Tropentag 2013, Stuttgart, Germany
September 17-19, 2013

Conference on International Research on Food Security, Natural Resource Management and Rural Development
organised by the University of Hohenheim

Effects of Crude Oil on the Physicochemical and Microbial Properties of Agricultural Soils grown with *Vigna unguiculata* and *Ammaranthus* sp.

Olukunle[a], O. F., Djossou[a], A. N. and Ewulo[b], B. S.

[a]Department of Microbiology, Federal University of Technology, P.M.B 704, Akure, Nigeria
[b]Department of Crop, Soil and Pest Management, Federal University of Technology, P.M.B 704, Akure, Nigeria

Abstract
An experiment was conducted in the screen house to ascertain the effects of crude oil on the physicochemical and microbiological characteristics of agricultural soil grown with cowpea *(Vigna unguiculata)* and leafy vegetables *(Ammaranthus* sp.). The response of *Vigna unguiculata* and *Ammaranthus* sp. to the contamination with crude oil, as well as the slight alteration of the physicochemical, physical and microbial characteristics show the deleterious effects of crude oil on agricultural soil and the negative impacts it has on our environment in general. Comparing the physicochemical, physical and microbial results; it was deduced that cowpea reacted almost instantly; (three days) after the introduction of the crude oil. This shows that cowpea has little or no ability to withstand crude oil spillage while *Ammaranthus* still exhibited some level of resistance to the crude oil especially from the least 5% (w/v) to the highest 11% (w/v) concentration. Microorganisms identified and isolated from soil samples were Bacillus cereus, *Bacillus megaterium, Clostridium sporogenes, Micrococcus luteus, Aspergillus fumigatus, Trichoderma viride, A. saprophyticus, Methylococcus capsulatus, Pseudomonas areoginosa, Acinetobacter calcoaceticus, Vibrio anguillarum, Penicillium notatum, Sporobolomyces salmonicolor* and *Rhbizopus nigrican*. However, the contamination had no significant effect on pH but rather on phosphorus, sodium, potassium, magnesium, calcium, organic carbon and organic matter content of the contaminated soils for *Vigna uiguiculata* compared to the uncontaminated, whereas; for soils with *Ammaranthus* sp; there were no significant effects for sodium but rather on phosphorus, pH, potassium, magnesium, calcium, organic carbon and organic matter content of the contaminated soils compared to the uncontaminated soil samples.

Keywords: Crude oil, agricultural soil, physicochemical parameters, microbial community, *Vigna unguiculata* and *Ammaranthus species.*

Introduction
Man's technological and scientific advances have caused environmental changes that are impossible to evaluate and fully comprehend. Pollution of the environment is one of the major effects of man's technological advancement. Pollution results when a change in the environment harmfully affects the quality of human life, animals, microorganisms and plants ((Okoh, 2006). Accidental and deliberate crude oil spills are significant sources of environmental pollution,

posing serious environmental problem such as damage to vegetation, soil-borne microorganisms and soil fertility. (Nweke and Okpokwasili, 2004).

The objectives of this study are to isolate and identify bacteria associated with agricultural soil before and after pollution with crude oil; determine the physicochemical properties of the agricultural soil before and after pollution with crude oil; and determine the morphological parameters of *Vigna unguiculata and Ammaranthus* sp. cultivated on polluted soils.

…

Material and Methods

Collection of soil samples

Agricultural soil samples were collected from the premises of the University Farm and transported to the Research Laboratories of the Departments of Microbiology and Crops, Soil and Pest Management for microbial and physicochemical analyses respectively according to (Holt *et al*., 1994; Barnett and Gibbons, 1982; and A.O.A.C, 2003).

Contamination of the soil samples

The soil samples were weighed (10kg each) into the planting pots of 20 litres capacity and arranged in two groups. Each group contained 12 pots each for cowpea and *Ammaranthus sp*. The contamination of the soil samples with crude oil was done at the concentrations of 5%, 8% and 11% while soil samples without contamination were used as control. Microbial and physicochemical analyses were determined before and after pollution.

Physical characteristics of the plants

The physical characteristics of both contaminated and uncontaminated plants (plant height, leaf area, number of leaves and branches and stem girth) were measured with the aid of vernier calliper, tape rule and ruler.

Cowpea seeds and *Ammaranthus seeds*

The cowpea seeds were obtained from the Agricultural Development Programme, (ADP), Alagbaka , Akure, Nigeria while the seeds of the *Ammaranthus sp.* were obtained from the Seeds Section of the Ondo State Ministry of Agriculture, off Ondo Road, Akure, Nigeria. Only viable seeds were planted and the seedlings were later thinned to two seedlings per plastic pot after three weeks to avoid overcrowding.

Statistical analysis of data obtwained

Data obtained were subjected to a single factor analysis of variance (ANOVA) while the significant means were separated with Duncan’s multiple range test (DMRT) at 5% confidence level (P = 0.05) using SPSS.

Results and Discussion

The microbial analysis of the soil before and after pollution shows that there were alterations in the microbial community after pollution. It was observed that *Bacillus megaterium, Clostridium sporogenes and Micrococcus luteus* were no longer present after two weeks of pollution with crude oil. This could be as a result of the competition for nutrients, carbon and energy source in the polluted soil. This finding corroborates that of Ijah and Antai (2003) who carried out similar research. However, more organisms were identified from the polluted soil. These organisms are likely to possess enzymatic capacity to degrade the crude oil. This same finding was reported by Olukunle and Boboye (2013).

Number of leaves, plants' height, number of branches, stem girth for both plants except leaf area (*Vigna unguiculata*) increased within the first two weeks of planting and decreased steadily after the pollution. *Vigna unguiculata* dropped its leaves after three days of introduction of crude oil, while *Ammaranthus* sp maintained its leaves but there were no increase in plant height for both plants, which correlates with the findings of Vavrek and Campbell (2000).

Pollution of the soil based on different treatment levels had negligible difference in pH values. The results obtained are at variance with that of Dennis (2009). The soil nutrients (N.P.K) were reduced after the pollution in case of *Vigna unguiculata* which may be responsible for the dropping of leaves after three days of pollution. According to Xu and Johnson (1997); oil spill can significantly reduce the availability of plant nutrients in the soil.

Conclusions and Outlook

The microbial community of unpolluted soil indicates that the soil has a stable and unaltered ecological pattern while the pollution with crude oil shows its harmful effect on the plants and altered the soil ecological pattern. The results obtained from soil physicochemical analysis imply that crude oil creates adverse condition to the soil composition which made oil spilled soils unsuitable for cropping. The microorganisms obtained from this research and *Ammaranthus* sp. are potential candidates for bioremediation and phytoremediation respectively.

References

1. AOAC, (2003), Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Washington, DC.
2. Barnette, H.L. and Gibbons, N.E. (1982). Illustrated Genera of Imperfect Fungi (4th ed.) Bingess Publishing Company, Minneapolis Minnesota pp. 241.
3. Dennis, H. (2009). The availability of minerals and soil pH influence growth in plants. Longman Group Limited, London pp. 26 -31.
4. Holt, J., G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T. and Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology*. Williams and Wilikins, Baltimore, USA. pp 786 -788.
5. Ijah, U. J. J. and Antai: S.P (2003) Research on Nigerian light Crude Oil in soil over 12 Month period. *International Bioremediation and Biodegradation* 51: 93-99.
6. Nweke, C. O and Okpokwasili, G.C. (2004). Effects of bioremediation treatments on the

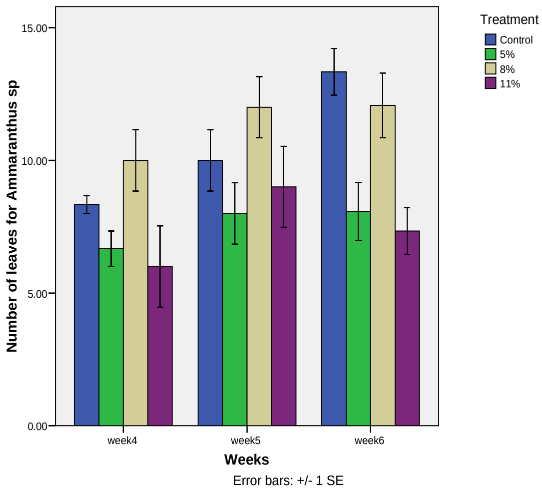
bacterial and fungi population of soil depths. *Nigeria Journal of Microbiology*,18 : 363-372.

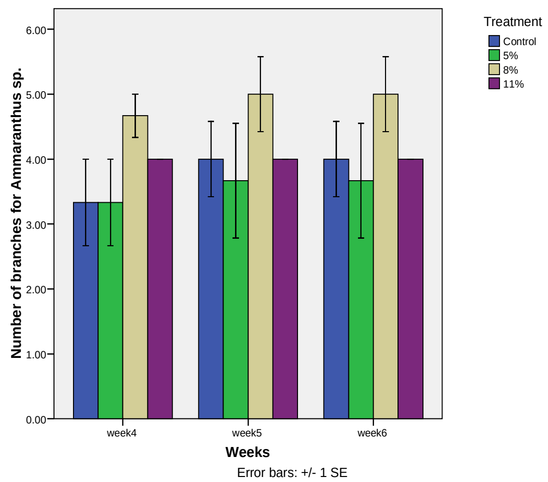
7. Okoh I. A. (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnology and Molecular Biology*. Revised 1 (2): 38-50.
8. Olukunle, O. F. and Boboye, B. (2013). The Molecular Succession of Bacterial Community of Crude Oil Polluted Soil and Water Samples from the Niger Delta, Nigeria. *British Journal of Applied Science & Technology* 3(4): 777-788.
9. Vavrek, M. C. and Campbell, W. J. (2000). Phytoremediation of petroleum: identification of plant traits that enhance degradation. Lousiana Applied Oil Spill Research and Development Program, OSRADP, Technical Reports Series 00 – 012
10. Xu, J.G. and Johnson, R.L.(1997). Nitrogen dynamics in soils with different hydrocarbon contents planted to barley and field pea. *Canadian Journal of Soil Science.* 77: 453- 458

Plate 1: Picture showing the wilting of Ammaranthus sp. and Vigna unguiculata along with the controls in the screen house on Day 7.

Table 1: Microbial analysis of unpolluted and polluted agricultural soils

Unpolluted soil	Polluted soil
Bacteria	**Bacteria**
Bacillus cereus	*Pseudomonas aeruginosa*
Bacillus megaterium	*Bacillus cereus*
Clostridium sporogenes	*Methylococcus capsulatus*
Micrococcus luteus	*Acinetobacter calcoaceticus*
Fungi	*Vibrio anguillarum*
Aspergillus fumigatus	***Fungi***
Trichoderma viride	*Trichoderma viride*
Aspergillus saprophyticus	*Aspergillus saprophyticus*
	Penicillum notatum
	Sporobolomyces salmonicolor
	Rhizopus nigrican

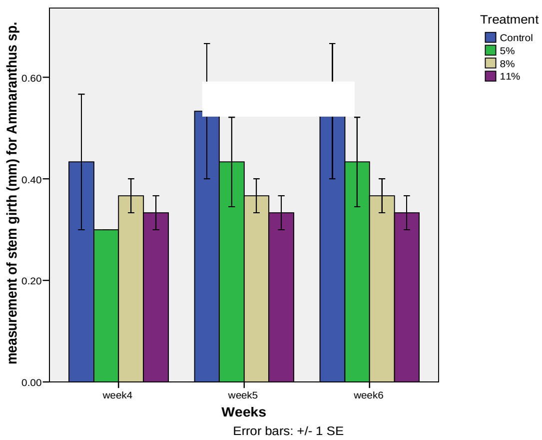




Fig

ure 1: Number of leaves for *Ammaranthus* sp. growing on polluted soil samples

Figure 2: Number of branches for *Ammaranthus* sp. growing on polluted soil samples



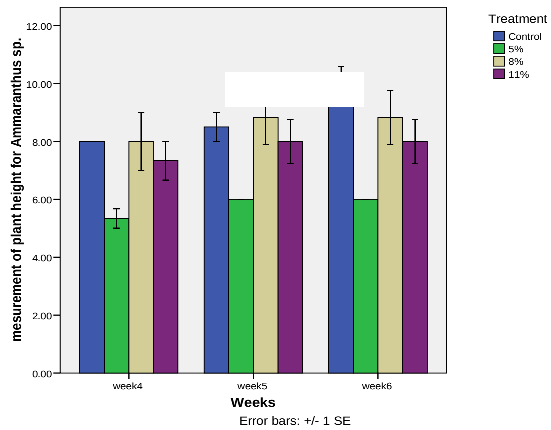
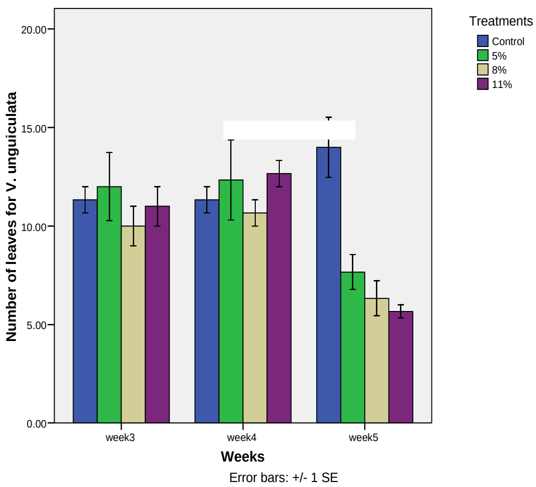


Figure 3: Stem girth for *Ammaranthus* sp. growing on polluted soil samples

Figure 4: The height of *Ammaranthus* sp. growing on polluted soil samples



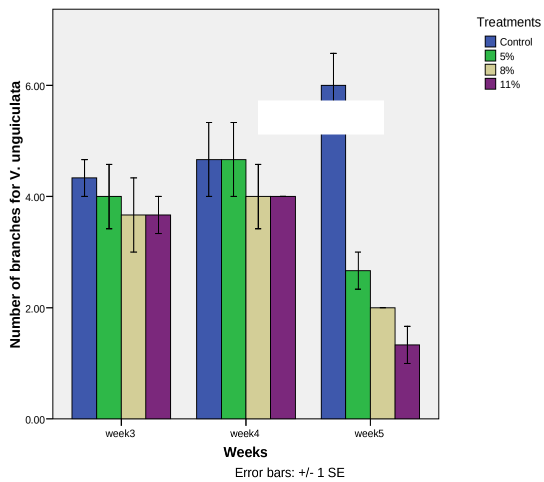


Figure 5: Number of leaves for *Vigna unguiculata* growing on polluted soil.

Figure 6: Number of branches for *Vigna unguiculata* growing on polluted soil.

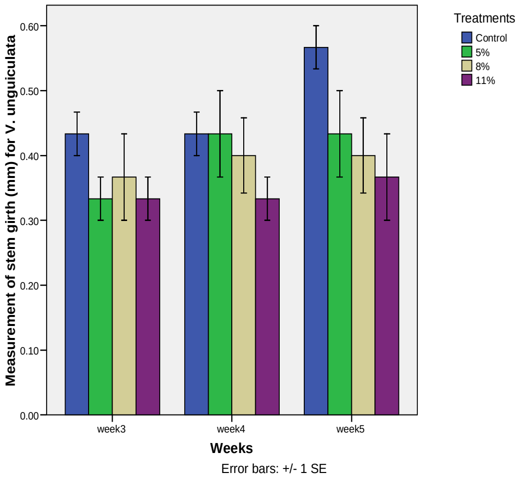


Figure 7: Stem girth for *Vigna unguiculata* growing on polluted soil.

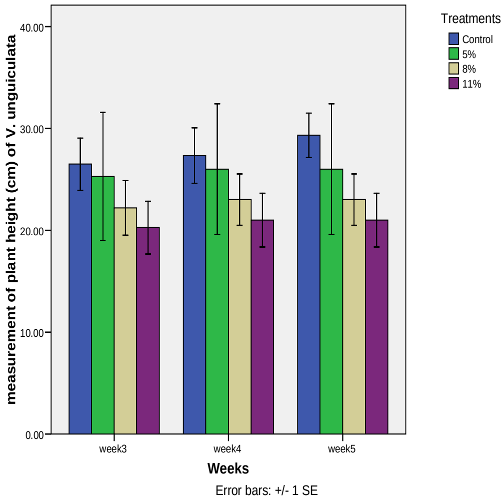


Figure 8: Plant height for *Vigna unguiculata* growing on polluted soil.