Cowpea Induced Physicochemical and Biological Rhizosphere Changes in Hydrocarbon Contaminated Soil

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Abstract

Purpose

To understand the influence of cowpea on its rhizosphere physicochemical and biological conditions.

Methods

Pristine soil samples were contaminated with Bonny-Light crude oil and viable seeds of cowpea were planted to establish rhizosphere soil. Cowpea root exudates were collected and characterized while soil metabolic activities, physicochemical properties and rhizosphere effect were monitored following plant emergence.

Results

Cowpea root exudates were composed of organic acids, phenolics, carbohydrates and hydrocarbons. High rate of soil respiration and microbial biomass carbon were observed in the contaminated rhizosphere reaching its peak on 12th week (70.56 $mgCO_2g^{-1}day^{-1}$) and 10th week (23.18mg/Kg) respectively. Lower rates of soil respirations and microbial biomass carbon were observed in contaminated (10.28 $mgCO_2g^{-1}day^{-1}$; 1.24 mg/Kg) and uncontaminated (0.23 $mgCO_2g^{-1}day^{-1}$; 0.37 mg/Kg) non-rhizosphere control soils respectively. The metabolic properties were positively correlated with soil organic matter contents and microbial size ($r = 0.98$; $p < 0.05$). There was considerable improvement in soil physicochemical properties in the cowpea rhizosphere as compared to non-rhizosphere soil Microbial populations were generally improved with positive rhizosphere effect values (>1) presumably due to the presence of compounds in exudates that promote microbial growth.

Conclusion

The results highlighted the influence of cowpea on its rhizosphere conditions which is a good indication for its ability to promote plant growth and environmental cleanup. Therefore, there is the need to further understand the microbial community dynamics in cowpea rhizosphere using culture-independent techniques.

Introduction

Soil serves as an interface for sustaining life on Earth providing myriads functions to ecosystem (Ragnarsdóttir and Banwart 2015). The inability of human to properly manage natural resources have adversely affected soil quality (Bünemann et al. 2018). Soil contamination with persistent organic pollutants is a major problem and one of the leading causes of environmentally induced displacements (Terminski 2012). Although soil contamination has been described as a hidden reality (Rodríguez-Eugenio

et al. 2018), its adverse effects are gradually manifesting especially that contaminations above critical threshold are incessantly emerging. Soil contamination affect food, water and air quality in addition to land degradation (Food and Agriculture Organization; FAO 2020). Soil pollution significantly reduces food security, not only by reducing crop yields due to toxic levels of contaminants, but also by making crops produced from polluted soils unsafe for animal and human consumptions. Increased soil contamination puts food safety and food security at risk (FAO 2018). It has been estimated that over 70% of soil contaminations are due to petroleum hydrocarbons (Marinescu et al. 2001) which result from oil exploration, transportation and consumption (Riazi 2021).

Spillage of petroleum hydrocarbons into the soil can alter the natural soil environment and causes minor and major biotic community changes (Khan et al. 2018). Due to the fact that petroleum is rich in carbon and trace amount of nitrogenous compounds, it can change the composition and structure of soil organic matter and impact the C/N, C/P, salinity, pH and conductivity of soil (Li et al. 2009). Similarly, its low density, higher viscosity and low emulsification ability, make it easily adsorbed on soil surface, affecting the permeability and porosity of soil (Wang et al. 2018). The presence of Nickel and Vanadium in crude petroleum increases the risk of pollution due to heavy metals (Efsun et al. 2015). Decrease in soil water retention capacity at high potential as a result of oil succeeding water in the competition for pore spaces and reduction in water film thickness around macro-aggregates, are also identified as effects of oil in soil environment (Udom et al. 2011).

Due to increasing interest in maintaining soil health and sustainability, there has been tremendous efforts towards cleaning polluted environments in the last few decades. Different types of technologies have been in use since the occurrence of major world oil spills (Michel and Fingas 2016; Riazi 2021). Technologies involving physical, chemical, thermal and biological processes are widely used in the treatment of soil contamination (de Souza et al. 2013; Wang et al. 2017). Phytoremediation which uses the ability of plants to thrive in polluted soil and remove contaminants have been deployed in treating different environmental contaminants including organic (Atagana and Anyasi 2017; Hussain et al. 2018), inorganic (Zhuang et al. 2007; Placek et al. 2016) and radionuclear residues (Singh et al. 2009; Sharma et al. 2015; Yan et al. 2021). Different plants serve as naturally occurring systems capable of containing, destroying, or extracting contaminants from an environment (Frick et al. 1996). In remediation of hydrocarbon contaminants, rhizodegradation is the major phytoremediation mechanism employed. Rhizodegradation which was defined as degradation of contaminants in the rhizosphere (Gerhardt et al. 2009), is a microbe-assisted phytoremediation mechanism. The process employs plant-microbe synergism in which microbes degrade contaminants whereas, the plants boost microbial activity and population through nutrients supplementation within the root zone (Correa-Garcıa et al. 2018).

The ability of plants to grow in contaminated media varies from one species to another, and this variation is key to petroleum hydrocarbons remediation (Alarcón et al. 2019). Weather conditions, most especially precipitation is among the major determinants of the success of remediation (Abdallah et al. 2020), since plants require water as a medium of transporting growth requirements and also, most spills are in large scale beyond irrigation (Michel and Fingas 2016). In addition, edaphic factors have a large impact on the

entire plant growth and performance (Interstate Technology & Regulatory Council; ITRC 2009). Soil composition, conditions (e.g. pH, conductivity, porosity, and nutrients) and biomes are the cornerstone for plant growth and consequently affect phytoremediation (Chakravarty et al. 2017). Soil environment is highly dynamic due to the influx and outflow of organic and inorganic substances. There is strong interconnectivity between factors dictating soil physicochemical status; where by, a slight shift in one factor may cause drastic changes in one or many other factors that may have negative consequences on plants and organisms living in the soil (Wang et al. 2013).

Petroleum hydrocarbon contaminants cause serious soil perturbations. The primary contributing factor towards positive changes in soil during reclamation is root exudation within the rhizosphere (Rohrbacher and St-Arnaud 2016). Rhizosphere although poorly defined, has an important role in the phytoremediation of organic contaminants via plant-microbe interactions (Ismail et al. 2021). Apart from nutrient addition that increase the microbial population and activity, plant root exudates also increase hydrocarbon bioavailability through chemical and mechanical processes, promote degradation by releasing contaminant analogs and enhance contaminants removal by secreting compounds that serve as primary substrates for co-metabolism (Farrell and Germinda, 2002). Presence of exudates in the soil induces physicochemical changes that affect plant growth and microbial activity (Wang et al. 2013; Wang et al. 2017; Baumert et al. 2018). Some changes happen in favor of microbial communities which in turn promote plant growth through the alleviation of contaminants phytotoxicity (Siciliano and Germida 1998). On the other hand, physiochemical properties of soils have a direct influence on specific microbes and plant root exudates (Dumbrell et al. 2010). Unless plants and microbes are able to adapt to the soil extremes or optimum soil conditions are attained, the remediation process may be halted, poorly implemented or take longer time than necessary (Ho et al. 2017; Fortin-Faubert et al. 2021).

Despite the rapid growth of phytotechnologies in the last three decades, information on the soil dynamics affecting plant-microbe interaction within the rhizosphere is limited. There are few studies that highlighted the influence of root exudates on soil physicochemical properties *vis a vis* hydrocarbon phytoremediation. Investigations in this context have revealed astonishing findings that need to be expanded in all ramifications with a view to elucidating the interplay of various components in plant rhizosphere. As root exudation is significantly dependent on plant type, age, and induced-abiotic stress (Liu et al. 2019; Hoang et al. 2021), investigation into exudates composition of different plant species and its influence on soil characteristics is desirable. Although some previous studies have indicated the phytoremediation potentials of cowpea (*Vigna unguiculata* Walp.), the effects of its exudates on soil physicochemical and microbial conditions still remain sketchy. Recently, Mohan et al. (2020) characterized cowpea root exudates and demonstrated their potential use in the control of plant-nematodes via the enhancement of hyper-parasitic bacterial population. Although this finding, among others (Jidere et al. 2012; Ismail et al. 2019) have demonstrated the role of the exudates in influencing microbial population in the rhizosphere, their role in shaping the soil conditions have not been exploited. It is against this backdrop, the present study investigated the induced-rhizosphere physicochemical and biological changes in order to unravel the interplay of root exudates and soil conditions in shaping the microbial populations through rhizosphere effect.

Materials And Methods

Sampling

Cowpea seeds (IT07K-318-33 cultivar) were collected from the Institute for Agricultural Research (IAR) Ahmadu Bello University Zaria. The seeds were identified at the Usmanu Danfodiyo University Sokoto (UDUS) herbarium. Bonny light crude oil was obtained from Kaduna Refinery and Petrochemical Company, Kaduna. Pristine soil samples were collected from the Botanical garden UDUS.

Experimental procedure

Setup

Pristine soil samples (6000g) placed in 6 plastic pots were prepared and contaminated with 450ml Bonny Light crude oil. Another 6 plastic pots containing the same amount of soil were also prepared but left uncontaminated. The setup was left for two weeks undisturbed to enable acclimatization of soil conditions. Following that, three (3) pots from both contaminated and uncontaminated soil were randomly separated with a view to establishing a rhizosphere soil. The remaining 3 pots from each group were left as controls. To establish rhizosphere conditions in the soil, cowpea seeds were planted in both contaminated (CR) and uncontaminated (UR) soil. The pots were irrigated every other day with equal volume of distilled water to maintain proper cowpea growth over a period of 12 weeks. The unplanted pots were left as non-rhizosphere contaminated (CNR) and uncontaminated (UNR) controls. Rhizosphere and non-rhizosphere soil samples were collected at 2 weeks interval for soil physicochemical, metabolic and microbial analysis.

Physicochemical analysis of soil

Determination of soil particles size

Soil particle size was determined using the method of IITA (1979). Air dried soil was sieved and 51 grams was transferred into 1liter shake mix cup. Fifty (50) milliliters of 5% sodium hexametaphosphate was added followed by 100ml of distilled water. The soil suspension was stirred thoroughly using glass stirrer for 15 minutes and transferred into a cylinder containing hydrometer (QC2300, HG. Japan). Distilled water was added to the lower blue line of the cylinder. The volume changed to 1130ml and the hydrometer was removed. The top of the cylinder was covered with hand and inverted several times until all soil was in suspension. The cylinder was placed on a flat surface and time was noted.

Hydrometer was placed in the suspension and the first reading (H_1) was noted immediately after 40 seconds. Subsequently, the temperature (T_1) was recorded after the hydrometer was removed. The suspension was allowed to stand for 3 hours and the second reading was taken for hydrometer (H_2) and the temperature (T_2).

The percentage sand, clay and silt were calculated thus:

Sand = 100.0-[H_1+0.3(T_1-20)-2.0]2

Clay = H_2+0.3(T_2-20)-2.0

Silt = 100.0-(% sand + % clay).

Determination of soil pH and temperature

The soil pH was determined according to the methods reported by Nazir et al. (2015). Sample (20g) of air-dried soil was sieved and placed in 50 mL capacity beaker. To it, a 20mL of distilled water was added and allowed to stand for 30 mins, while occasionally stirring with a glass rod. The pH meter was calibrated with a buffer of pH 7.0 before use. The electrode of the pH meter was inserted into the partly settled suspension, and the reading on the pH meter would be noted and recorded accordingly. The temperature of the soil was measured immediately after taking soil sample using a garden thermometer (Smart Choice, UK).

Determination of moisture content

An empty crucible was weighed (W_0), and 2 g of soil was added and weighed again (W_1). The soil were then dried in hot air oven at 105°C for 24 h until constant weight was achieved (W_2) (Prasad and Bhaskara Rao 2011). Both the crucible and the dried sample were weighed again. The moisture content was calculated as:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Determination of total organic carbon

The soil sample was ground and sieved. One gram of soil samples was weighed in duplicates and transferred into 250 mL Erlenmeyer flasks. Aliquots (10 ml) of potassium dichromate solution and 20 mL of concentrated sulphuric acid were added, and the contents of the flasks shaken gently until properly mixed. One hundred millilitres of distilled water was added and allowed to stand for 30 min. This was followed by adding 3 drops of an indicator and titrating against 0.5N ferrous sulphate solution (Mitchell and Gu 2010). The percentage carbon was calculated according to the formula:

$$\% \text{ Organic Carbon} = (\text{Me } K_2Cr_2O_7 - \text{Me } FeSO_4) / (\text{Wet soil} - \text{dry soil}) \times 100$$

Where Me = mole equivalent.

Determination of total nitrogen

Five grams of soil samples was weighed into a dry 500 mL macro-Kjeldahl flask, and 20 mL of distilled water were added. The content was swirled for few minutes and allowed to stand for 30 min. One tablet of mercury catalyst and 10 g of K_2SO_4 were added, and 30 mL of concentrated H_2SO_4 were also added through an automated pipette. The content of the flask were heated gently in the digestion stand. After

cooling, 100 mL distilled water was added and transferred to another clean macro-Kjeldahl (750 mL) apparatus and the sand residue was washed four times with 50 ml of distilled water. All the contents were transferred into the same flask. After that 50 ml, H_3BO_3 indicator solution was added into 500 mL Erlenmeyer flask which was placed under the condenser of distillation apparatus and 150 mL of 10N NaOH was poured. This was followed by distillation, and the condenser remained cooled (30°C) to allow sufficient cold water to flow and also minimize frothing. Ammonium was determined in the distillate by treating with 0.01N standard H_2SO_4 using a 25 mL burette graduated at 0.1 mL interval. The colour changed at the end point from green to pink (Mitchell and Gu 2010). Percentage nitrogen was calculated using the formula:

% Nitrogen = $(N \times 0.014 \times Vd \times 10) / (A \times \text{Wt of sample}) \times 100$

Where: N = normality of acid, Vd = volume of the digest, A = aliquot of the digest.

Determination of phosphorus

Aliquot (2g) of soil and one teaspoon of carbon black were added to 40 ml of extracting solution in 125 mL Erlenmeyer flask. The flask was shaken for 30 min and filtered through the filter paper. More carbon was added to obtain a clear filtrate, after that 2mL of the clear supernatant was dispensed in a 20 ml test tube, and 5 mL of distilled water plus 2 mL of ammonium molybdate (NH_4 $6MO_7O_{24}.4H_2O$) were added. The contents were mixed properly, and 1 mL of dilute stannous chloride ($SnCL_2.2H_2O$) solution was added and mixed again. After 5 min, percentage absorbance on the spectrophotometer at 660 nm wavelength was measured, and the phosphorous content was calculated using the formula (Mitchell and Gu, 2010):

P (mg/Kg) = $(\text{Reading} \times 0.61 \times \text{dilution factor}) / (\text{Atomic weight of phosphorus}).$

Determination of electrical conductivity

To determine soil electrical conductivity (EC), 25 g of air dried soil sample was placed into a 250 ml beaker. Distilled water was added slowly drop by drop uniformly over the entire soil surface until the soil appears to have been wetted. A stainless steel spatula was used to form a homogeneous soil saturated paste. The beaker was then covered with a petri-dish. 50ml distilled water was added and shaken for 1hour. Aliquot 40ml of the diluted extract was placed into 100ml beaker and the conductivity meter was inserted and the electrical conductivity of the soil recorded in $uScm^{-1}$.

Collection of root exudates

Root exudates were collected according to the description of Da Silva et al. (2014) with modifications. A 14 days old cowpea plantlets were removed from the pots and their roots were immersed in glass tubes filled with 50 mL of 0.01 molar KOH for 5 min to remove organic anions adhering to the root surfaces. It was then thoroughly washed with tap water followed by a final rinse in distilled water. Complete root systems of the plantlets from a single pot were inserted in a conical flask filled with 80 mL of methanol in which the

root exudates were collected. After 24 hr, the suspensions containing root exudates were collected and filtered through double layer whatman filter paper to remove root detritus and microbial cells.

Analysis of root exudates

The root exudates suspensions were analyzed using 5977B GC-MS (Agilent Technologies USA) coupled with HP-5MS column (30 m × 0.25 mm × 0.25 mm, Agilent Co., United States) connected to a triple axis HED-EM 5975C mass spectrometer (Agilent Co., United States) and nitrogen as the carrier gas. Other operating conditions are the same with Mohan et al. (2020). Compound identification was based on comparisons of mass spectra with the NIST library database (http://www.nist.gov/srd/nist1a.cfm), published spectra, and real standards.

Soil metabolic activities

Determination of soil respiration

Respiration of indigenous microorganism was determined as CO_2 evolution. Carbon dioxide (CO_2) was determined by adopting the method of Hegazy et al. (2014). Using 2g of soil sample from each treatment, it was transferred into a well labeled plastic vial. The plastic vial was placed in closed 1 litre glass jar. A glass vial containing 10 ml 0.2 N NaOH was placed in each jar to trap CO_2 resulting from substrate mineralization. The NaOH trap was allowed to stay for one hour. Ten millilitres of 0.5 N $BaCl_2$ was added to the NaOH trap and the amount of CO_2 produced by each treatment was determined by titrating with 0.1 N HCl and the values expressed as mg CO_2 $g^{-1}h^{-1}$ (Moebius-Clune 2016).

Determination of microbial biomass carbon (MBC)

Microbial biomass carbon was determined using fumigation extraction procedure (Vance et al. 1987). Ten gram of soil samples were sterilized in 250 ml flask and later fumigated with ethanol free chloroform for 24 hrs. Then, both fumigated soil and un-fumigated soil samples were extracted for 45 mins by 50 ml of 0.5 M K_2SO_4 and filtered using Whatman no. 1 filter paper. Soil microbial biomass carbon was estimated using the relationship:

Biomass carbon = EC/KEC

Where:

EC is (organic carbon extracted from fumigated soil) minus (organic carbon extracted from non-fumigated soil)

KEC = 0.45 (microbial extraction efficiency) (Wu et al. 1996).

Evaluation of rhizosphere effect values (RE)

The number of total heterotrophic bacteria and fungi, and hydrocarbon utilizing bacteria and fungi in the samples were estimated from the number of colonies formed on nutrient agar, sabouraud dextrose agar and oil agar plates using a colony counter and expressed as CFU/g. Data obtained from enumeration of cultivable bacteria and fungi were used to calculate the RE of cowpea on soil microbial communities within the rhizosphere using the following relation.

$$\text{RE: } \frac{\text{Microbial population in the rhizosphere soil}}{\text{Microbial population in non-rhizosphere soil}}$$

Statistical analysis

Data generated were subjected to appropriate statistical analysis using GraphPad Prism version 9.1.0 at 95% level of significance.

Results

Initial soil condition

Soil physicochemical conditions were determined immediately after sampling prior to crude oil contamination. The results showed that, the soil was a sandy soil made up of 89.4% sand, 8.6% silt and 2.0% clay particles. The pH of the soil was slightly acidic (6.8 ± 0.60) at 27℃ and its electrical conductivity and cation exchange capacity were determined to be 167.0 ± 0.07 µs/cm and 12.55 ± 0.10 mol/kg respectively. Organic matter (0.45 ± 0.12%) and Carbon (0.22 ± 0.50%) contents of the soil were low just as the Nitrogen (0.04 ± 0.10%) and other ions like Phosphorus (0.61 ± 0.05 ppm), Calcium (0.33 ± 0.01 mg/Kg), Magnesium (0.43 ± 0.20 mg/Kg), Potassium (0.55 ± 0.8 mg/Kg) and Sodium (0.36 ± 0.03 mg/Kg) were.

Root exudates

Organic compounds released from roots as exudates were summarized and are presented in Table I (supplementary). Gas chromatography - mass spectroscopic analysis showed that the exudates were primarily made up of hydrocarbons, organic acids, phenolics, carbohydrates and N-containing organic molecules. Other compounds are coumarin, thiazepine and terpenoids. Figure 1 shows the percentage occurrence of the various groups of the organic compounds identified. The results also showed that plants grown in uncontaminated soil (UR) had abundant (n = 57) but less diverse compounds than contaminated rhizosphere (CR) which is more diverse but with fewer compounds (n = 51). Majority of the compounds in UR and CR were hydrocarbons consisting both saturated and unsaturated compounds. Organic acids and phenolics were next in abundance but terpenoids and thiazepines occurred only in CR. Statistical analysis revealed that the composition of the root exudates in UR and CR are not distinctly different at 99% level of significance. Using equivalence test, Figure 2 highlighted that the compounds in the two plants were not different and also not equivalent.

Changes in soil metabolic activities

Metabolic activities of the rhizosphere and non-rhizosphere soil samples was investigated. The results showed that soil respiration was more pronounced in rhizosphere soil than non-rhizosphere soil. Highest activity was observed in CR soil especially from week 8 through week 12 at the rate of 60.5 $mgCO_2g^{-1}day^{-1}$, 69.99 $mgCO_2g^{-1}day^{-1}$ and 70.56 $mgCO_2g^{-1}day^{-1}$ respectively. In contrast to the rhizosphere soil, soil respiration in the non-rhizosphere soil is generally at a lower rate. Respiration in uncontaminated non-rhizosphere (UNR) soil was averagely 0.22 $mgCO_2g^{-1}day^{-1}$, thus making the lowest throughout the study. In the contaminated non-rhizosphere, the rate was observed to increase with increasing hydrocarbon concentration but remained almost constant throughout the study period as shown in Figure 3.

Results for microbial biomass carbon (MBC) is presented in Figure 4. There was gradual increase in the MBC from week 2 through week 10 from where a drastic decline in biomass was observed. Soil in CR contained more biomass carbon (22.2 mg/Kg) due to microbial activities after 12 weeks period. However, lower MBC were observed in all non-rhizosphere soils irrespective of hydrocarbon contamination.

Changes in physicochemical parameters

Changes in soil physicochemical properties due to plant growth was investigated. Soil pH, temperature, and moisture were stable throughout the study period. The pH was slightly acidic to neutral in the range of 6.00 and 7.10 as shown in Table 1a. A more acidic condition (5.80) was however observed in CNR soil in the 4th week of the experiment which differed significantly from that of the UR soil ($p > 0.05$). It was also observed that the soil temperature of CNR was significantly higher than that of the rhizosphere especially after plant emergence. The temperature of the rhizosphere soil ranged between 27°C and 37°C (Table 1a). The soil moisture contents was generally low ranging from 2.0% to 3.5% with higher values recorded in the first and 8th week (Table 1b). Although CNR was observed to have slightly lower moisture, no significant difference was observed. The soil temperature and pH did not significantly differ from those during sampling and pre-planting periods.

For soil electrical conductivity (Table 1b), uncontaminated soils had higher conductivity than the contaminated soil during the pre-planting period. Soil samples from UR and UNR had 168.5 µS/cm and 165.9 µS/cm respectively whereas, those from CR and CNR had 62.7 µS/cm and 45.6 µS/cm respectively. Highest conductivity values were observed in the 12th weeks for all treatments. Statistical analysis using ANOVA showed that there was significant difference in electrical conductivity between contaminated and uncontaminated soils ($p > 0.05$).

Table 2a presents the organic carbon contained in the soil samples. Carbon content of the soil was higher in contaminated than uncontaminated soils in the earlier stages of the experiment where significant differences were observed. Higher carbon contents were observed in CNR (1.97%) and CR (1.08%) prior to planting, whereas UR (0.28%) and UNR (0.34%) had lower contents respectively during the same period. Highest percentage of carbon were recorded after four weeks of plant growth except in UR and UNR which occurred after 8 weeks. Furthermore, increase in organic matter contents (Table 2a) of the soil was related to increase in carbon contents, in which highest values were recorded in the 4th and 8th weeks of the experiment. Soil in UNR was poor in organic matter and was observed to differ significantly from all other

treatments including UR at 95% confidence limit. In the 12th week of the experiment, CR (2.22%) recorded the highest organic matter contents respectively among the rhizosphere soils.

The results of soil Nitrogen (N) and Phosphorus (P) contents are presented in Table 2b. The N and P contents of the soil were generally low especially before planting which did not differ from that of the soil samples before contamination. However, the N and P in the soils gradually increased through the 8th week except in UNR and CNR where lower quantities were observed. In the rhizosphere soil, N and P contents ranged from 0.04% to 0.28% and 0.50 ppm and 2.35 ppm respectively. Averagely, CR had highest N whereas UR had highest P contents. Analysis of variance showed that the N and P contents of different soil treatments were not statistically significant ($p > 0.05$).

Results in Table 3a shows the Calcium and Magnesium contents of the soil samples. Calcium (Ca) contents of the soil were higher in contaminated soil than uncontaminated soil. Soil samples from CR had higher Ca contents while lowest Ca content was observed in UNR which was shown to differ significantly from other treatments ($p < 0.05$). Similarly, soil Magnesium (Mg) contents were higher in soil with hydrocarbon contamination. Uncontaminated soil recorded lower Mg contents especially in the rhizosphere (UR). However, the Mg contents decreased overtime and least (0.20 ppm) Mg content was observed in UR after 12 week period. UNR maintained highest Mg contents throughout the study period (Table 3a). There was no significant difference in Mg contents between the treatments except UN and CNR in week 12 ($p > 0.05$).

Moreover, soil Potassium (K) and Sodium (Na) contents were evaluated and the result presented in Table 3b. The K and Na contents of the soil samples from CNR and UR was the highest and lowest respectively throughout the study period. Slight changes in these contents were observed in each treatment till the end of the experiment. Potassium contents of UNR and UR differ significantly with other treatments in the pre-planting and four weeks of the experiment respectively. However, Na contents of the soil did not significantly differ ($p > 0.05$). Results for cation exchange capacity (CEC) show that it was higher in contaminated than uncontaminated soil and increased with increasing hydrocarbon concentration (Table 4). Uncontaminated non-rhizosphere soil had lowest CEC contents and was observed to differ significantly ($p < 0.05$) from all the treatments. High CEC values were observed 4 weeks after plant emergence with CR having the highest value (14.66 mol/Kg).

Table 1a: Changes in soil pH and Temperature of cowpea rhizosphere

Treatment	pH				Temperature (°C)			
	PRE	M1	M2	M3	PRE	M1	M2	M3
UR	6.87 ± 0.2	6.73 ± 0.01	6.66 ± 0.21	6.44 ± 0.12	32 ± 0.51	28 ± 0.23	28 ± 0.12	29 ± 0.25
UN	6.91 ±0.30	7.00 ± 0.55	6.80 ± 0.12	6.90 ± 1.10	30 ± 0.11	28 ± 0.25	28 ± 0.50	27 ± 0.11
CR	6.80 ± 1.01	6.40 ± 0.60	7.00 ± 0.33	6.90 ± 0.10	32 ± 0.01	28 ± 0.30	28 ± 0.42	29 ± 0.6
CNR	6.52 ± 0.01	6.21 ± 0.33	5.80 ± 0.22*	6.00 ± 0.50	32 ± 0.32	37 ± 0.25*	35 ± 0.12*	36 ± 0.23*

* The value is significantly different from others in a particular column ($p \leq 0.05$)

[a] Values with the same superscript in a column are significantly different ($p \leq 0.05$)

UR: Uncontaminated rhizosphere (control for plant growth); UNR: Uncontaminated non-rhizosphere; CNR: Contaminated non-rhizosphere; CR: Contaminated rhizosphere. RE: Pre-planting; W4: four weeks after planting; W8: 8 weeks after planting; W12: Twelve weeks after planting

Table 1b: Changes in soil moisture and Electrical conductivity of cowpea rhizosphere

Treatment	Moisture (%)				Electric conductivity (µ/mg)			
	PRE	W4	W8	W12	PRE	W4	W8	W12
UR	2.5 ± 0.22	3.5 ± 0.11	3.0 ± 0.50	2.5 ± 0.12	168.5 ± 2.00	177.2 ± 3.33	235.2 ± 1.22[a]	288.0 ± 0.22
UN	2.5 ± 0.10	2.5 ± 0.22	2.3 ± 0.12	2.5 ± 0.50	165.9 ± 3.00	228.8 ± 0.50[a]	208.1 ± 1.00	281.6 ± 0.30
CR	2.5 ± 0.33	2.5 ± 0.11	3.0 ± 0.13	2.5 ± 0.10	62.7 ± 0.20	143.2 ± 0.22	166.0 ± 1.33	186.0 ± 2.00
CNR	2.0 ± 0.12	2.3 ± 0.50	2.0 ± 0.22	2.1 ± 0.25	45.6 ± 1.33	67.3 ± 0.50[a]	89.5 ± 1.22[a]	98.8 ± 0.122*

* The value is significantly different from others in a particular column ($p \leq 0.05$)

[a] Values with the same superscript in a column are significantly different ($p \leq 0.05$)

UR: Uncontaminated rhizosphere (control for plant growth); UNR: Uncontaminated non-rhizosphere; CNR: Contaminated non-rhizosphere; CR: Contaminated rhizosphere. RE: Pre-planting; W4: four weeks after planting; W8: 8 weeks after planting; W12: Twelve weeks after planting

Table 2a: Soil nutrient contents (Carbon and organic matter) of cowpea rhizosphere

	Carbon contents (%)				Organic matter (%)			
Treatment	PRE	W4	W8	W12	PRE	W4	W8	W12
UR	0.28 ± 0.21[a]	0.36 ± 0.12 [a]	1.34 ± 0.02	1.27 ± 0.20	0.46 ± 0.22	1.57 ± 01.2	2.11 ± 0.12	2.10 ± 0.22
UN	0.34 ± 0.50[b]	0.40 ± 0.11[b]	0.42 ± 0.33	0.39 ± 1.20	0.66 ± 0.02	0.68 ± 0.15*	0.65 ± 1.12*	0.67 ± 0.01*
CR	1.08 ± 0.22	1.17 ± 0.12	1.12 ± 0.33	0.86 ± 0.22	1.862 ± 0.10	2.31 ± 0.03	2.31 ± 1.00	2.22 ± 0.22
CNR	1.97 ± 0.01	1.87 ± 0.33[b]	1.19 ± 0.50	0.88 ± 0.05	2.20 ± .22	2.18 ± 0.10	2.19 ± 1.33	2.01 ± 0.22

* The value is significantly different from others in a particular column ($p \leq 0.05$)

[a] Values with the same superscript in a column are significantly different ($p \leq 0.05$)

UR: Uncontaminated rhizosphere (control for plant growth); UNR: Uncontaminated non-rhizosphere; CNR: Contaminated non-rhizosphere; CR: Contaminated rhizosphere. RE: Pre-planting; W4: four weeks after planting; W8: 8 weeks after planting; W12: Twelve weeks after planting

Table 2b: Soil nutrient contents (Nitrogen and Phosphorus) of cowpea rhizosphere

Treatment	Nitrogen contents (%)				Phosphorus (ppm)			
	PRE	W4	W8	W12	PRE	W4	W8	W12
UR	0.04 ± 0.12	0.19 ± 0.22	0.28 ± 0.22	0.51 ± 0.20	0.51 ± 0.12	0.65 ± 0.12	0.66 ± 0.12	0.69 ± 0.20
UN	0.04 ± 0.01	0.05 ± 0.01*	0.04 ± 0.01*	0.04 ± 0.01*	0.54 ± 0.10	0.55 ± 0.01	0.48 ± 0.02	0.45 ± 0.01
CR	0.06 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.67 ± 0.20	0.50 ± 0.01	0.57 ± 0.002	0.71 ± 0.01	0.50 ± 0.03
CNR	0.06 ± 0.01	0.03 ± 0.01*	0.05 ± 0.01*	0.09 ± 0.01*	0.51 ± 0.05	0.49 ± 0.01	0.45 ± 012	0.41 ± 0.12

* The value is significantly different from others in a particular column ($p \leq 0.05$)

[a] Values with the same superscript in a column are significantly different ($p \leq 0.05$)

UR: Uncontaminated rhizosphere; UNR: Uncontaminated non-rhizosphere; CNR: Contaminated non-rhizosphere; CR: Contaminated rhizosphere. RE: Pre-planting; W4: four weeks after planting; W8: 8 weeks after planting; W12: Twelve weeks after planting

Table 3a: Soil mineral contents (Calcium and Magnesium) of cowpea rhizosphere

Treatment	Calcium (mol/Kg)				Magnesium (mol/Kg)			
	PRE	W4	W8	W12	PRE	W4	W8	W12
UR	0.35 ± 0.01	0.56 ± 0.02	1.34 ± 0.01	1.37 ± 0.10[a]	0.40 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.2 ± 0.02[a]
UNR	0.34 ± 0.02	0.30[a] ± 0.01[b]	0.32 ± 0.01[a]	0.33 ± 0.02[a]	0.40 ± 0.10	0.40 ± 0.02	0.38 ± 0.01	0.34 ± 0.01
CR	0.88 ± 0.10	1.12 ± 0.02[a]	1.11 ± 0.10	1.06 ± 0.10	1.15 ± 0.02	1.13 ± 0.01	0.63 ± 0.10	0.36 ± 0.01
CNR	0.97 ± 0.10	0.96 ± 0.10	0.99 ± 0.02	0.89 ± 0.01	1.02 ± 0.10	0.93 ± 0.02	0.85 ± 0.02	0.90 ± 0.10[a]

The value is significantly different from others in a particular column ($p \leq 0.05$)

[a] Values with the same superscript in a column are significantly different ($p \leq 0.05$)

UR: Uncontaminated rhizosphere; UNR: Uncontaminated non-rhizosphere; CNR: Contaminated non-rhizosphere; CR: Contaminated rhizosphere. RE: Pre-planting; W4: four weeks after planting; W8: 8 weeks after planting; W12: Twelve weeks after planting

Table 3b: Soil mineral contents (Potassium and sodium) of cowpea rhizosphere

	Potassium (mol/Kg)				Sodium (mol/Kg)			
Treatment	PRE	W4	W8	W12	PRE	W4	W8	W12
UR	0.56 ± 0.22	0.35 ± 0.22*	0.45 ± 0.12	0.41 ± 0.20	0.30 ± 0.22	0.50 ± 0.12	0.41 ± 0.22	0.31 ± 0.22
UNR	0.55 ± 1.2*	0.48 ± 1.22	0.50 ± 0.22	0.53 ± 0.30	0.30 ± 1.22	0.30 ± 0.33	0.31 ± 1.12	0.31 ± 1.50
CR	1.15 ± 2.0	1.15 ± 1.50	1.17 ± 0.33	1.01 ± 1.50	0.65 ± 2.00	0.61 ± 1.20	0.63 ± 0.50	0.62 ± 1.22
CNR	1.51 ± 0.33	1.59 ± 1.12	1.61 ± 0.50	1.51 ± 1.22	0.65 ± 0.30	0.62 ± 0.42	0.59 ± 1.20	0.61 ± 0.02

* The value is significantly different from others in a particular column ($p \leq 0.05$)

[a] Values with the same superscript in a column are significantly different ($p \leq 0.05$)

UR: Uncontaminated rhizosphere; UNR: Uncontaminated non-rhizosphere; CNR: Contaminated non-rhizosphere; CR: Contaminated rhizosphere. RE: Pre-planting; W4: four weeks after planting; W8: 8 weeks after planting; W12: Twelve weeks after planting

Table 4: Soil mineral contents (Cation Exchange Capacity) of cowpea rhizosphere

	CEC (mol/Kg)			
Treatment	PRE	W4	W8	W12
UR	11.21 ± 0.22	11.60 ± 0.2	11.05 ± 0.2	12.98 ± 0.20
UNR	10.93 ± 0.55*	11.03 ± 1.2	10.8 ± 0.33*	10.99 ± 0.11*
CR	14.66 ± 1.00	14.66 ± 0.50	14.44 ± 2.0	14.39 ± 0.66
CNR	13.95 ± 1.20	13.90 ± 0.30	13.86 ± 0.84	13.86 ± 0.50

The value is significantly different from others in a particular column ($p \leq 0.05$)

[a] Values with the same superscript in a column are significantly different ($p \leq 0.05$)

UR: Uncontaminated rhizosphere; UNR: Uncontaminated non-rhizosphere; CNR: Contaminated non-rhizosphere; CR: Contaminated rhizosphere. RE: Pre-planting; W4: four weeks after planting; W8: 8 weeks after planting; W12: Twelve weeks after planting

Rhizosphere effect of cowpea on microbial population

The influence of plant roots on the abundance of microorganisms in the soil samples was evaluated and expressed as rhizosphere effect (RE) as shown in Table 5. Total heterotrophic bacteria (THB), hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing Fungi (HUF) populations were positively influenced by about 1.5 to 4.6 folds after two weeks of plant emergence. With regards to the total heterotrophic fungi (THF) however, low rhizosphere effect was observed especially in CR (0.3) where negative effects were observed during the same period. Increased RE was noticed in the following weeks (4 weeks) with UR having the highest effect (14.2) on the THB population while CR has 7.6 RE values respectively. For the HUB, CR had the highest (9.0) effects during the same period. Low effect on fungal populations was observed especially with respect to THF where 1.0 RE values were observed across all the treatments.

Similarly, the THB, HUB and HUF were positively influenced especially after six weeks of plant growth. More effect on THB was observed in UR (12.9) whereas THF populations were not affected much positively. Similar trend was observed in week 8 with the exception of THB in CR which recorded highest effect. Rhizosphere effects in HUF were generally low during this period which was contrary to the previous weeks. During the last four weeks, decrease in RE values of THB and HUB was generally observed except in UR. The same phenomenon was observed in THF except in CR where the population was not affected. For the oil utilizing fungi, there was increase in the RE in the week 12 as compared to week 10.

Table 5: Rhizosphere effect values during plant growth in contaminated and uncontaminated rhizosphere soil

Treatment	Rhizosphere effect*											
	2 weeks				4 weeks				6 weeks			
	THB	HUB	THF	HUF	THB	HUB	THF	HUF	THB	HUB	THF	HUF
UR	4.1	4.6	1.0	2.5	14.2	4.0	1.0	1.5	12.9	1.2	1.5	1.5
CR	2.7	4.4	0.5	4.0	7.6	9.0	1.0	1.0	6.8	5.6	1.2	1.0
	8 weeks				10 weeks				12 weeks			
UR	6.5	5.0	1.0	0.6	8.2	2.5	1.5	2.0	8.8	4.2	1.0	2.5
CR	10.0	6.4	1.0	1.0	6.9	3.1	1.0	1.0	4.9	1.5	1.0	2.0

* Values are obtained using this relation RE = R/S where RE: rhizosphere effect; R: microbial counts (CFU/g) of rhizosphere soil (treatment); S: microbial counts (CFU/g) of corresponding non-rhizosphere

(control) soil.

THB: Total heterotrophic bacteria; HUB: Hydrocarbon utilizing bacteria; THF: Total heterotrophic fungi; HUF: Hydrocarbon utilizing fungi.

Discussion

Based on the results for initial soil conditions prior to hydrocarbon contaminations, the soil was sandy, with low nutrients and mineral compositions. The physical properties were in the normal prevailing soil conditions obtainable in the area. Based on its physicochemical properties, the soil could best be described as marginal, porous and highly aerated. Thus, the soil is ideal for the growth of cowpea (Davis et al. 1991; Omoigui et al. 2018). In addition, the soil conditions make hydrocarbon contaminants less bound, easy to migrate and readily bioavailable (Cunningham et al. 1996). The capacity of soil to filter, retain, or release hydrocarbons is fundamental in determining the nature and fate of the contaminants. Previous studies have shown that retention, volatilization, and transport of hydrocarbons is affected by soil physical and chemical properties (Fine et al. 1997). For faster and efficient hydrocarbon cleanup, sandy soils are more desirable than other soil textural classes because it supports more robust and dynamic microbial species and allows better interaction with hydrocarbon contaminants. Amellal et al. (2001) and Huesemann et al. (2004) showed that sandy textured soils showed higher microorganism's populations and demonstrated higher degradation rates of hydrocarbons than fine silt and clay soils. Other physicochemical properties like nutrient, moisture, temperature, EC, and pH of the soil were also ambient for plant growth and microbial activities (Hajabbasi 2016; Koshlaf and Ball 2017). Therefore, the soil supports rapid aerobic microbial activities and degradation of hydrocarbons.

Changes in soil properties were observed; which were assumed to be due to root exudates secreted in the rhizosphere. Different compounds were identified and could have the capacity to promote microbial growth. Majority of the exudates identified in the treatment and control plants were hydrocarbons (21% and 20% respectively). The hydrocarbon compounds observed in this study might not be from the soil contaminants since the compounds did not significantly differ between the treatment and control plants. Recently, Mohan et al. (2020) identified a number of organic compounds contained in the root exudates of tomato, potato and cowpea grown in pristine garden soil. Majority of the compounds identified in their study were C_6 to C_{34} hydrocarbons molecules which are similar to those identified in this study. The presence of the hydrocarbons compounds might played significant role in the biodegradation of pollutants. Singer et al. (2003) proposed that some exudates specifically trigger enzymatic pathways for degradation of particular hydrocarbon compounds because they act as analogues to particular molecules especially if they have related chemical structures.

Organic acids and phenolics were also identified to be part of the plants root exudates. Organic acids provide substrates for microbial metabolism and serve as intermediates for biogeochemical reactions in soils. Organic acids serves as a readily available carbon source that microbial species have easy access to. In a comparative study by Shi et al. (2011), organic acids were reported to have significant role in shaping soil bacterial communities and impact on plant growth than sugars. Some phenolics act as

inducers of genes for degradation pathways in rhizospheric microbes due to their resemblance with contaminants (Hoang et al. 2021). A number of studies have revealed the presence of phenolics in root exudates. Ray et al. (2018) observed that phenolics in root exudates played a key role in communication between *Abelmoschus esculentus* and microbes in the rhizosphere. Some unsaturated hydrocarbons in combination with organic acids in cowpea root exudates may have a regulatory effect on the cuticle aging process (Mohan et al. 2020).

Coumarin and thiazepine were among the compounds identified in the root exudates. Coumarins and thiazepines are group of secondary metabolites produced by many plants species with properties similar to alkaloids and lactones respectively. Earlier studies have shown that coumarins play an important role in iron mobilization and uptake in iron-deficient soil. Recent works reported by Stringlis et al. (2019) have shown that coumarins act as antimicrobial agents, anticoagulants, anticancer; and used in abiotic stress management and plant-microbe interaction. This is also supported by the work of Lundberg and Teixeira (2018) who reported that coumarin was involved in plant iron starvation response and also protect plants from pathogenic fungi. Voges et al. (2018) have also observed the role of coumarins derived from *Arabidopsis thaliana* in shaping its rhizobiome by limiting the growth of *Pseudomonas* strain. Bisht et al. (2015) have reported that plant metabolites such as catechin and coumarin may serve as co-metabolites for polychlorinated biphenyl (PCB) degrading bacteria. More so, thiazepines which are substituted thiepins have been reported to act as phytoalexins (Pedras and To 2015). Mohammed et al. (2014) opined that thiazepine type alkaloids are rare in nature and most of the known thiazepine rings occur in 1,4-form and generally fused with other substituents like benzene. This work also support the findings considering the fact that the thiazepine identified in this study is a 1,4-type. Pedras and To (2016) described thiazepine as an important class of therapeutic drugs used as antifungals, calcium channel blockers and antidepressants in addition to its use in plant defense mechanisms.

Although not equivalent, the compounds present in the root exudates of treatment and control plants were also not different; which indicated that growth under hydrocarbon stress did not affect exudation. Correa-Garcia et al. (2018) and Duan et al. (2020) have shown that hydrocarbon and nutrient stress does not affect root exudation respectively. This however contradicted the works reported by Sasse et al. (2018) in which abiotic stress were observed to play a key role in modulating root exudates composition. Stringlis et al. (2018) have also observed increased secretion of fluorescent phenolic compounds in *Arabidopsis thaliana* rhizosphere which result from iron-starvation conditions.

Soil metabolism was enhanced in rhizosphere than non-rhizosphere; and also in contaminated than uncontaminated soil. This might be attributed to the fact that abundant nutrients are provided by root exudation and hydrocarbon contamination. This agrees with the findings of Hegazy et al. (2014) and Uba et al. (2018) who observed that nutrient addition and aeration increase soil respiration (SR) and microbial biomass carbon (MBC). Soil respiration and MBC were related and gave an idea on the microbial activity in soil and dictate the rate of substrates mineralization. Soil respiration is a key ecosystem process that releases organic carbon present in soil resulting from its utilization by plants, bacteria, fungi and animals. Root exudation stimulate microbial activity, which further contributes to SR. Experimental data have shown

that high rate of exudates addition affect microbial community composition most especially in C-poor subsoil (Baumert et al. 2018). Studies by Sun et al. (2017) have shown that, in spite of variations in flux rates of respiration and exudation along different segments of *Quercus* spp. rhizosphere, root exudation was observed to be in direct proportion to soil respiration.

Results in Figures 3 and 4 show that UR and CR had higher SR and MBC respectively throughout the experimental period. This might be due to abundant carbon in form of exudates released from the plant roots and hydrocarbon contaminants; which translate to higher soil organic matter (SOM) observed in the treatment. A positive linear correlation was established between SOM, SR ($r = 0.89$; $p < 0.05$) and MBC ($r = 0.98$; $p < 0.05$) in the control. Increase in the SOM resulted to increased microbial biomass and subsequent utilization of hydrocarbons leading to higher respiration rates. More so, the relationship between exudation, SR and MBC observed in this study might suggested that the exudation also influenced the physiological performance of the plant roots and also rhizospheric microorganisms. This is in accordance with the work of Yuste et al. (2007) who observed that SR were higher in soil with active than dead vegetation. Active plants are continuously exuding organic material to soil in the form of easily decomposable substrates such as simple sugars, amino acids and organic acids. Bais et al. (2006) also opined that root exudates keep roots healthy and active by providing lubrication for proliferation, defense against pathogens and attracting for mycorrhizal fungi and growth promoting bacteria. In the present study also, higher respiration rates were observed in treatment with more plant density which might have received more exudates than those with fewer ones.

Some of the soil physical properties were stable which might indicate that the cowpea plant did not exert so much influence on some of the soil characteristics like soil temperature and moisture. However, soil pH and electrical conductivity were observed to increase over time. Soil pH and moisture are closely related and influenced each other especially in field environment. Zárate-Valdez et al. (2006) reported that increase in soil moisture leads to increase in pH within minutes after addition of water to dry soil. This is consistent to the findings of this research in which the 8th week of the experiment was characterized by high pH due to slight increase in soil moisture. In addition, the increase in pH could be due to root exudation especially that higher pH was more prominent in the rhizosphere than non-rhizosphere soil. This agreed with the work of Zhu et al. (2020) who observed that high concentration of *Stellera chamaejasme* root exudates significantly increased soil pH due to their alkalescence. The occasional decrease in soil pH results from respiration and root exudates – which contained more acidic components. Root exudation and respiration can contribute some proportion of rhizosphere pH decrease due to accumulation of CO_2 in the root zone. The reports of Hinsinger et al. (2003) have shown that the build-up of the CO_2 concentration in the rhizosphere forms carbonic acid which dissociate and result in some pH decrease especially in neutral to alkaline soils. The multifactorial effects exerted on soil pH might have been the main reason for its relative stability in the study.

Results of soil electrical conductivity indicated that soil with higher levels of hydrocarbon contaminants have lower electrical conductivity (EC). Hydrocarbon reduces soil EC because of its non-polar substrates. Plant growth facilitates reduction of hydrocarbon concentration and release polar substrates. This leads to

the decrease in soil pH which results to increasing EC as observed during the post-planting period. This is consistent with the work of Doerge (2001) who made similar observations. Although EC in soil is dynamic and subject to several other factors, EC in soil solution strongly affect soil pH. This agreed with the work of Carmo et al. (2016) who made similar observation.

Soil organic matter (SOM) and organic carbon (SOC) from contaminated rhizosphere and non-rhizosphere soil differed significantly from uncontaminated non-rhizosphere. The difference observed were attributed to the absence of not only plants but also hydrocarbon contaminants. This can be justified when contaminated non-rhizosphere treatment is taken into consideration as it also significantly differed from the uncontaminated non-rhizosphere. Addition of hydrocarbon contaminants have been linked to increased SOM and SOC especially from the temporal perspectives. This agreed with the opinion of Osuji and Nwoye (2007) who stated that, following the addition of hydrocarbons, soil SOM and SOC normally increase.

On the other hand plants secrete soluble organic compounds in form of exudates which are believed to be responsible for higher SOM and SOC observed in the rhizosphere soil. Studies have shown that addition of root exudates is generally linked to increased SOM contents (Baumert et al. 2018). Although SOM decreases hydrocarbon bioavailability, it may also stimulate microbial growth especially that the soil is poor in nutrients and also not aggregated. This is consistent with the work of Masakorala et al. (2014) who observed that almost all bacterial populations in rhizosphere soil become active and vigorous in most contaminated soils due to dual effects of SOM addition and hydrocarbon contaminants. Wang et al. (2013) have also shown that hydrocarbons in soil could increase the SOC, which might affect nutrients and microbial equilibria.

Nitrogen (N) and phosphorus (P) contents of the rhizosphere soil were significantly different from those in the non-rhizosphere soil. Initial N contents in the soil were generally low indicating that the soils of the study area are scarce in N to support robust growth of plants and microbes. The N deficiency could be attributed to low SOM which might have result from low input of plant residues in to the soil. This finding is in agreement with the work of Kebede et al. (2021) who observed a strong correlation between SOM and soil N content. Following contamination and subsequent plant growth, N and P contents in the rhizosphere soil increased beyond its critical limits especially during the 12 week period. Okalebo et al. (2002) suggest that high N values (> 0.25%) decrease nodule formation and N fixation. The higher values observed might have resulted from concomitant non-symbiotic nitrogen fixation and decreased N assimilation due to decrease microbial population (See Table 6).

The soil N and P contents might have remained stable during the 4th and 8th week due to dual effects of the plant and microbial activities respectively. The leguminous nature of the plant used in this study greatly played a role in elevating nitrogen contents of the rhizosphere soils. However, the available N which is limited in polluted soil become readily utilized by microbial population in the rhizosphere, thus lowering its concentration. Many studies have singled out leguminous plants as most efficient in phytoremediation due to their ability to fix nitrogen (Sugiyama and Yazaki 2012).

Upon hydrocarbon contamination, soil P decreased and subsequently increased during plant growth. Hydrocarbon contamination stimulates microbial communities capable of hydrocarbon utilization which deplete available P. In addition, the increase in pH caused by contamination makes P less soluble and unavailable. This agrees with the findings of Wang et al. (2013) and Ebuehi et al. (2005) who made similar observation. With the influence of plant growth, the P was observed to increase steadily. Zhang et al. (2016) observed that *Vicia faba* (Faba beans) increased soil P contents leading to a better growth of *Zea mays* and confirmed that it was due to root exudation of citrate and acid phosphatase by faba bean. This is also supported by the work of Dakora and Phillips (2002) who pointed out that P bound to organic molecules are released due to the effect of extracellular enzymes produced by microbial cells and plant roots.

Chemical properties of the soils were slightly affected; although Ca and CEC were significantly increased. Calcium represents 75–85% of exchangeable bases in soils (Vidonish et al. 2016) and reports indicated that oil pollution has been shown to be associated with accumulation of exchangeable bases including Ca^{2+} (Robson 2003; Wang et al. 2013). In addition, the presence of exudates might have contributed to more Ca in the rhizosphere. This agreed with the findings of Ohta and Hiura (2016) who observed significantly higher Ca and Mg in the soils under the influence of *Camellia japonica* exudates. However, it disagreed with the finding of Abii and Nwosu (2009) who observed decrease in soil Ca contents of hydrocarbon contaminated soil. The high CEC observed in this study might have been influenced by a number of factors including pH, SOM and soluble cations. Earlier studies have shown that CEC depends on the amounts and kinds of clay and organic matter present; and increase in organic matter, leads to corresponding increase in CEC increase (Parker 2009).

Rhizosphere effect (RE) values were generally positive which indicated the plants' effect on microbial growth and activities. The increased microbial numbers and activities in the rhizosphere are due to the release of organic carbon by the plant roots. It has been reported by Bakker et al. (2013) that loss of root cap and border cells, insoluble mucilage, soluble root exudates, volatile organic carbon, flow of carbon to root associated symbionts, and death and lysis of root cells are the major drivers of microbial population in the root zone. Soil microorganisms are chemotactically attracted to the plant root exudates, after which they proliferate in the environment. It has been estimated that bacterial populations in the rhizosphere, are 10–100 times higher than in bulk soil (Pinton et al. 2007). Diab (2008) and Olahan et al. (2016) have also observed that positive rhizosphere effect results from plants' root exudation which plays a crucial role in microbial degradation of pollutants.

In some instances, especially during first-two weeks of plant growth, the RE values for THF were < 1 indicating negative influence of the plant on fungal abundance. This might be due to the inability of some of the fungal species to withstand the toxicity of the hydrocarbon contaminants which resulted to low effect. Low RE in the subsequent weeks however, could be attributed to inhibitory effects of some compounds in the root exudates against specific fungal population. The work of Nóbrega et al. (2005) have demonstrated that cowpea exudates contain defense proteins which exert inhibitory effects on the growth of the fungus, *Fusarium oxysporum.* Broeckling et al. (2008) have also reported that certain

components of alfalfa root exudate inhibited the growth of *Fusarium*. To the contrary, presence of phenolic compounds in the root exudates might have resulted to positive RE in most of the plant growth period. Clocchiatti et al. (2021) have shown that phenolic root exudates may serve as stimulants for saprotrophic fungi in the rhizosphere. There is increasing evidence that root exudates regulate fungal community composition and diversity in the rhizosphere (Broeckling et al. 2008 Zhalnina et al. 2018).

Conclusion

Crude oil contamination caused dramatic changes in the soil physicochemical conditions. Production of root exudates as a result of plant growth gradually affected the soil properties including the physical, mineral and microbial population. Little fluctuations in soil pH and moisture affected EC and CEC. Increase in organic matter and carbon resulted to corresponding increase in the rhizosphere effect. Although bacterial population were overall positively affected, fungal population were limited at certain points in time which was assumed to be due to the presence of some compounds in the exudates that are known to be for biocontrol. The findings strongly point out that root exudates of cowpea caused a significant shift in soil conditions and promote the size and activity of rhizosphere microbial communities. This property is essential for rhizodegradation of petroleum hydrocarbons and therefore the phytoremediation potentials of cowpea is further supported. In order to properly understand the microbial dynamics in the cowpea rhizosphere, culture-independent microbiological investigation is necessary.

Declarations

Conflict of interest: The authors declare that there is no competing interest.

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