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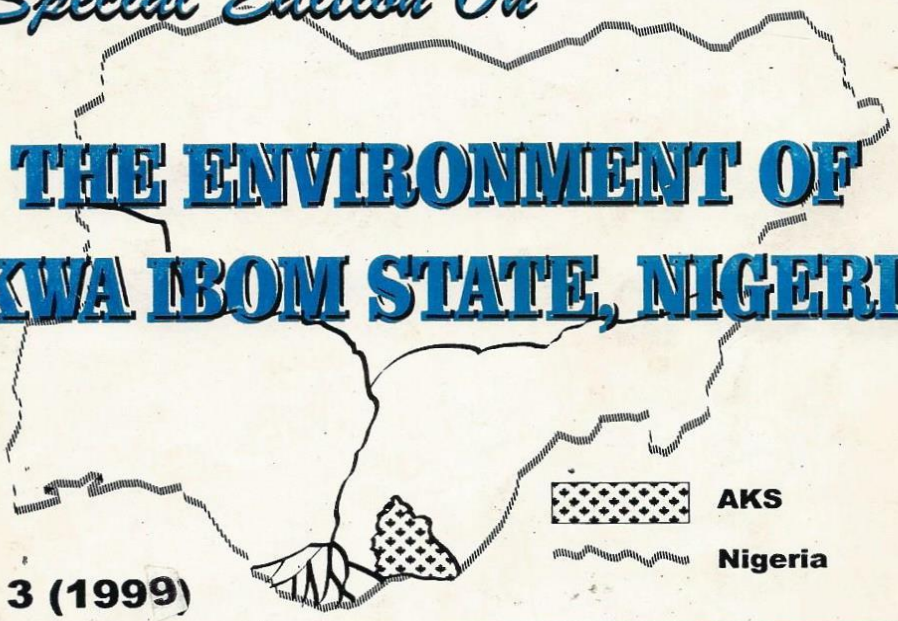

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Variation In Rhizosphere Microbiological Properties Of Vegetables Grown On Oil Contaminated Ultisol

by

J. P. Essien and I. R. Udotong
Department of Microbiology, University of Uyo, Uyo.

ABSTRACT

Analysis of the rhizosphere microflora of Capsicum annuum (pepper), Telfairia occidentalis (Fluted Pumpkin) and Abelmoschus esculentus (okra) grown on crude oil contaminated Akwa Ibom ultisol revealed a vast community of microorganisms. The density of the aerobic heterotrophs, actinomycetes, diazotrophic bacteria and fungi varied with the type of crop, and were slightly lower in oil contaminated than uncontaminated ultisols. The number of rhizosphere microflora was observed to increase with age of all the test crops except fluted pumpkin. The root system of fluted pumpkin tended to suppress the proliferation of aerobic heterotrophs and diazotrophic bacteria. Slight variations were also noticed on the species diversity of the isolates associated with the plant roots. Very few diazotrophic bacteria; Bacillus, Pseudomonas and Klebsiella species were isolated from the crops' root system. The significance of rhizosphere microflora to plant growth and soil fertility in stressed environment is highlighted in this report.

INTRODUCTION

Microorganisms are important for soil quality and fertility. They play a major role in the decomposition of organic matter, degradation of chemical pollutants and mineralization of nutrients in soil (Balloni and Favilli, 1987; Brussard, 1994). Microbes also influence the soil structure and aggregate stability (Gupta and Germida, 1988; Lynch and Bragg, 1985). The size of microbial mass is considered important, as a large biomass can store nutrients and is capable of cycling more nutrients through the system (Stenberg *et al,* 1998).

The root system of higher plants is associated not only with an inanimate environment which is composed of organic and inorganic substances but also with a vast community of metabolically active microorganisms referred to as rhizosphere organisms (Rovira and Davey, 1975; Alexander, 1977). The microbial community that responds to the presence of living roots is distinctly different from the characteristic soil community. The plants in this case create a unique subterranean habitat for microorganisms, and are in turn markedly affected by the population or activities of the microbes since the root zone is the site from which nutrients are obtained and through which pathogens must penetrate. Consequently interactions between the macro and microorganisms in this locule can be of considerable significance for crop production and soil fertility (Rovira and Davey, 1975).

Although products of microbial metabolism in the rhizosphere may be detrimental to plants (Neal *et al,* 1970), rhizosphere microflora are also known to favour plant development by producing growth stimulating substances. Rhizosphere microflora contribute to the formation of

stable soil structure, releasing nutrients through the mineralization of organic complexes, and by entering into symbiotic root association as in the case of diazotrophic (N-fixing) bacteria (Alexander, 1977). Phosphorus availability to plant is greatly influenced by microscopic rhizosphere inhabitants (Neal *at al,* 1975; Alexander, 1977). Because crops require appreciable quantities of phosphorus, changes in the assimilable phosphate concentration are of considerable consequences. Rhizosphere micro-organisms may also alter the level of sulphur toxicity in soil (Neal *et al,* 1979).

Contamination of soil by crude oil could lead to a depression of microbial density and activities even in cases of relatively light contamination (Odu 1972a; Odu, 1972b). Apart from its phytotoxicity, excess oil in soil may also limit the availability of nitrogen (a major plant growth element) in soil. The extent of the effects depends on the original soil properties and the plants exposed to contaminated soil. Assessing microbial response to pollution stress may provide basic information for the improvement of microbial activities in order to promote soil fertility and plant growth. This investigation was conducted to assess the variation in the rhizosphere microbiological properties of crops grown in oil contaminated ultisol in Akwa Ibom State.

MATERIALS AND METHOD

Test Soil:
The test soil, an acidic sandy loam soil classified as ferralitic sandy loam ultisol (D'Hoore, 1964) was collected from the botanical garden of University of Uyo, in Akwa Ibom State. The soil was obtained from an area not previously exposed to petroleum hydrocarbons.

Analysis of Soil:
The soil samples were air dried and passed through a 2mm sieve. The particle size distribution was determined by the pippette method (Gee and Bauder, 1986). Three size classes were estimated: <0.002mm (Clay) 0.002-0.2mm (Silt) and 0.2 - 3mm (Sand). The pH was determined in a 1:2 soil/water volume ratio. Total C and total soil N were determined after combustion at 1400°C using CNS 2000 Analyser (Leco Equipment Corporation, England). Total C was corrected to carbonate to give organic carbon (C org). Available P, K, Ca and Mg were extracted with the acetate ammonium lactate method (AL) (Black, 1965; Rhoades, 1982). The total hydrocarbon content (THC) of the soil was estimated by Spectrophotometry at 420nm wavelength after extraction with hexane.

Soil Treatment:
Each experimental unit was 3kg of soil contained in porous bottomed wooden boxes (60cm side) simulated with Qua Iboe Light (QIL) crude oil collected from Qua Iboe Terminal of Mobil Producing Nigeria Unlimited, Ibeno, Akwa Ibom State. The oil sample contained in sterile glass containers was added at a rate of 50.0gkg^{-1} soil, which is the range considered optimal for bioremediation (Dibble and Bartha, 1979). The boxes in three sets of triplicates (totaling 9 wooden boxes with oil simulated soil) were labeled and exposed to ambient conditions for one week. Another three sets of wooden boxes containing uncontaminated soil samples were also prepared to serve as a control.

Cultivation of Test Plants

Local cultivars of *Abelmoschus esculentus* (Okra), *Capsicum annuum* (Pepper) and *Telfairia occidentalis* (Fluted pumpkin) belonging to the families Malvaceae, Solanaceae and Cucurbitaceae, respectively, were obtained from Akwa Ibom State Agricultural Development Project (AKADEP). Apparently healthy seeds were sorted out and 10 seeds of each test crop were dibbled in the oil simulated soils. The seeds on germination were thinned to 6

seedlings per wooden box to create space for vigorous growth.

Control Experiment
Wooden boxes containing the garden ultisol but with no oil supplement were also cultivated with the test plants seeds, thinned on germination and allowed to grow. This treatment served as the control experiment.

Microbiological Analysis:
Enumeration of rhizosphere microflora:
The rhizosphere microorganisms of the vegetables were enumerated by the viable plant count method using surface spreading technique (Zuberer, 1994). Three samples of rhizosphere soil (plant root plus adhering soil) were carefully obtained per plant for each treatment (oil contaminated and uncontaminated (control) ultisols) during sampling, pooled and a 10g subsample was ground in a sterile mortar. These subsamples diluted to 100ml were considered to be 10^{-1} dilution. Further, serial ten-fold dilutions ranging from this 10^{-1} to 10^{-4} of the oil contaminated and uncontaminated soil samples were prepared. Volumes (0.1ml) of each dilution were plated on appropriate microbiological media.

The Bacto-plate count agar (Difco) plates, into which three drops of fungizone (50µg ml^{-1}) had been incorporated to inhibit fungal growth, were used for estimation of the number of aerobic bacteria. Triplicate plates from each dilution (10^{-1} to 10^{-4}) were prepared and incubated at 28±2°C for 48h before enumeration.

Bacto-Actinomycete agar (Difco) was used for the enumeration of actinomycetes in the rhizosphere of the crops. The pH of the medium was adjusted to 5.5 by the incorporation of lactic acid to suppress the growth of non-filamentous bacteria. The inoculated plates were incubated at 28± 2°C for 48h before enumeration. The number of diazotrophic bacteria was estimated on Bacto-nitrate agar (Difco). Inoculated nitrate agar plates were incubated at 37°C for 4 days before enumeration. Uninoculated nitrate agar plates were also incubated to serve as control.

The rhizosphere fungal flora of the vegetables were enumerated on Sabouraud dextrose agar (Difco) plates into which three drops of streptomycin (50µg ml^{-1}) has been incorporated to suppress bacterial growth. The fungal plates were incubated at 28 ± 2°C for 4 days before enumeration.

Isolation of Rhizosphere microorganisms
Discrete bacterial colonies, which developed on the plates were randomly picked and purified by sub-culturing onto fresh nutrient agar using the streak-plate technique. Isolated colonies, which appeared on the plates were then transferred onto nutrient agar slants and stored as stock cultures for further tests.

The fungi present in the rhizosphere of the crops were isolated using the same procedure as for bacterial isolation above but employing Sabouraud Dextrose Agar plates fortified with streptomycin. A portion of each fungal colony, which developed was picked using a sterile inoculating needle and aseptically sub-cultured onto fresh Sabouraud Dextrose Agar plates. The plates were kept as stock cultures for identification tests.

Characterization and Identification of the Isolates
The bacterial isolates were Gram stained, examined for motility, presence or absence of spores and ability to assimilate sugars. The probable identities of the isolates were determined using methods described by Skerman (1967) and Holt *et al* (1994). The fungal isolates were examined macroscopically and then microscopically using the needle mount method. Their identification was performed according to Hunter and Benneth (1975), Domsch *et al* (1980) and Samson *et al* (1984).

RESULTS AND DISCUSSION

Some properties of the test soils are listed in Table 1. The soil an acidic sandy loam soil (ultisol) with very low concentration of total hydrocarbon (94.2ppm) also contained considerable amount of N (0.42%), avail-P (8.62ppm) and organic C (1.10%). On simulation with 50.0g kg^{-1} soil of Qua Iboe Light Crude Oil, the avail - P and organic C contents of the ultisol were raised to 10.12ppm, and 4.23% respectively. Also, the total N content as well as the concentrations of Ca, K and Mg in the soil were slightly reduced to 0.13%, 0.4 meq/100g, 0.98 meq/100g and 7.2 meq/100g, respectively, within one week after simulation. The simulated soil had THC of 178.4 ppm.

Microbiological analyses of the rhizosphere of the crops cultivated in the oil contaminated and uncontaminated ultisol revealed the presence of a vast community of microorganisms in the surrounding and on the surface of roots and root hairs. The slight change in the properties of the soil simulated with crude oil affected the rhizosphere microbial counts of the vegetables. The properties however varied with the different types of crops cultivated on the soil (Table 2). There was a remarkable increase in the total aerobic bacterial counts of the rhizosphere of pepper and okra with age of plant. On the other hand, a gradual reduction in the total aerobic bacterial density was observed in the rhizosphere of fluted pumpkin grown in contaminated ultisol. These results are in agreement with earlier results by Odu (1972a; 1972b) who reported a selective increase or decrease in microbial population indices of crude oil contaminated soils. The high incidence of aerobic bacteria in the rhizosphere of the crops was expected and may be ascribed to high level of accumulation of metabolic wastes in the root zone of the plants. Rovira and Davey (1975) and Alexander (1977) have reported similar observations. It has also been reported that the root zone of plants is the site which accumulates more nutrients in soils, and the increase in rhizosphere microbial population of plants corresponds to increase in the amount of nutrients accumulated in the site (Gupta and Germida, 1988). Thomas-Banzon *et al* (1982) reported selective enhancement of certain categories of bacteria by plant root systems. In an earlier investigation, Brown (1975) observed that the plant rhizosphere is a highly favourable habitat for the proliferation and metabolism of various microbial types.

However, the comparatively low count of aerobic bacteria observed in the rhizosphere of fluted pumpkin is not in agreement with reports by other investigators. The low counts may be attributed to certain changes in the physiological conditions of the rhizosphere environment of the plant. Odoemena and Essien (1995) reported the presence of certain antibacterial compounds such as glycosides, triterpenes, alkaloids and saponins in pumpkin root tissue. These anti-oxidative secondary metabolites may be released as components of root exudates, especially by plants under stress.

The population of actinomycetes in the rhizosphere of the crops also varied with the type of plant. In this case, okra plant root system harboured the highest number of filamentous bacteria. The relatively low counts of actinomycetes encountered in the crops rhizosphere were not unexpected. The low counts may be attributed mainly to the period of sampling. This investigation was conducted during the rainy season, while actinomycetes are known to be more numerous and common in dry soils than in wet soils (Alexander, 1977). The counts of N-fixing bacteria in the crops rhizosphere were also low despite the high numbers of aerobic bacteria enumerated in both the oil contaminated and uncontaminated (control) soils. The values obtained for aerobic bacteria although positively related with diazotrophic bacterial counts were about six times higher than the counts of the later. Thomas-Bouzon *et al* (1982) reported a 5%

contribution to total bacterial count by N-fixing bacteria in the rhizosphere of rice. A few species of diazotrophic bacteria that would probably participate in the process of nitrogen fixation in soil were encountered (Table 4).

In contrast to the bacterial counts the fungal counts in the rhizosphere of the crops did not vary markedly between crop types. However the values observed were generally higher in the rhizosphere of all the crops except okra, grown on oil-contaminated soil. This result further confirmed reports on the selective enrichment effect of plants on microorganisms in the root system. The number of bacterial species associated with the root systems of the crops appeared to have been considerably influenced by the presence of crude oil in soil (Table 3). The effect was more marked in the rhizosphere of fluted pumpkin grown on oil-contaminated soil. This result may be attributed partly to the antimicrobial potential of pumpkin root (Odoemena and Essien, 1995) and partly to the absence or presence of microbial inoculum in the root vicinity. The individual genetic traits of the isolates may have also influenced their survival and availability in the stressed rhizosphere.

The role of microorganisms as organic matter decomposers, pollutants degraders, and in the mineralization of nutrients in soils (Brussard, 1994; Lynch and Bragg, 1985) make their presence in plant root systems very important and as a sign of plant well being and improved soil fertility. The rhizosphere flora obtains their carbon and energy source from the plant. The organisms have been reported to utilize about 20% of the carbon dioxides assimilated by the plants thereby playing a role in the carbon allocation from plant to soil (Jakobsen and Rosendahl, 1991). These plants receive in return mineral nutrients like P, N, K, Ca, Mg, Zn and Cu from the organisms. It therefore implies that crops (e.g pepper) with rich rhizosphere microbiological endowments are highly favoured to grow better in crude oil contaminated ultisol (with limited concentrations of N and P) than those (e.g fluted pumpkin) with poor rhizosphere microbiological quality. This is obvious in the poor performance of fluted pumpkin in most oil-contaminated communities of the Niger Delta.

Table 1: Some physicochemical properties of the Akwa Ibom ultisol investigated

Soil Properties	Oil contaminated ultisol	Uncontaminated ultisol (control)
pH	6.56	6.80
K (Meq/100g)	0.98	1.13
Mg (Meq/100g)	0.72	1.01
Ca (Meq/100g	1.4	1.9
Available Phosphorus (ppm)	10.12	8.62
Organic Carbon (%)	4.23	1.10
Total Nitrogen (%)	0.31	0.42
C/N ratio	13.65	10.1
Sand (%)	80.1	80.0
Silt (%)	14.4	14.5
Clay (%)	5.5	5.5
THC (ppm)	178.4	94.2

Values are means of duplicate determinations

Table 2: Microbial density in the rhizosphere of vegetables grown in oil contaminated (P) and uncontaminated (C) ultisols.

Crop	Treatment	Age (days after germination)				
		14	28	42	56	72
		(a) Aerobic bacterial count (x 10^3 CFU/g)				
Pepper	P	15.3	16.4	18.5	20.1	21.4
	C	12.0	14.5	17.4	20.4	26.2
Pumpkin	P	9.6	9.6	6.2	6.4	5.8
	C	6.6	6.8	5.8	5.7	7.5
Okra	P	7.2	14.5	18.0	18.8	20.3
	C	6.5	9.5	16.4	19.4	24.5
		(b) Actinomycete count (x 10^2 CFU/g)				
Pepper	P	1.5	2.0	2.0	2.0	1.8
	C	2.4	3.0	2.4	3.0	3.5
Pimpkin	P	1.5	2.0	2.0	1.5	3.0
	C	2.0	3.0	2.5	3.0	3.0
Okra	P	1.0	1.5	2.0	3.0	2.5
	C	3.0	4.2	2.5	3.6	3.5
		(c) Diazotrophic bacterial count (x 10^1 CFU/g)				
Pepper	P	0	0.5	1.4	1.6	1.5
	C	0.5	2.0	1.5	0.5	1.0
Pumpkin	P	1.5	2.0	1.5	2.0	1.5
	C	1.0	3.0	1.2	2.0	1.5
Okra	P	2.0	1.0	1.5	1.0	1.5
	C	1.5	1.0	2.0	2.0	2.0
		(d) Fungal count (x 10^3 CFU/g)				
Pepper	P	1.5	1.8	2.0	3.4	3.6
	C	2.0	2.5	2.5	3.0	3.2
Pumpkin	P	1.5	2.6	1.8	2.0	3.4
	C	2.0	2.0	2.0	1.8	2.8
Okra	P	2.0	3.0	2.0	1.8	3.0
	C	3.0	4.0	3.0	2.0	4.0

Values are based on the mean count of colony forming unit (CFU) per gram of three determinations of rhizosphere soil.

Table 3: Microbial types isolated from the rhizosphere of crops grown in oil contaminated and uncontaminated ultisol

	Contaminated Ultisol			Uncontaminated Ultisol (Control)		
Microbial Isolate	**P**	**F**	**O**	**P**	**F**	**O**
1. BACTERIA						
Aeromonas sp	+	-	+	+	-	+
Acinetobacter sp	+	-	-	+	+	+
Bacillus subtilis	+	+	+	+	+	+
Bacillus megaterium	+	+	+	+	+	+
Enterococcus faecalis	+	-	+	+	-	+
Escherichia coli	-	-	-	+	-	+
Klebsiella sp	-	-	+	+	+	+
Lactobacillus sp	+	+	-	+	+	+
Micrococcus sp	+	-	+	+	-	+
Pseudomonas sp	+	-	+	+	-	+
Serratia indica	+	-	+	+	-	+
Staphylococcus sp	-	-	+	+	-	+
Xanthomonas sp	+	+	+	+	+	+
Total	**10**	**4**	**10**	**13**	**6**	**13**
2. ACTINOMYCETES						
Micrononospora sp	-	-	+	-	-	+
Nocardia sp	+	+	-	+	+	+
Streptomyces sp	+	+	+	+	+	+
Streptosporangium sp	-	-	-	-	-	-
Total	**2**	**2**	**2**	**2**	**2**	**2**
3. FUNGI						
Aspergillus flavipes	+	+	-	+	+	+
A carbonarius	-	-	+	-	+	+
A. terreus	+	+	+	+	+	+
A. niger	+	+	+	-	-	+
A. versicolor	-	-	-	+	-	-
Candida tropicalis	-	+	-	-	-	+
C. pseudotropicalis	-	+	-	+	-	-
Cephalosporium sp	-	-	+	+	-	+
Fusarium moniliforme	+	-	+	-	-	+
Fusarium roseum	+	-	+	+	-	+
F. tricinctum	-	-	+	-	-	+
Penicillium fequentans	+	+	-	+	+	+
P. notatum	-	+	-	+	+	+
Mucor hiemalis	-	-	+	+	-	+
Total	**6**	**7**	**7**	**9**	**5**	**11**

+=Present; - = Absent or present at very low counts; P = Pepper; F = Fluted Pumpkin; O = Okra.

Table 4: Probable diazotrophic bacteria isolated from the rhizosphere of crops grown in oil contaminated and uncontaminated ultisols

	Contaminated soil			Uncontaminated soil		
Isolate	**P**	**F**	**O**	**P**	**F**	**O**
Bacillus megaterium	+	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+	+
Pseudomonas sp	+	-	+	+	-	+
Klebsiella sp	-	-	+	+	+	+
Total	**3**	**2**	**4**	**4**	**3**	**4**

+ = Present; - = Absent or present at very low counts; P = Pepper; F = Fluted pumpkin; O = Okra

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