Scientific Research and Essays Vol. 8(2), pp. 99 -107, 11 January, 2013
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE12.731
ISSN 1992-2248 ©2013 Academic Journals

Full Length Research Paper

Effects of Bonny light crude oil contamination on the germination, shoot growth and rhizobacterial flora of *Vigna unguiculata* and *Arachis hypogea* grown in sandy loam soil

Eze C. N.*, Maduka J. N., Ogbonna J. C. and Eze E. A.

Department of Microbiology, University of Nigeria, Nsukka.

Accepted 17 December, 2012

This study was carried out to evaluate the effects of different levels of Bonny light crude oil contamination on the germination, shoot growth and rhizobacterial flora of *Vigna unguiculata* (cowpea) and *Arachis hypogea* (groundnut) grown in potted sandy loam soil samples in Nsukka, Nigeria. Crude oil at a level of 2.5% increased germination time in cowpea by 24 h but at higher doses (5 to 20%) germination of cowpea seeds was totally inhibited. On the contrary, germination of groundnut seeds occurred at all levels of crude oil pollution although at different rates. The germination time was 7 days in soils with 0.5 to 2.5% oil concentration and an average of 9 days in those with 5 to 20% levels of the pollutant. Even though groundnut germinated and grow in all concentrations of crude oil tested, significant ($p<0.05$) shoot growth retardation still occurred in both legumes consequent on crude oil toxicity. Rhizobacterial population also diminished with increase in crude oil concentration. The study reveals both the vulnerability of cowpea and the resistance of groundnut to crude oil, marking groundnut out as a promising phytoremediation candidate.

Key words: Crude oil, pollution, rhizobacteria, legume, phytoremediation, rhizosphere.

INTRODUCTION

Since crude oil was discovered as energy source, it has remarkably stepped up the rate of civilization. However, in spite of its enormous benefits, it has also wreaked much havoc on the ecosystem due mainly to its toxicity. It has been shown to be harmful to both the biotic and abiotic components of the ecosystem (Chaineau et al., 2000; Wyszkowska et al., 2001; Bamidele, 2010). Petroleum hydrocarbons can reach the soil from many sources including oil spillages by transportation vessels, pipeline leakages, automobile servicing stations, fuel stations and other anthropogenic activities. These activities cause serious pollution problems to the natural environment through the release of petroleum hydrocarbons.

Soil and water contamination by crude petroleum and refinery products is an ever growing problem in Nigeria as oil mining and refining activities increase steadily. This problem manifests particularly in the loss of fertility of agricultural lands and death of plants, including crops, in the oil producing areas of Nigeria. A report by Adoki and Orugbani (2007) on the same subject shows that it prevents crop growth and yield in those areas for varying periods of time. Crude oil negatively affects the germination, shoot growth and yield of most plant species including seashore plants (Bamidele and Igiri, 2011) and field grasses (Debojit et al., 2011).

Crude oil and its products are made up of aliphatic, oleic, naphthenic and aromatic hydrocarbons, which change the physical and chemical properties of soil and its structure (Chi Yuan and Krishnamurthy, 1995). These compounds are largely responsible for altered fertility of soil. Affected soils lose their biological activity and many

*Corresponding author. E-mail: chibuzoreze@gmail.com.

require up to ten years to recover it (Wyszkowska et al., 2001).

One of the indices of loss of biological activity of soils as a result of crude oil pollution is the reduction or inhibition of microbial activity. Microorganisms of particular interest in this study are the rhizobacterial flora (rhizosphere bacteria), due to their many beneficial roles. They have been shown to be important in the degradation of pollutants, biofertilization through nitrogen fixation, phytostimulation and biocontrol of soil-borne plant diseases (Chin-A-Woeing et al., 1998). This area of soil around plant roots, known as the rhizosphere contains higher populations and greater diversity of microorganisms than soil with no plant (Nichols et al., 1997). This is because plant roots release exudates into the soil that increase microbial activity by supplying nutrients to the organism. These exudates consist of enzymes, aliphatics, aromatics, amino acids, sugars and low molecular weight carbohydrates (Burken and Schnoor, 1996).

Even though it is already established that crude oil negatively affects seed germination and growth of seedlings (Bamidele and Igiri, 2011; Debojit et al., 2011; Bamidele 2010; Lin and Mendelsshohn, 2009), no two plants are expected to exhibit the same degree of resistance or susceptibility to crude oil. What will make the difference is the inherent property of each plant, and seed phytochemistry can be a good pointer to these properties. The two plants used in this study (cowpea and groundnut) are staple leguminous crops in different parts of Nigeria. Legumes play an important role in the restoration and sustenance of soil fertility through their ability to fix atmospheric nitrogen in partnership with certain bacterial species.

Owing to this overriding role of legumes in agriculture, their growth and survival in arable lands become paramount, particularly now that many farm lands are fast losing their agricultural value due to pollution and over-use. For this reason and also considering the enormous benefits of rhizosphere bacteria, this study was undertaken to evaluate the effects of crude oil pollution of sandy loam soil on these components of ecological flora. The study is also aimed at extrapolating the phytoremediation potentials of the two legumes (cowpea and groundnut) from their respective degrees of resistance to petroleum hydrocarbon toxicity.

MATERIALS AND METHODS

Sources of Materials

Crude oil

Bonny light crude oil (specific gravity = 0.81; API gravity = 43.2°) was obtained from Nigerian National Petroleum Corporation (NNPC) Port Harcourt Refinery, Alesa – Eleme, Rivers state, Nigeria. The crude oil was unweathered, having been obtained fresh from the production plant.

Plant seeds

Viable seeds of *Vigna unguiculata* (cowpea) and *Arachis hypogea* (groundnut) were purchased at Ogige market, Nsukka and stored at room temperature (25 to 30°C) for not more than 24 h.

Soil sample

Pristine sandy loam soil was collected from the Botanical Garden, University of Nigeria, Nsukka.

Soil pollution with crude oil and sowing of plant seeds

The soil sample was air-dried, sieved and dispensed in 3 kg weights into thirty-six (36) plastic pots (20 cm deep × 20 cm diameter) perforated at their bases. The pots were divided into two groups of eighteen each, and each group was used for one of the crop plants. Each pot in a group, apart from the control, was contaminated with one of eight different levels of Bonny light crude oil (0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0 or 20.0% v/w). All control samples were not polluted. Thereafter, seeds of the plants (cowpea and groundnut) were sown, which consisted of three seeds of each plant sown in duplicate pots to achieve six replicates of each plant. All pots were kept in a green house at the Botanical Garden, University of Nigeria, Nsukka and watered every four days by spraying.

Phytochemical analysis of plant seeds

Determination of Phytate

Procedure: The plant material was extracted with 0.2 N HCl. A 0.1 ml volume of the extract was pipetted into a test tube. Two millilitres of solution A (as shown below) was added and the test tube covered. The tube was heated in boiling water for 30 min and later cooled to room temperature. Four milliliters of solution B was added and mixed very well. Absorbance was measured at 519 nm (Onwuka, 2005).

Solution A = 0.2 g of ammonium iron III sulphate mixed with 100 ml of 2 N HCl and the volume made up to 1000 ml with distilled water.

Solution B = 10 g of bipyridine and 10 ml thioglycolic acid in distilled water and the volume made up to 1000 ml.

Alkaloid determination

A 5.0 g weight of the plant material was measured into a 250 ml beaker. Two hundred millilitres of 10% acetic acid ethanol (10% acetic acid in 100 ml of ethanol) was added, covered and left to stand for 4 h (Habourne, 1973).

The extract was filtered and to it was added NH_4OH drop-wise until the precipitation was completed. The whole solution was allowed to settle and afterwards the precipitate was collected, washed with dilute NH_4OH solution and then filtered. The residue was weighed and reported as the crude alkaloid.

Saponin determination

A 20 g weight of the sample was put into a flask containing 200 ml of 20% ethanol. The mixture was heated in a water bath for 4 h with continuous stirring at about 55°C. Filtration and re-extraction of the residue were carried out using another 200 ml of 20% ethanol. The

combined extract was reduced to 40 ml over water bath at 90°C and the concentrate transferred into a 250 ml separator funnel and washed with 20 ml of diethyl ether. The aqueous layer was recovered while the other layer was discarded and the purification process repeated. Sixty milliliters of 17-butanol was added and the extract washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was heated in a water bath, evaporated and oven-dried to a constant weight (Obadori and Ochuko, 2001).

Calculation: $$\% \text{ Saponin} = \frac{\text{Weight of residue}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Flavonoid determination

Ten grams of the sample were put in a flask and extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was filtered using Whatman filter paper and the filtrate transferred into a weighed crucible. Finally, the content of the crucible was evaporated to dryness over a water bath and later weighed to a constant weight (Bohan and Kocipia, 1974).

Calculation: $$\% \text{ Flavonoid} = \frac{\text{Weight of residue}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Determination of tannins

Procedure: A 1 g weight of the test sample was put into a flask and 10 ml distilled water was added. This was allowed to stand for 30 min at room temperature with gentle shaking at 5 min intervals, at the end of which the mixture was centrifuged. Exactly 2.5 ml of the supernatant and 2.5 ml of standard tannin solution were measured into separate 50 ml volumetric flasks. One millilitre of Folin-Dennis reagent was added into each flask followed by 2.5 ml of saturated Na_2CO_3 solution (Pearson, 1976).

The solution was made up to the mark and later incubated for 90 min at room temperature. The absorbance was read at 250 nm

Calculation: $$\text{Percentage tannin} = \frac{An}{As} \times C \times \frac{100}{W} \times \frac{Vf}{Va}$$

Where:

An = Absorbance of test sample
As = absorbance of the standard
C = Concentration of standard
W = Weight of sample used
Vf = Total volume of extract
Va = Volume of extract used for the analysis

Analytical techniques

Seed germination

Germination of seeds was assessed daily for 56 days as positive or negative; it was positive if there was a visible cracking of the seed coat with measurable root or shoot production (Maila and Cloete, 2002). The germination time (in days) was recorded for seeds in every pot.

Plant growth evaluation

Plant shoot growth was measured initially fourteen days after seed sowing and subsequently done weekly throughout the eight-week experiment. Measurement was carried out using a calibrated 30 cm transparent plastic rule.

Microbiological analyses

Measurement of the population of bacteria in the rhizosphere of the two legumes and that of the surrounding bulk soil was carried out using the total plate count technique (Wistreich, 1997). Top soil samples (0 to 3 cm deep) (Adoki and Orugbani, 2007) were collected both from the rhizosphere and bulk soil at two-weekly intervals from each pot and put into sterile labeled polythene bags. The samples were immediately taken to the laboratory for analysis. One gram of each sample was serially diluted using sterile distilled water and the 10^{-8} dilution inoculated by spread plating on sterile nutrient agar plates; incubation was for 24 h at room temperature (25 to 30°C).

Statistical analysis

Data analysis was carried out using a two–way analysis of variance (ANOVA), and the difference was done by comparing tests with $p < 0.05$.

RESULTS

Seed germination

Results of the effects of crude oils on the germination of cowpea and groundnut seeds are presented in Tables 1 and 2. Low doses of crude oil (0.5 to 2.0%) did not affect seed germination of the legumes. However, at higher concentrations (2.5% or beyond), germination was delayed in both legumes. In cowpea, 2.5% crude oil concentration increased germination time by 24 h, and beyond that level germination of cowpea seeds was totally inhibited (Tables 1 and 2). In groundnut, germination occurred at all crude oil levels but the process was delayed by 48 and 72 h at crude oil concentrations of 5 to 15 and 20% respectively. There was 100% germination in groundnut up to 2.5% crude oil level (Table 2). In cowpea, 100% of the seeds germinated only in the control (0% crude oil) and test sample with 0.5% oil concentration. Beyond 2.5% oil level, there was 0% germination in cowpea.

Result of the quantitative phytochemical analysis of seeds of the crops is presented in Table 3. The analysis was carried out to investigate the probable causes of the difference in resistance to crude oil observed between groundnut and cowpea, as shown in Table 3. Seeds of both legumes lacked saponin but contained phytate, tannins, flavonoids and alkaloids. Groundnut had a much lower percentage of phytate but higher percentage of

Table 1. Effects of different levels of crude oil on germination time of the plants.

Crop plant	Crude oil levels (%)								
	0.0	0.5	1.0	2.0	2.5	5.0	10.0	15.0	20.0
	Germination time (days)								
Groundnut	7±0.5	7±0.5	7±0.5	7±0.5	7±0.5	9±0.5	9±0.6	9±0.6	10±0.6
Cowpea	4±0.5	4±0.5	4±0.5	4±0.5	5±0.5	*	*	*	*

*No germination.

Table 2. Percentage germination of the plants in sandy loam soil polluted with varying concentrations of crude oil.

Crop plant	Crude oil levels (%)								
	0.0	0.5	1.0	2.0	2.5	5.0	10.0	15.0	20.0
	Germination (%)								
Groundnut	100	100	100	100	100	83	67	67	50
Cowpea	100	100	83	50	50	*	*	*	*

*No germination.

Table 3. Phytochemical contents of seeds of the crop plants.

Crop plants	Phytate (%)	Tannin (%)	Flavonoid (%)	Alkaloid (%)	Saponin (%)
Groundnut	0.95 ± 0.1	0.040 ± 0.003	20.0 ± 0.70	8.5 ± 0.35	_
Cowpea	2.85 ± 0.13	0.023 ± 0.002	11.0 ± 1.06	5.5 ± 0. 49	_

tannins, flavonoids and alkaloids than cowpea.

Shoot growth and rhizobacterial flora

Figures 1 and 2 show the effects of the Bonny light crude oil on the shoot lengths of the crops. The shoot lengths of the crops were significantly ($P<0.05$) retarded by the oil. This is easily seen when the shoot lengths of the test samples are compared to the control crops (Figures 1 and 2). For instance, at the eighth week, the mean maximum shoot lengths of cowpea plants in the control and 2.5% crude oil – polluted soils were 57.0 cm and 48.5 cm (Figure 2) respectively.

In groundnut, the mean maximum shoot lengths at the 8^{th} week, of the control plants and plants grown in soils with 2.5, 5, 10, 15 and 20% crude oil contamination varied from 35.5 cm to 19.9 cm, 8.7 cm, 7.6 cm, 5.3 cm and 5.1 cm respectively (Figure 1). Even though groundnut germinated and grew in all the levels of crude oil pollution, there was growth depression and subsequent stagnation at high doses.

Results of the effect of the oil on rhizosphere and bulk soil bacteria are presented in Figures 3 and 4. Growth of rhizosphere and bulk soil bacteria in soil samples with low concentrations of crude oil (0.5 and 1%) was enhanced in both groundnut and cowpea plants. At crude oil concentrations above 1%, there was significant ($P<0.05$) retardation of bacterial growth. In groundnut for instance, from 2% crude oil level bacterial numbers decreased steadily with increase in oil concentration (Figure 3). Plate counts taken 14 days after planting showed bacterial numbers in cowpea rhizosphere varying from 3.1×10^{10} cfu/g in the unpolluted control to 2.9×10^{9} cfu/g in the 2.5% polluted sample (Figure 4). A significant ($P<0.05$) difference was observed between the effects of the crude oil on the rhizosphere and bulk soil bacterial population in both crops. Higher populations of bacteria in the range of up to one order of magnitude were observed in the rhizosphere than in the adjacent bulk soil.

DISCUSSION

The depression of germination of seeds by crude oil is in line with previous reports on related research (Bamidele and Igiri, 2011; Debojit et al., 2011; Malek-Hossein and

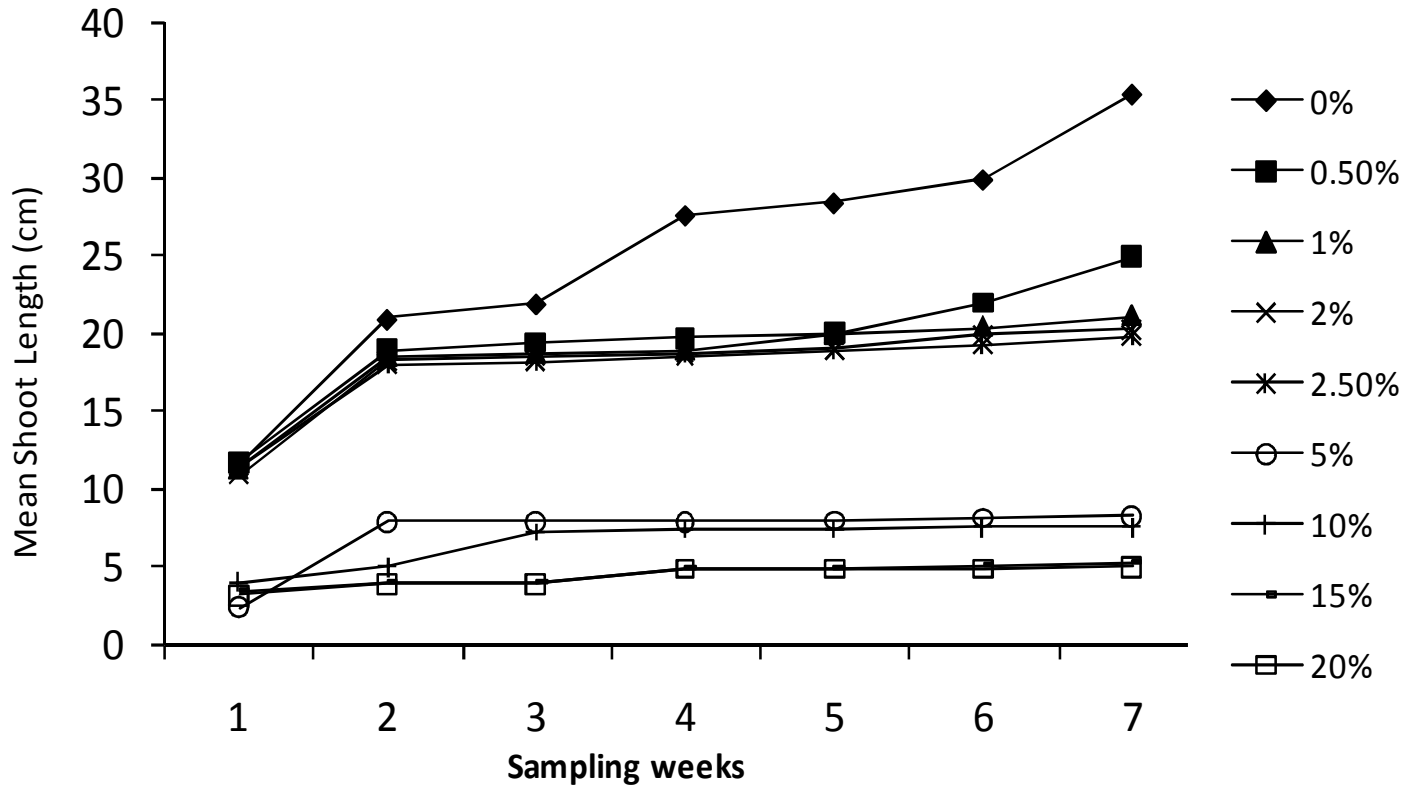


Figure 1. Effects of different levels of crude oil on vegetative growth of groundnut.

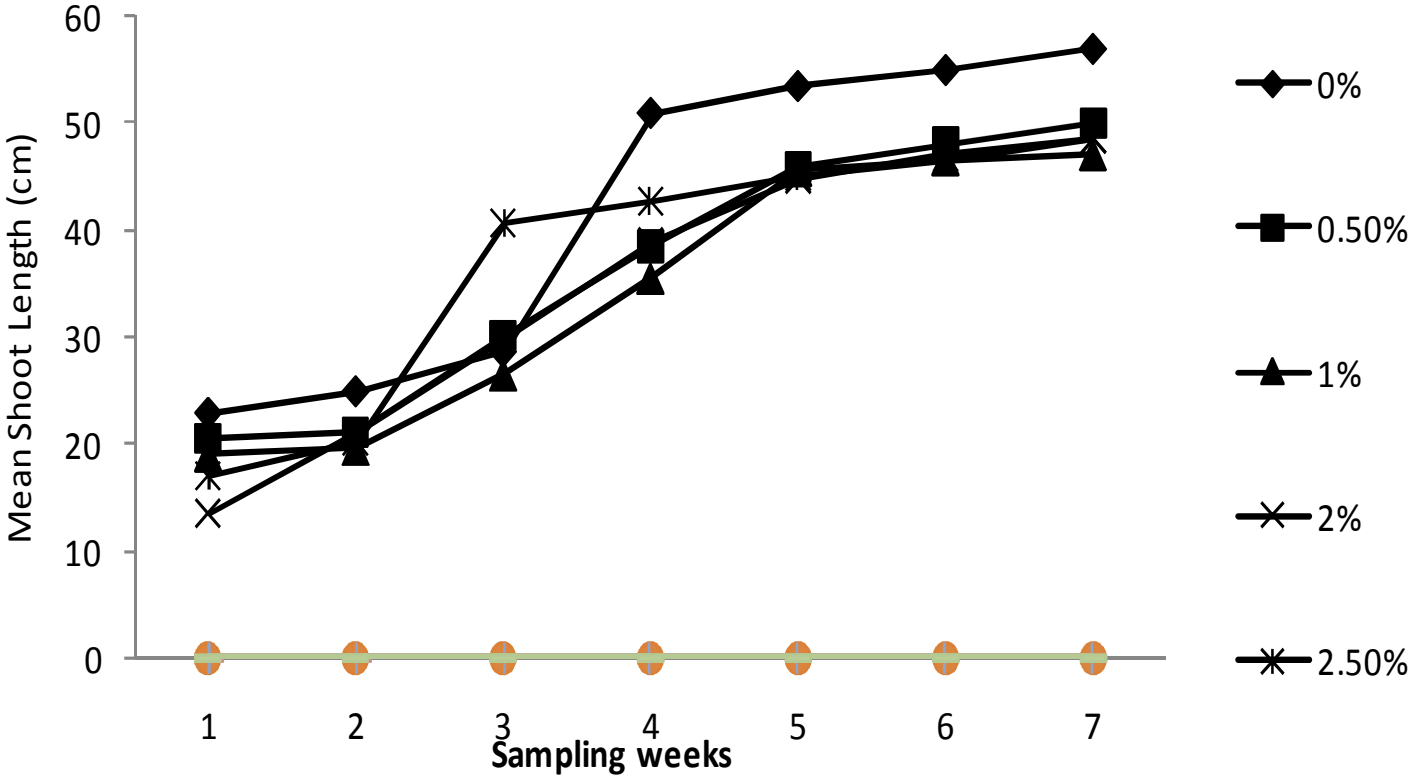
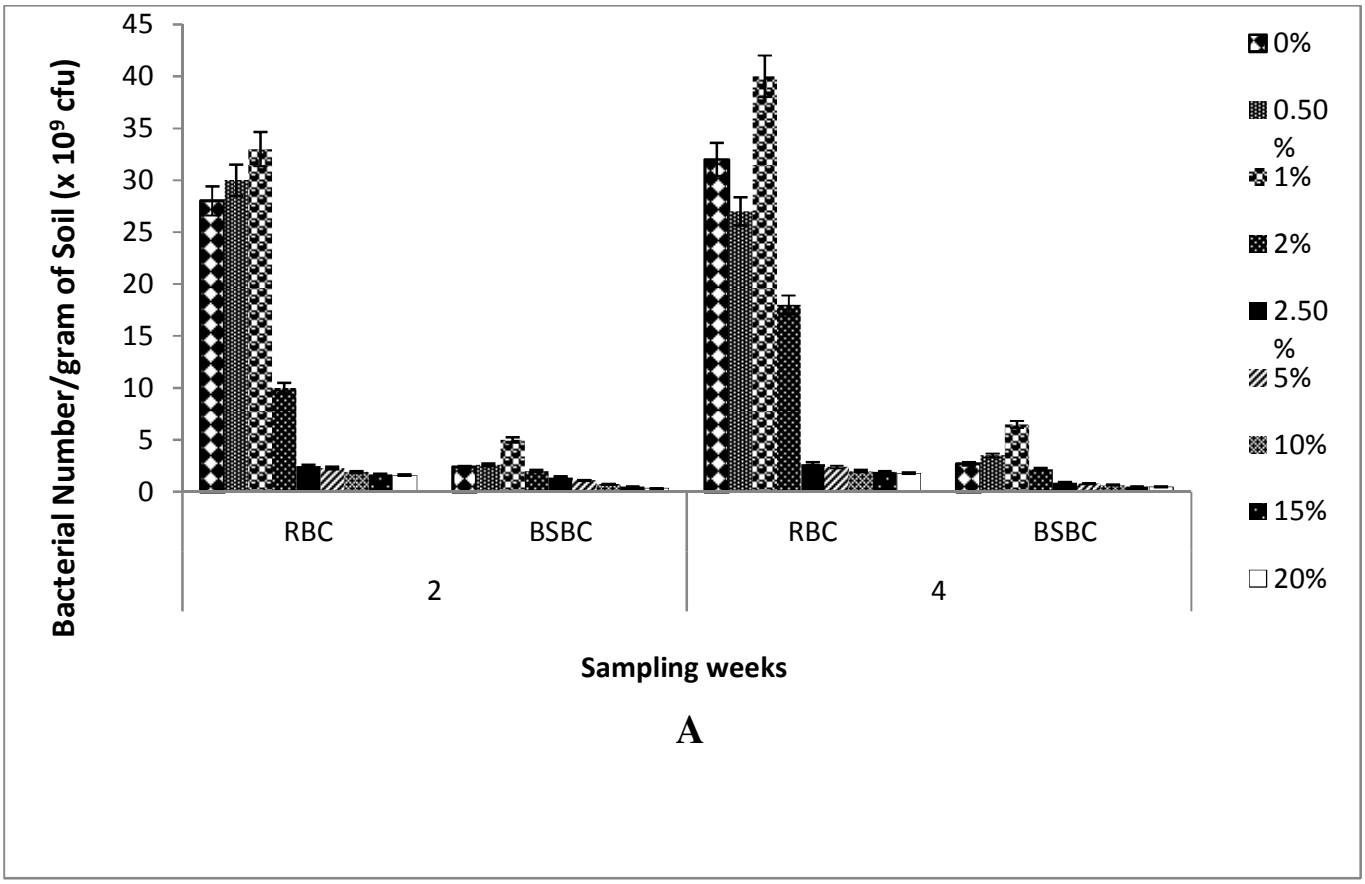


Figure 2. Effects of different levels of crude oil on the vegetative growth of cowpea.



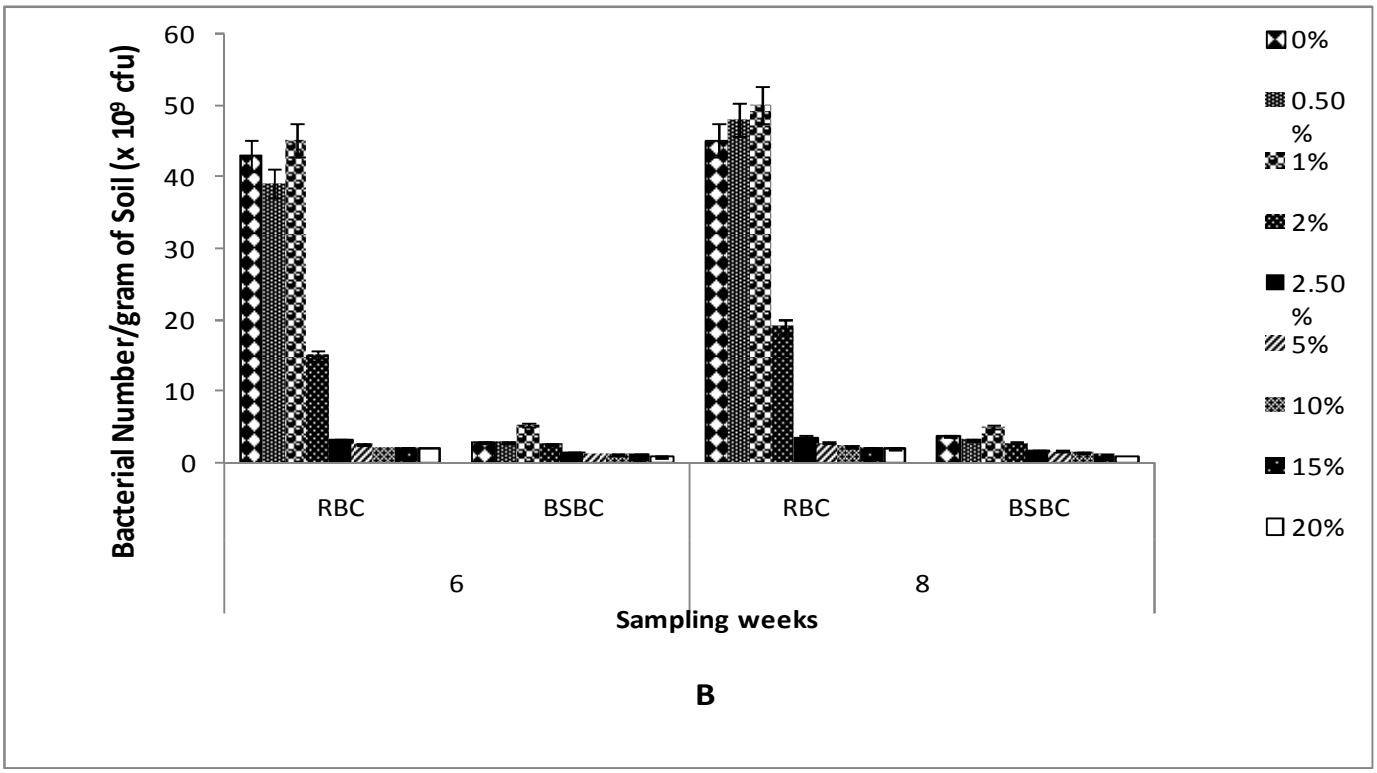


Figure 3(A and B). Effects of varying levels of crude oil pollution on bacterial numbers in groundnut rhizosphere and surrounding bulk soils. RBC = Rhizosphere bacterial count, BSBC = Bulk soil bacterial count.

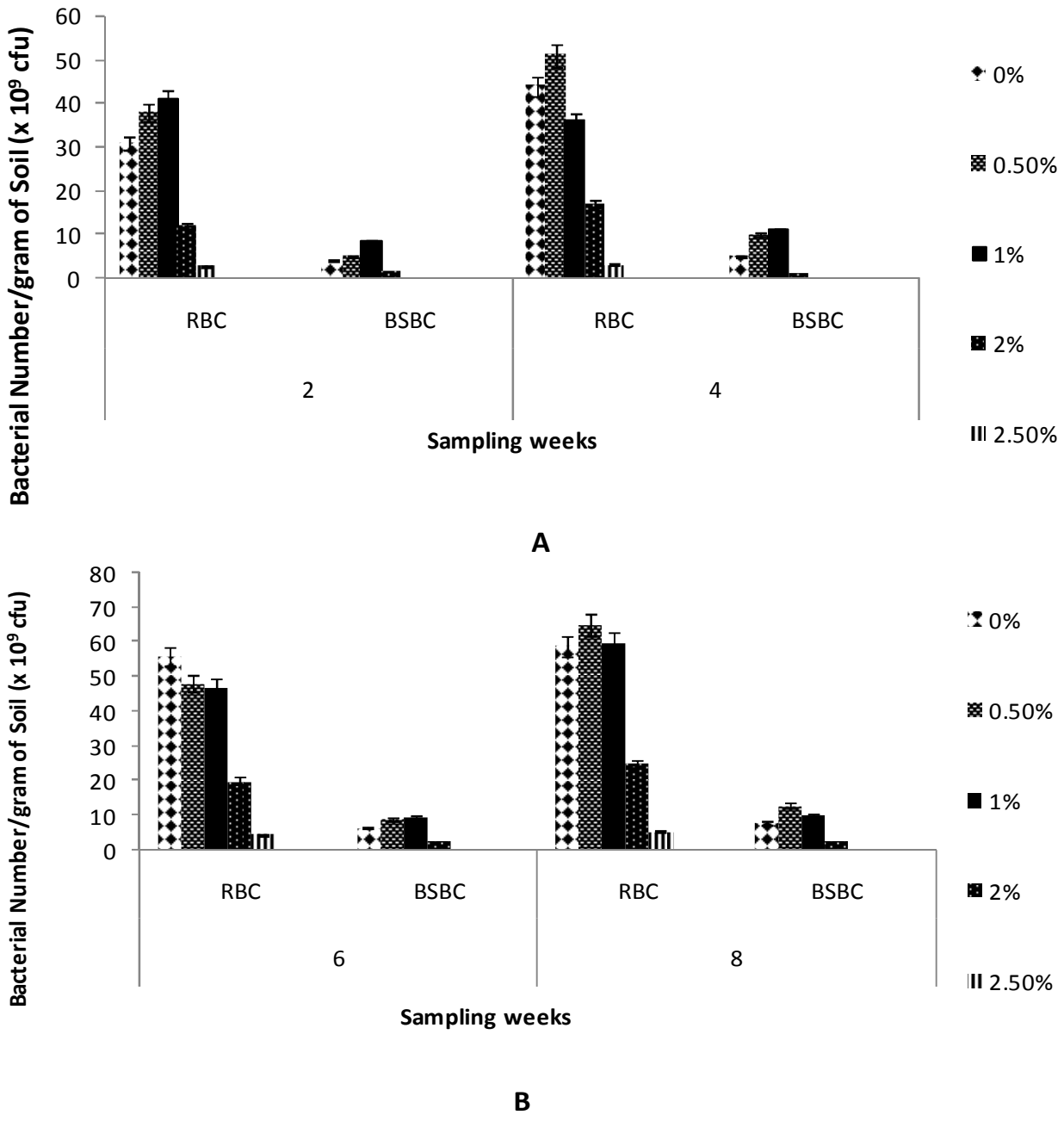


Figure 4(A and B). Effects of varying levels of crude oil pollution on bacterial numbers in cowpea rhizosphere and surrounding bulk soils. RBC = Rhizosphere bacterial count, BSBC = Bulk soil bacterial count.

Gholamreza, 2007; Amadi et al., 1996; Sparrow and Sparrow, 1988). Crude petroleum is able to interfere with seed germination by coating the seeds with oily substances thereby limiting water-air movement within the seed and directly through toxic actions. According to Table 2, groundnut seeds had higher percentage germination than cowpea at 1.0% crude oil level and above. This was probably caused by innate resistant qualities of the groundnut seeds. The remarkably low phytate content of groundnut seed might have enhanced its germination and growth at all the levels of crude oil used, since high levels of phytate (an anti-nutrient) inhibit mineral nutrients absorption in both plants and animals (Raboy, 2002; Urbano et al., 2000). Some of these mineral elements (for example, Ca, P and Mg) are needed for seed germination. When the phytate level is low, seeds sown in crude oil polluted soil will probably have only the external crude oil factor to contend with during germination.

Shoot growth retardation in plants due to petroleum

pollution as observed in this work had been reported by different workers on related studies (Debojit et al., 2011; Bamidele and Igiri, 2011; Bamidele, 2010; Lin and Mendelsshohn, 2009; Adoki and Orugbani, 2007). Adoki and Orugbani (2007) during their study with three vascular plants (fluted pumpkin, maize and okro) reported retardation in their shoot growth as a result of crude oil contamination. When crude oil coats plant parts with hydrophobic substances, it reduces respiration and cell membrane permeability in the affected parts. Reduction in cell membrane permeability consequently reduces nutrient absorption, metabolism and growth in the plants.

The slight increase in the growth of rhizosphere and bulk soil bacteria in those samples with low concentrations (0.5% and 1%) of crude oil might have occurred as a result of the establishment of a favourable carbon-nitrogen (C:N) ratio which made it easy for hydrocarbon degraders to utilize the oil as carbon source. According to Jobson et al. (1974), bacteria require about 10 parts of carbon to 1 part of nitrogen for good growth. If the ratio increases to up to 100:1 or 1000:1 as a result of oil spillage, bacterial growth and utilization of carbon source will be retarded, and there will also be nitrogen deficiency in such oil-polluted soil. Atlas and Bartha (1973) reported that nutrients, especially nitrogen and phosphorus, are the main limiting factors to the occurrence of petroleum degrading microorganisms. A favourable C:N ratio, which can occur at low petroleum concentrations in the soil, may therefore enhance the growth of hydrocarbon degraders in such oil-contaminated soil. Population decreases were observed in the rhizosphere and bulk soil bacteria at crude oil levels above 1%. These decreases occurred in bacterial numbers because high concentrations of crude oil in addition to causing nitrogen deficiency by creating an imbalance in the C:N ratio, undermine the growth of the organisms through toxicity effects and reduction in cell membrane permeability.

The higher populations of bacteria which occurred in the rhizosphere than in the adjacent bulk soil agrees with previous reports that there is increased microbiological activity within the rhizosphere (Nichols et al., 1997; Clegg and Murray, 2002; Kuiper et al., 2002). This increase is caused by exudates and sloughed-off tissues from the plants which served as nutrients to the microorganisms.

Conclusions

According to results of this study, groundnut resisted the toxic effects of crude oil more than cowpea. This is evidenced by its ability to germinate and grow in crude oil concentrations high enough to cause mortality in cowpea. This property marks it out as a promising candidate for the phytoremediation of crude oil-polluted soils since the usefulness of any plant in the phytoremediation of a polluted habitat is determined by its ability to grow in the polluted habitat in question. Also shown in this work is that crude oil pollution of soil at low concentrations (0.5 to 1%) enhances rhizobacterial growth in sandy loam soil containing the two leguminous plants but high concentrations of oil leads to growth depression.

We are not aware of any other comparative study revealing the effects of Bonny light crude oil contamination of sandy loam soil on the germination, shoot growth and rhizobacterial flora of *Vigna unguiculata* and *Arachis hypogea*.

REFERENCES

Adoki A, Orugbani T (2007). Influence of nitrogenous fertilizer plants effluents on growth of selected farm crops in soils polluted with crude petroleum hydrocarbons. Afr. J. Agric. Res. 2(11):569-573.

Amadi A, Abbey SD, Nma A (1996). Chronic effects of oil spill on soil properties and microflora of rain forest ecosystem in Nigeria. Water Air Soil Pollut. 86:1-11.

Atlas RM, Bartha R (1973). Degradation and mineralization of petroleum in sea water: Limitations by nitrogen and phosphorus. Biotechnol. Bioeng. 14:309-317.

Bamidele JF, Igiri A (2011). Growth of seashore paspalum (*Paspalum vaginatum*) in soil contaminated with crude petroleum oil. J. Appl. Sci. Environ. Manage. 15(2):303-306.

Bamidele JF (2010). Threats to sustainable utilization of coastal wetlands in Nigeria. J. Nig. Environ. Soc. 5(3):217-225.

Bohan BA, Kocipia AC (1974). Flavonoids condensed from leaves of Hawain *Vaccinium vaticulatum and Vaccinium calycinium.* Pac. Sci. 48:458-463.

Burken JG, Schnoor JL (1996). Phytoremediation: Plant uptake of atrazine and role of root exudates. J. Environ. Qual. 29:549-578.

Chaineau CH, Morel JL, Oudot J (2000). Biodegradation of fuel oil hydrocarbons in the rhizosphere of maize. J. Environ. Qual. 29:549-578.

Chin-A-Woeng TEC, Bloemberg GV, Van der Bij AJ, Van der Drift K.M, Schripsema J, Kroon B, Scheffer RJ, Thomas JE, Luternberg BJ (1998). Biocontrol by Phenazine-I-carbonxamide-producing *Pseudomonas Chlororaphis* PCL 1391 of tomato rot caused by *Fusarium oxysporum F. Sp. Radicis-lycopercisi.* Mol. Plant Microbe Interact. 11:1069-1077.

Chi Yuan F, Krishnamurthy M (1995). Enzymes for enhancing bioremediation of petroleum contaminated soils: A brief review. Air Waste Manage. Assoc. 45:453-460.

Clegg C, Murray P (2002). Soil microbial ecology and plant root interactions. Iger Innovations.

Debojit B, Jitu B, Sarada KS (2011). Impact of Assam petroleum crude oil on the germination of four crude oil-resistant species. Asian J. Plant Sci. Res. 1(3):68-76.

Habourne JB (1973). Phytochemical Methods. Chapman and Hall, London pp. 110-113.

Jobson AM, McLaughlin FD, Cook FD, Westlake DWS (1974). Effects of amendments on the microbial utilization of oil applied to soil. Appl. Microbiol. 27:166-171.

Kuiper I, Kravchenko I, Bloemberg GV, Lutenberg BJJ (2002). *Pseudomonas putida* strain PCL 1444, selected for efficient root colonization and naphthalene degradation, effectively utilizes root exudates components. Mol. Plant-Microbe Interact. 15:734-741.

Lin Q, Mendelsshohn IA (2009). Potential of restoration and phytoremediation with *Juncus roemerianus* for diesel-contaminated coastal wetlands. Ecol. Eng. 35:85-91.

Maila MP, Cloete IE (2002). Germination of *Lepidium sativum* as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil. Int. Biodeter. Biodeg. 50:107-113.

Malek-Hossein S, Gholamreza S (2007). Study of growth and germination of *Medicago sativa* (Alfalfa) in light crude oil–contaminated soil. Res. J. Agric. Biol. Sci. 3(1):46–51.

Nichols TD, Wolf DC, Rogers HB, Beyrouty CA, Reynolds CM (1997). Rhizosphere microbial Populations in contaminated soils. Water Air Soil Pollut. 95:165-178.

Obadori BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci. 8:203-208.

Onwuka GI (2005). Food Analysis and Instrumentation Theory and Practice. Naphth Ali Prints, Lagos, Nigeria pp. 144-145.

Pearson DA (1976). The chemical analysis of foods (7th ed.) Churchill and Livingstone, New York, pp. 6-19.

Raboy V (2002). Progress in breeding low phytate crops. Am. Soc. Nutr. Sci. J. Nutr. 67(6):2649-2656.

Sparrow SD, Sparrow EB (1988). Microbial biomass and activity in a subarctic soil ten years after crude oil spills. J. Environ. Qual. 17:304-309.

Urbano G, Lopez M, Aranda P, Vidal-valverde C, Tenorio E , Porrs J (2000). The role of phytic acids in legumes: Antinutrient or beneficial function? J. Physiol. Biochem. 56(3):283-294.

Wistreich GA (1997). Microbiology Laboratory: Fundamentals and Applications. Prentice-Hall Inc. New Jersey, USA pp. 144-186, 194-259.

Wyszkowska J, Kucharski J, Jastrzebska E, Illasko A (2001). The biological properties of the soil as influenced by chromium contamination. Polish J. Environ. Stud. 10:37-42.