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Impact of Crude Oil Water Soluble Fraction on Soil Physiochemical Properties and Bacteria

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ABSTRACT: Owing to the underlying negative impact of crude oil to the ecosystem, this study investigated the effect of water soluble fraction (WSF) of crude oil on selected soil physiochemical properties and bacteria using appropriate standard methods. The investigation was carried out using control (without WSF), 2, 5, 10 and 20% concentration for 42 days and analysis was carried out at day 0, 14, 28 and 42 using standard protocol. The result showed that WSF treatment increased the concentration of physicochemical properties especially for day 0 while as the time of exposure increased their concentration decreased. A significant ($p<0.05$) increase in THC, nitrate, electrical conductivity and sodium ion at day 0 across from control to 20% and a similar result at day 14 for nitrate, however as concentration increased with time a significant decrease was observed except for sodium which had little or no changes. pH decrease with time from day 0 to day 14 and maintained a steady concentration afterwards. Potassium, calcium and magnesium ion conc. decreased significantly ($p<0.05$) for each time (Day 0, 14, 28 and 42). Bacteria count decreased as exposure time increased, and increased with concentration within specific time. Bacteria count significantly decreased at day 0, increased significantly at WSF 20% for day 14 and 28 while an insignificant decrease was observed at day 42 when compared with control. This result suggests that consistent contamination with WSF could adversely alter the soil physiochemical properties and decrease soil bacteria count leaving just hydrocarbon degrading bacteria such as *Pseudomonas spp.* and *Bacillus subtilis* to thrive. Oil spillage site should be prevented or promptly remediated to maintain soil physicochemical properties and indigenous microbes essential for soil fertility and Nutrient availability to plants.

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The oil and gas industry is the major source of revenue for most countries with millions of barrels of crude oil in their reserve. This is because on a yearly bases billions of tons of crude oil are exported and processed into refined products such as fuel oils, diesel fuel, kerosene and aviation liquefied petroleum gas (Olajire, 2014; Ngene *et al.,* 2016). Technological and Anthropogenic activities that are applied in crude oil exploration includes refining and transportation for distribution to producers and consumers. Besides the useful products produced from the industry, some negative impacts are associated with the exploration process and the major consequence is environmental pollution through drilling cutting, drilling effluents, gas flaring and Oil spillage (Olajire, 2014; Ngene *et al.,* 2016). Oil spillage is the most common source of environmental pollution and it is caused by accidental leakages and bursting of old pipelines, blow out wells, sabotage and transportation (Khosravi *et al.*, 2013; Agbonifo, 2016). Apart from the physical impact on the ecosystem, the underlying effects of crude oil on ~~L~~living organism results from its toxic components. This has caused detrimental effects to the abiotic and biotic environment components. The physiochemical components of the soil influences plant nutrients and fertility and changes in these properties are indicatives of soil pollution. Oil spillage impacts the soil physiochemical properties by causing limitations in their optimal availability and a good number of research have demonstrated these changes (Abii & Nwosu, 2009; Bello and Anobeme, 2015). Crude oil, which is hydrocarbon containing complex mixture can be classified into four classes and they include saturates, aromatics, asphaltenes and resins. The most toxic component are the aromatics and they include polycyclic aromatics (PAHs) and alkyl aromatics like Benzene, toluene, ethyl benzene and xylenes (Ite *et al.*, 2013). It also contains non-hydrocarbon components like sulfur, nitrogen, oxygen and trace amount of nickel, copper, vanadium and iron (Sivansankar, 2008). However, the toxic hydrocarbons are seen to negatively impact plants (Firi Appah *et al.*, 2014), animals Odo *et al.*, 2012; Ubani *et al.*, 2012; Ordinioha and Brisibe, 2013) and humans (Aziz and Rahman,

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2010 and Ngene *et al.*, 2016) depending on the duration and concentration of exposure (Millioli *et al.*, 2009; Das and Chandran, 2011). Biological methods that involves the role of soil microbes in hydrocarbon degradation through a process called bioremediation have been found to be more effective, environmentally friendly, versatile and cheaper compared to physical and chemical methods (Margesin and Schinner, 1997; Das and Chandran, 2011). This process is based on the ability of soil microorganisms to detoxify or degrade hydrocarbon-containing soils to compounds that are less toxic and this is dependent on; the individual metabolic capability of the microorganism to remove environmental organic contaminant, creating an optimal environmental condition for biodegradative activity to be stimulated, the nature and the type and molecular composition of hydrocarbon present in the environment (Marques-Rocha e al., 2000; Mehrasbi *et al.*, 2003).

The impact of a complex hydrocarbon mixture such as Crude oil directly influences the microbial structure of the Soil. The susceptibility of hydrocarbons to microbial attack differs and can be generally ranked as follow; linear alkanes > branched alkanes > small aromatics > cyclic alkanes (Perry, 1984; Das and Chandran, 2011). Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all (Atlas and Bragg, 2009). The microorganism with degrading abilities include Bacteria, fungi and yeast with bacteria as the most effective. Studies have showed that the following bacteria strains were isolated from crude oil polluted site *Pseudomonas, Arthrobacter, Mycobacterium, Burkholderia, Sphingomonas, and Rhodococcus.* Also *Pseudomona fluorescens, Bacillus subtilis, Bacillus* sp*., Alcaligenes* sp., *Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus, P. aeruginosa, and Corynebacterium* sp. were isolated from the polluted stream which could degrade crude oil (Adebusoye *et al.*, 2007). Meanwhile *Bacillus Badius*, *Micrococcus varians*, *Corynebacterium amycolatum* and *Corynebacterium ulcerans* were isolated from contaminated soil compost (Omotayo *et al.*, 2012). Owing to the negative impact of crude oil on the living biota and the quest to provide a very effective solution, this study was carried out to investigate the impact of water soluble fraction of crude oil on soil physicochemical properties and bacteria population in contamination cases.

MATERIALS AND METHODS

Sample collection: The study was carried out in the department of pharmacological microbiology in the University of Benin with a two months period. Sandy and loamy soil was collected from capitol region (an area with much reduced vehicular disturbance) of the University of Benin, Benin City, Nigeria. The soil was left to air dry and then 500g was measured into a nursery bags.

Crude oil Fractionation: Bonny light Crude oil of American petroleum institute gravity of 37 was obtained from the Refinery and Petrochemical Company in Warri, Delta state, Nigeria. A portion was fractionated according to the method of Anderson *et al.* (1974) into water soluble and water insoluble fraction using 1:2 dilution; 400ml of crude oil and 800mls of distilled water was put in a 1 litre conical flask and constantly stirred with a magnetic stirrer for 48h. A separating funnel was then set up for 48hrs and Water soluble Fraction (WSF) was separated from Water insoluble fraction (WIF) and collected in a conical flask.

Soil contamination: Range finding test was carried out using the following concentration; 0, 2%, 5%, 10%, 20%, 30% and 50%, but 0, 2, 5, 10 and 20% were used for soil contamination with water soluble crude oil fraction for the following time intervals day 0, day 14, day 28 and day 42.

Physiochemical Analysis: The physicochemical analysis was carried out with 2 gram of control and WSF treated samples which were taken to the laboratory, air dried and sieved through a 2mm sieve and then stored in plastic bags for analysis. The pH, Total Hydrocarbon contents (THC), Nitrate, Phosphate, Electricity Conductivity, Sodium ion (Na^+), Potassium ions (K^+), calcium ion (Ca^{2+}) and Magnesium ion (Mg^{2+}). The pH of the soil was carried out according to the method of Bates (1954). All nutrient and ion was determined colorimetrically according to AOAC, (1980) and AOAC, (1984).

Bacteria Analysis: The nursery bags were purchased sterilized and enclosed in a sterilized container, glassware were treated for 2 h at 160^0C in a hot-air oven while distilled water and growth media were autoclaved for 15mins at 121^0C. Concentrated solution was prepared by mixing 1 gram soil sample suspended in 10mls of sterile water and suspension was diluted serially from 10^2 to 10^{10} and 10^6, 10^8, and 10^{10} was used in estimating aerobic heterotrophic bacteria by pour plate method in triplicates each. Nutrient agar containing 0.015% (w/v) nystatin (to inhibit fungi growth) was used for bacteria isolation and incubation was done at ambient temperature for seven days. Pure isolates of representative communities were maintained on agar slant at 4^0C. Identification of bacteria isolates was based on microscopic, cultural and biochemical characteristics with reference to

Bergey's manual of determinative bacteriology (Brown, 1939).

Statistical Analysis: The data collected was analyzed using Microsoft excel and results were represented as Mean ±Standard error (SEM). Statistical analysis was carried out using statistical package for Social Scientists (SPSS version 20.0) for comparing two parameters one tailed t-test was carried out while for three and more parameter one way Anova was done. Tukey analysis was used for significant difference set at P<0.05.

RESULT AND DISCUSSION

Water soluble fraction of crude oil can detrimentally impact on soil physiochemical properties and bacteria population and the results of this study showed changes in physiochemical parameters, microbial community and also the relevance of indigenous soil bacteria in bioremediation process. These parameters are interrelated as hydrocarbon biodegradation in soil can the determined by a number of factors such as pH, soil properties, temperature, moisture, nutrients and concentration of the contaminant.

Physicochemical Parameter: The result for the physiochemical parameters were represented for day 0, 14, 28 and 42 at 0% as control, 2, 5, 10 and 20% concentration, for total hydrocarbon content a concentration dependent increase was depicted at day 0 as concentration increased, no significant changes were observed within control, while a significant decrease was observed across day 0 to 42 at 2%, the decrease observed in the other concentrations were significant comparing day 0 and all other days presented. The pattern for pH differed slightly, a significant decrease was observed from day 0 to 14, little or no changes were seen from day 14 to day 42 for all conc. including control as shown in figure 1. Electrical conductivity increased with time for day 0 from 2% to 5%, day 14 and 28, control to 2%, 2% to 5% and then a concentration dependent decrease afterwards. However, for day 42, a noticeable decrease was observed from control to 2% and then an increase as concentration increased followed by a decrease again, all changes were below control for 42. Day 0 had the greatest electrical conductivity for all concentrations as depicted in figure 1.

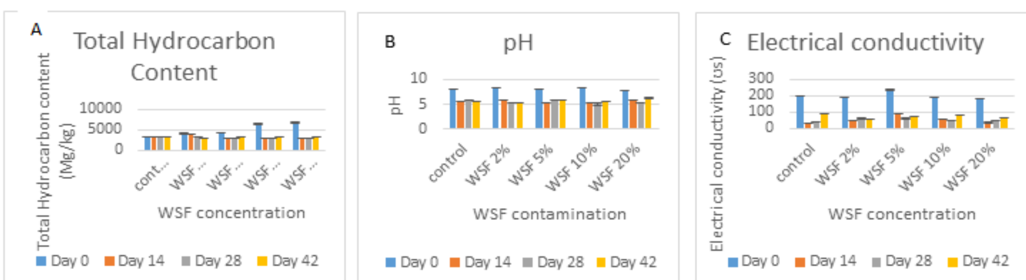

Fig 1. A. THC, B. pH and C. Electrical conductivity in WSF contaminated soil. Data represented as Mean±SEM.

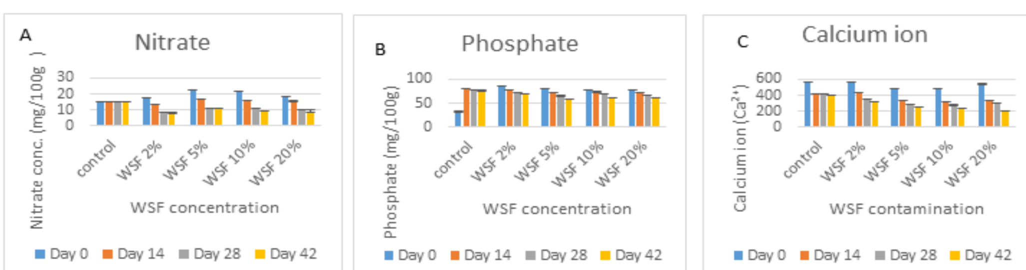

Fig 2. (A). Nitrate (B). Phosphate and (C). Calcium ion in WSF contaminated soil. Data represented as Mean±SEM.

A concentration dependent decrease was noticed for nitrate and phosphate across day 0 to 42 for all concentrations. And control for nitrate had little or no changes while control for phosphorus showed a significant increase which was noticeable from day 0 to day 14 as seen in figure 2. Calcium concentration decreased significantly in control samples from day 0 to 14 and no significant change was noticed from day14 through 42, meanwhile for all other concentrations a significant decrease was observed with time as seen in figure 2. On the other hand, sodium, potassium and magnesium ion concentrations had a similar pattern, sodium decreased with time across day 0 to 42 at each conc. and increased with concentration across from control to 20% in all the days for figure 3 graph. The result depicted as shown in figure 3 for potassium and magnesium were very similar, a drastic decrease was observed for each conc.

from day 0 to day 14, no other noticeable changes was recorded for potassium and the controls for magnesium. A time dependent decrease was revealed for all other concentrations according to figure 3.

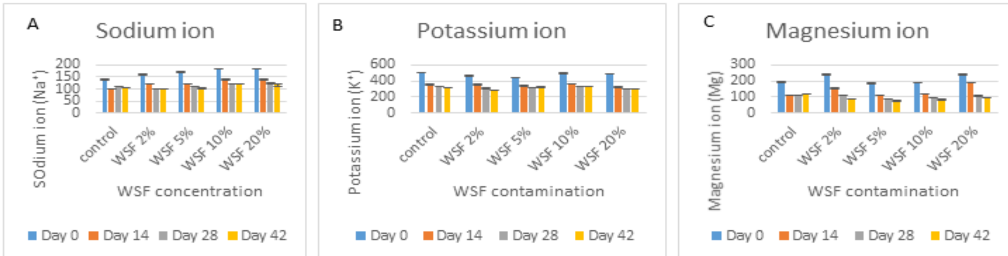

Fig 3. (A). Sodium (B). Potassium and (C). Magnesium ion in WSF contaminated soil. Data represented as Mean±SEM

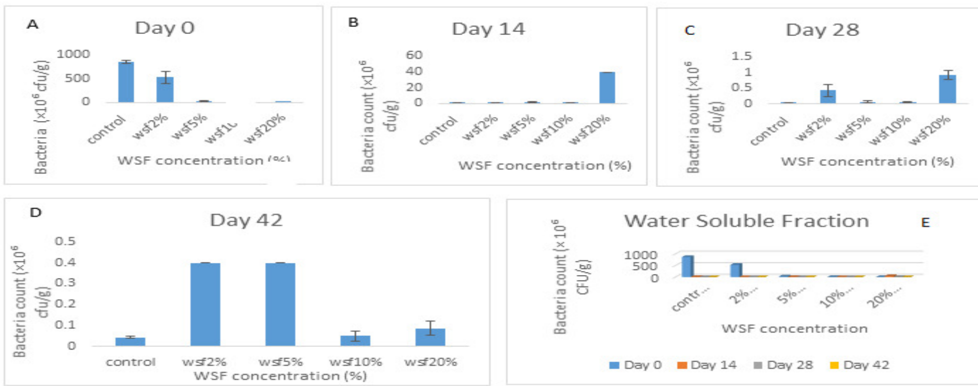

Fig 4. Bacteria count ($\times 10^6$cfu/g) in WSF contaminated soil at control, 2, 5, 10 and 20% concentration for (A) Day 0, (B). Day 14. (C) Day 28. (D). Day 42 (E). Summary of bacteria count for WSF treatment for time and concentration.

Oil spillage impacts the soil physiochemical properties by causing limitations in their optimal availability and a number of researchers have demonstrated these changes. pH and phosphate were observed to be increased in crude oil contaminated soil in the research conducted by Wang *et al.*, (2013). Similarly according to Abii and Nwosu, (2009), there were significant decrease in Ca, P, K, Mg and Cation exchange capacity and an increase in Na contents and sand fraction in crude oil polluted soil. Bello and Anobeme, (2015) also had similar reports, there was a significant decrease in Mg^{2+}, Ca^{2+}, K^+, ECEC, clay fraction, sand fraction content and silt fraction, in oil contaminated soils while a significant increase in Na^+ content, hydrocarbon content and electrical conductivity in affected soils compared to the oil free soil. Uquetan *et al.*, (2017) also investigated the effect of oil spillage on total hydrocarbons (THC) and soil physiochemical properties and reported a significant increase in THC while soil exchangeable ion such as Na^+, Ca^{2+} and K^+ and phosphate decreased significantly, suggesting that oil spillage results in depletion of available nutrient in the soil which is also in tandem to the present study. This seems to fit the Justification in the introductory section however the result from this study revealed a very similar result with previous studies on the effect of crude oil contamination on soil physicochemical properties.

Microbial parameter: The total count of heterogeneous bacteria ranged from 0.06×10^4 - 9×10^8 and the bacteria isolated from the soil samples in control and treated plants were *Pseudomonas spp, Staphylococcus aureus, Bacillus subtilis, Enterobacter spp* and *klebsiella spp* but just *Bacillus subtillis* and *Pseudomonas spp* were able to strive on day 42, a similar result was observed in the study carried out by Omotayo *et al.*, (2012) and Ekanem and Ogunjobi, (2017). In the later, *Bacillus subtillis* and *Pseudomonas spp* showed the highest hydrocarbon utilization compared to *Enterobacter spp*.

The consistent soil contamination with water soluble fraction resulted in a significant decrease with conc. for day 0, no noticeable changes and then a significant increase from 10 to 20% conc. for day 14, while a slightly similar result was observed for day 28. On the contrary at day 42 significant increase was noticed from control to 2% and sig. decrease from 5% to 10%, the increase from 10 to 20% was not significant. This result presented in Table 4 showed that the highest bacteria count was recorded for control samples (without WSF treatment) of day 0, however bacteria count decreased especially with time, day 0>14>28>42. This results suggests that WSF contamination reduced bacteria count as concentration increases and at some point adaptation may have taken place and so increase was observed with increasing concentration (day 14 and 28) however, as concentration increased with a longer time bacteria count decreased (day 42), this may imply that WSF became very toxic to the soil bacteria and so only the crude oil degraders of very high potentials (*Bacillus spp. and Pseudomonas spp.*) were able to survive. A similar result have been observed in previous studies (Sadoun *et al.*, 2008; Ekanem & Ogunjobi *et al.*, 2017 and Inieke *et al.*, 2018).

Conclusion: The study suggest that WSF contamination impacted soil fertility by reducing the levels of nutrient available to the soil and also bacteria count with consistent and prolong contamination, because it became toxic to the soil bacteria community, leaving to thrive the high potential hydrocarbon degraders. Therefore remediation of crude oil contaminated site should be swiftly given attention to prevent its detrimental effect on soil physiochemical properties (fertility) and microbial community essential for soil fertility and nutrient availability to plants.

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