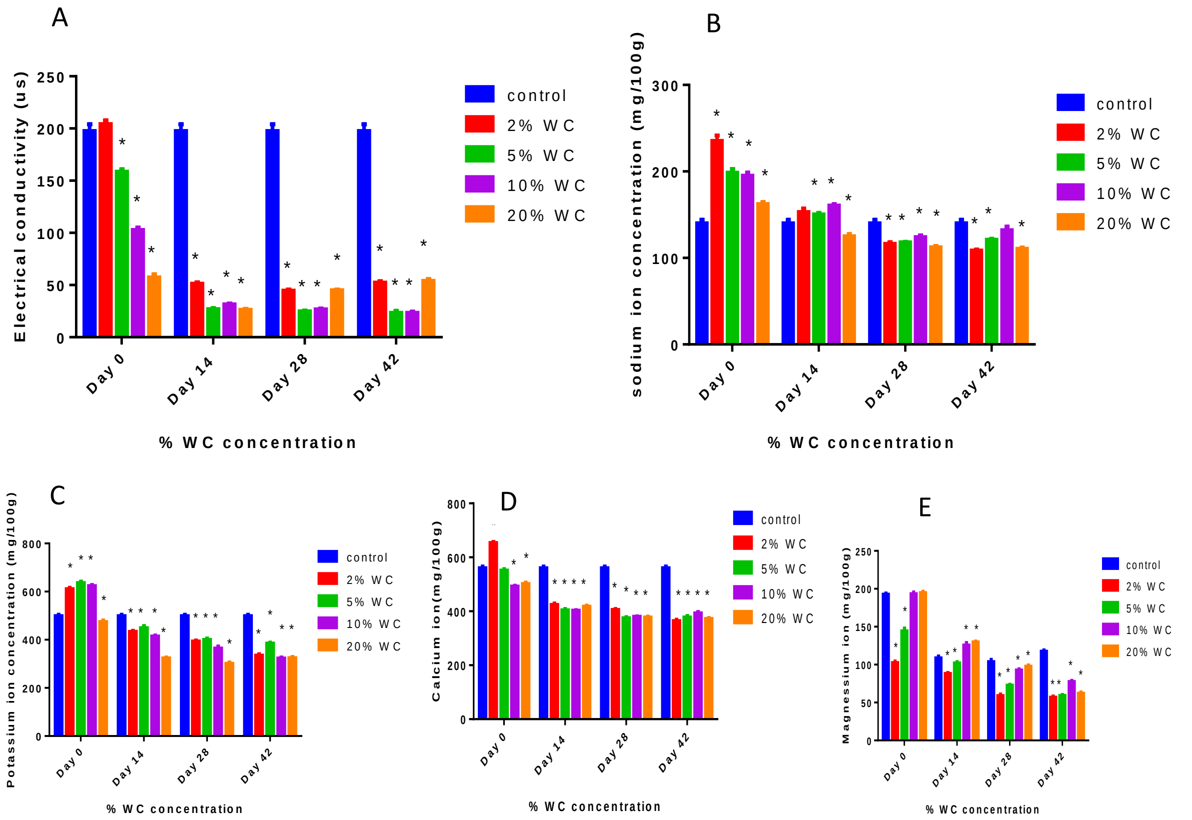

Figure 2: Changes in soil physicochemical and microbial properties of crude oil-contaminated soil at varying concentrations for day 0, 14, 28 and 42. (A). EC. (B). sodium ion. (C). potassium ion (D). calcium ion. (E). magnesium ion concentration. Results are presented as mean ± SEM of three determinations and * indicates significant difference at $p \leq 0.05$.

The data for microbial analyses reported in this study include the qualitative and quantitative parameters of bacterial and fungal population in the soil contaminated with 2, 5, 10 and 20% of crude oil on day 0, 14, 28 and 42. The data for bacterial analysis of crude oil contaminated soil are as presented in Figure 3 while that of fungal analysis are presented in Figure 4. The total count of heterogeneous bacteria ranged from 1.2×10^{4} to 4.3×10^{10} and bacteria isolated from the soil in control and treatment include *Pseudomonas* spp., *Bacillus subtilis, Staphylococcus aureus* and *klebsiella* spp. Bacterial count of the soil decreased significantly ($p \leq 0.05$) for all treatments when compared with the control. This was observed for crude oil-contaminated soil in Figure 3. Fungal count on the other hand ranged from 0. 01×10^{3} CFU/g to 9.0×10^{3} CFU/g. Similar result have been reported by Ebulue (2022). Soil treatment with WC resulted in a significant increase ($p \leq 0.05$) in fungi count at 5% concentration on all treatment days and at 20% on day 14. However, a significant decrease ($p \leq 0.05$) was observed at 2% on day 42 while no significant changes ($p \leq 0.05$) were seen at all other concentration (Figure 4). Different strains of fungi were isolated from control and contaminated groups including; *Penicillium, Aspergillus, Clasdospodium, Mucor, Neurospora, Streptomyces, Fusarium, Trichoderma, Chaonophora, Botrytis* and *Helminthosporium* (Dell'Anno *et al*., 2021). Shi *et al*. (2022) reported that crude oil contamination in the soil caused a decrease in soil bacterial and fungal count, however some oil degrading microorganisms were not affected, this again is in line with the present studies.

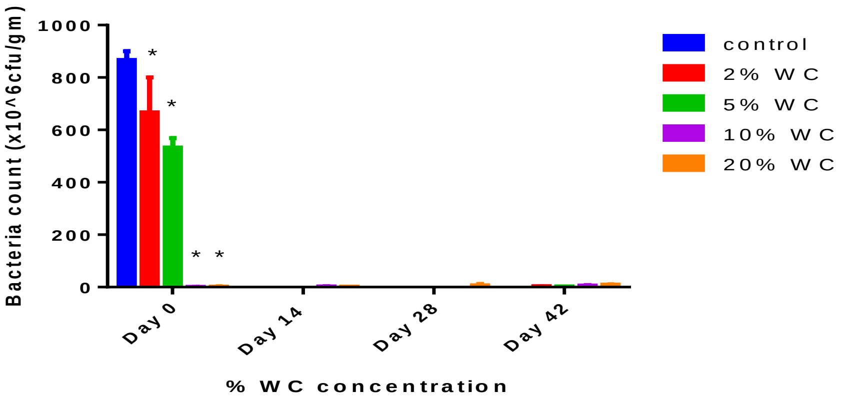

Figure 3: Bacterial count in soil contaminated with varying concentrations of crude oil. Results are presented as mean ± SEM of three determinations and * indicates significant difference at p ≤ 0.05

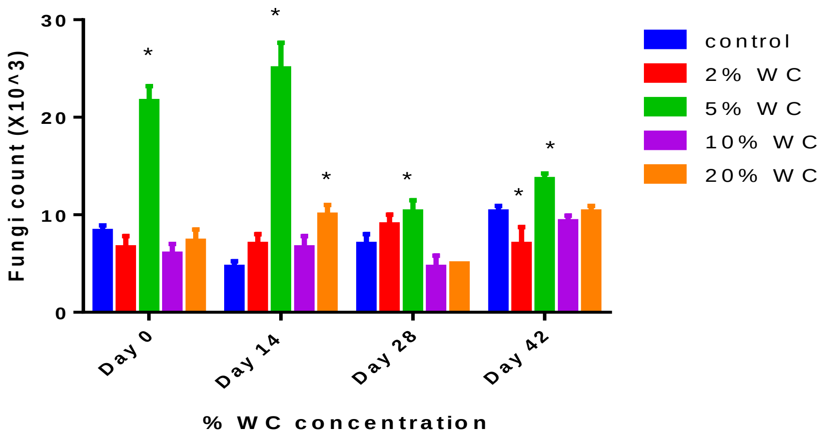

Figure 4: Fungi count of soil contaminated with varying concentration of WC. Results are presented as mean ± SEM of three determinations and * indicates significant difference at p ≤ 0.05.

CONCLUSION

The study highlights the need for proper management strategies to mitigate the detrimental impact of crude oil on soil and underscores the importance of environmental regulations in the oil industry. Overall, this research provides valuable insights for understanding and addressing the consequences of crude oil contamination on soil and contributes to the development of effective environmental protection measures.

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