Journal of Environmental Sciences Vol. 12, No. 3, pp. 296—302, 2000

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2000)03-0296-07 **CLC number**: X17 **Document code**: A

Distribution of actinomycetes in oil contaminated ultisols of the Niger Delta (Nigeria)

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Abstract: The distribution of actinomycetes in oil contaminated sandy loam ultisols of the Niger Delta region of Nigeria was studied to aid in understanding the effect of hydrocarbons on indigenous microbial population in tropical soils. The average total counts of actinomycetes in all the oil samples analysed was 10^3 cfu/g. Higher counts of actinomycetes were observed during the dry season than during the wet season. The counts of hydrocarbonoclastic actinomycetes correlated positively with the total count of actinomycetes. The actinomycetes were generally restricted to the top soil (0—10 cm soil depth) although a seemingly deeper (down to 40 cm soil depth) distribution was noticed in the dry season. The isolates included oil degrading species of *Actinoplanes*, *Norcadia*, *Streptomyces* and *Streptosporangium*. Their high oil utilization ability indicates their positive potential and role in the bioremediation of oil-spilled soils.

Key words: actinomycetes; oil contaminated ultisols

Introduction

Actinomycetes (filamentous bacteria) constitute a group of microorganisms that is widely distributed in soil. Plating estimates give values ranging between 10^5 and 10^8 colony forming units (cfu) per gram of soil in temperate zone although lower values have been recorded in the region of Antarctica, acid peat, the tundra and waterlogged soils (Sykes, 1973; Walker, 1975). When compared with the true bacteria, actinomycetes are less common in wet than in dry areas (Alexander, 1977). Their population is likewise greater in grassland, pasture soil than cultivated lands. Waterlogged soils and soils with pH less than 5.0 are unfavourable to the growth and proliferation of actinomycetes (Sykes, 1973; Cambell, 1982). Soils in warm climatic region are more conductive to extensive actinomycetes flora than those in colder areas, and the size of their community in temperate latitudes tends to increase as one comes close to the tropics (Walker, 1975). Their density in soil changes seasonally and are affected by cultural practices (Campbell, 1976; Alexander, 1977).

In Nigeria there are basically two season: the dry season which occurs between November and March and the wet or rainy season which occurs between April and October. The soil temperature is usually higher during the dry season (30—45℃) while the soil temperature during the wet season usually ranges between 20—30℃.

The soils of the Niger Delta region in Nigeria are generally acid soil with pH ranging from 3 to 5.1 (Kamalu, 1992). Lower pH values have been reported in the histosols (which comprised mainly of acid sulphate and organic salts of the mangrove deposits) and mineral soils (ultisols, entisols and inceptisols) of the coastal plane of the Niger Delta.

These soils are often contaminated with hydrocarbon pollutants resulting from accidental discharges through the activities of petroleum industries located in the region (Odu, 1972; Antai; 1989; Ijah, 1993). The possible effect of petroleum pollutants on soil properties include changes in redox potential, reduction in soil wetability, increase in organic matter content, increase in soil acidity, reduction in the bulk density of soil and increase in micropores (Odu, 1977; Roscoe, 1989). Such alterations in soil properties may lead to a depression of microbial number and

activities even in cases of relative light contamination(Odu, 1972; Rowel, 1977; Antai, 1989). On the other hand, increase in microbial count due to selective destruction of unresistant microorganisms by hydrocarbon contaminants have been reported by Raymond *et al*. (Raymond, 1980) and Harpal *et al*. (Harpal, 1982).

Few actinomycetes (*Endomyces*, *Nocardia* and *Streptomyces* species) are known to posses hydrocarbon degrading ability and could utilize crude oil as a source of carbon and energy(Harpal, 1982). In this study, the seasonality in the population dynamics of actinomycetes in hydrocarbon contaminated ultisols of the Niger Delta were examined. The study was aimed at ascertaining the oil degrading ability of the isolates obtained from oil spill areas.

1 Materials and methods

1.1 Study area

The study was carried out in Rumuekpe (R) in Emohua local government area of Rivers State and Ukpenekang (U) in Ibeno local government area of Akwa Ibom State. Both areas are oil producing communities located within the coastal (Niger Delta) region of Nigeria(Fig. 1). The region is characterized by bimodal rainfall pattern and acidic sandy loam soils classified as ferralitic sandy loam ultisols(D' Hoore, 1964). The investigation was conducted during the wet season(May to August 1996) and dry season(November 1996 to February 1997).

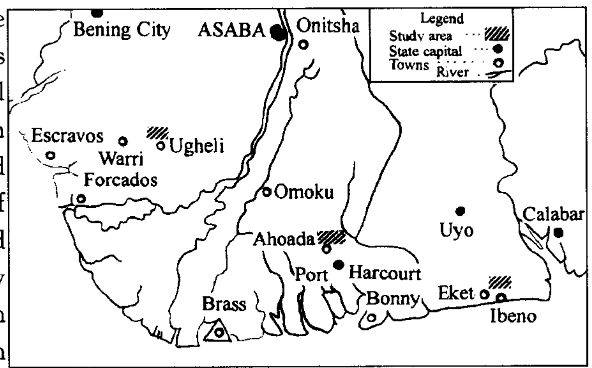

Fig. 1 The Niger Delta region showing the locations of the study areas

1.2 Soil characteristic

The chemical and physical characteristic of the soils(0—10 cm) are summarized in Table 1.

Table 1 Characteristics of oil contaminated soils (sandy loam ultisols) investigated

Properties	Study sites	
	Rumuekpe(R)	Upenekang(U)
pH	5.4	5.8
Organic C, %	4.64	4.74
Total N, %	0.06	0.07
Avail-P, ppm	0.035	0.033
C/N ratio	77.33	67.71
C/P ratio	13.0	14.1
Exchangeable cations, meq/100g		
K	3.3	3.3
Na	5.57	6.8
Mg	5.57	6.7
Particle size, %		
Sand	82.3	83.6
Clay	11.3	10.6
Slit	6.6	6.5
Oil content, %	15.6	14.6

Notes: values are mean of duplicate determinations

Soil pH was measured in 1:2 soil water suspension using a Pye Unicam pH meter. Particle size distribution of the soils were determined by combination of wet sieving and hydrometer technique in duplicate sample(Allison, 1965; AOAC, 1984; Duo, 1986).

The organic carbon was determined by the modified dichromate wet oxidation method of Allison(Allison, 1965). The total nitrogen was estimated by the microkjeldahl method. The *P*-value in the soil was determined after extraction by 0.5mol/L $NaHCO_3$, solution and exchangeable cations were determined after extraction by 1mol/L NH_4-acetate solution, using the flame photometer(AOAC, 1994; Udo, 1986). The oil content in the soil was determined by the soxhlets technique(Frais, 1972; Nyborg, 1975).

The carbon to nitrogen and carbon to phosphorus relation were based on the total content of nitrogen and phosphorus in the soil.

1.3 Microbial analysis

Soil samples(10 cm deep) obtained from the study sites were analysed with 8 hours after sampling, using appropriate analytical media.

1.3.1 Enumeration of actionomycetes

The total number of actinomycetes in soil were estimated by the viable plate count method using nutrient agar. The pH of the nutrient agar was adjusted to 5.5 with lactic acid to arrest the growth of non filamentous bacteria. Serial dilutions ranging from 10^{-1} to 10^{-10} of the soil samples were prepared. Quantities of each dilution(1 cm^3) were plated on nutrient agar. Triplicate plates from each dilution (10^{-3} to 10^{-10}) were prepared and incubated at 30℃ for 7 days before enumeration.

The variation in the distribution of actinomyteces with soil depth was assessed by the laboratory soil test column method described by Harpal *et al*. (Harpal, 1982). Soil samples obtained at 10, 20, 30 and 40 cm soil depths(in a 150 cm high and 30 cm diameter soil test column) were analysed.

1.3.2 Enumeration of hydrocarbonoclastic actinomycetes

The soil degrading actinomycetes in the soil samples were enumerated using the surface spreading technique. Serial dilutions of the soil were prepared as described above. One cm^3 of each diluent was plated on ZS agar(Zajic, 1972) to serve as controls. Triplacte plates from each dilution (10^{-3} to 10^{-10}) were prepared and incubated at 30℃ for one week. Counts of oil degrading actinomycete were obtained by subtracting the counts on ZS agar plates from the counts on oil agar plates. The differences in the counts represented the total number of oil degrading actinomycetes. This method has previously been used by Zajic and Supplisson (Zajic, 1972) and Antain and Mgbomo(Antia, 1989).

1.3.3 Characterization of isolates

The isolates were purified by subculturing on fresh nutrient agar plates. The colony morphology and pigmentation were determined by visual observation. The cells shape were examined microscopically, while the diameter of the cell(hypha) was measured by means of a calibrated ocular micrometer. The isolates ware then characterized based on the methods described by Alexander (Alexsander, 1977) and Halt *et al*. (Halt, 1994). Table 2 shows the major characteristics of the isolates.

Table 2 Colony morphology and biochemical characteristics of actinomycetes isolated from hydrocarbon contaminated soil

Characteristics	Isolate code			
	UR_1	UR_2	UR_3	UR_4
Colony morphology	Smooth moist	Smooth	Hard dripping	Dry and rough
Pigmentation cell shape	Hyaline filamentous	Hyaline filamentous branched no fragments	Whitish filamentous small rough	Translucent extensive hyphae no fragments
Aerial hyphae	+	+	+	+
Hyphal size, μm	0.2—2	1.4—2	1.6—2.1	1—1.5
Spores	Conidia in spore sac	Conidia exist singly	−	Chained conidia
Gram stain	+	+	+	+
Catalase test	−	−	+	+
Oxidase test	+	−	+	−
Nitrate reduction	+	+	+	+
Sugar fermentaion				
Adonitol	−	NA	−	−
Lactose	−	−	−	+
Galactose	−	−	−	−
Inositol	−	−	−	−
Maltose	+	+	+	+
Mannitol	±	+	+	+
Sorbitol	NA	NA	−	−
Xylose	−	−	−	−
Probable organism	*Actinoplanes*	*Micromonospora*	*Norcadia*	*Streptomyces*

Characteristics	Isolate code		
	U_5	U_6	R_7
Colony morphology	Smooth	Smooth	Dry and rough
Pigmenttion cell shape	Hyaline slender filaments	Hyaline filamentous branched with	Extensive no fragments
Aerial hyphae	+	+	+
Hyphal size, μm	1.2—1.5	1.4—2	1—1.5
Spores	Condia in sporangium	Condia in pairs	Macro chains
Gram stain	+	+	+
Catalase test	−	+	+
Oxidase test	+	+	+
Nitrate reduction	−	−	−
Sugar fermentaion			
Adonitol	−	NA	−
Lactose	±	−	+
Galactose	−	−	−
Inositol	−	−	−
Maltose	+	+	+
Mannitol	+	+	+
Sorbitol	NA	NA	NA
Xylose	NA	NA	+
Probable organism	*Streptosporangium*	*Microbispora*	*Microellobosporia*

Notes: + : positive reaction; − : negative reaction; ± : variable reaction; NA: not applicable

* Isolate codes depicts the site of isolation (R = Rumuekpe; U = Upenekang)

1.3.4 Estimation of oil degrading capabilities of the isolates

The rate and extent of oil degradation by hydrocarbonoclastic actinomycetes isolated from soil samples were measured according to the method of Odu(Odu, 1972). In assessing this, 0.1 cm^3 of the active inocula from each isolate was inoculated into 4.85 ml of ZS broth in tubes. A total of 8 tubes were set up for each organism. Tubes containing 4.85 cm^3 of ZS broth plus 0.5 cm^3 of crude oil but with no organisms served as control experiment. All the tubes were incubated for 25 days at 28℃. The amount of oil left in the tubes were determined every 5 days using the method (weight loss) described by Odu(Odu, 1972):

$$\frac{\text{weight of crude oil(control)} - \text{weight of crude oil(degraded)}}{\text{weight of crude oil (control)}} \times 100.$$

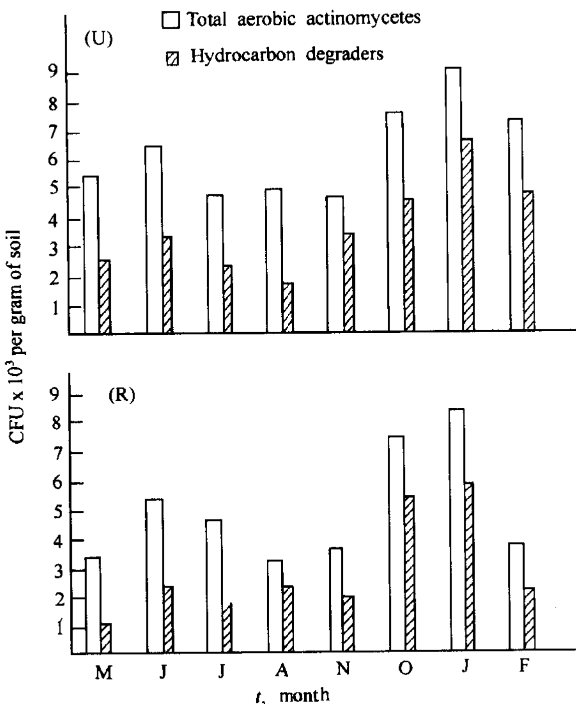

Fig. 2 Histograms showing counts of total aerobic actinomycetes and hydrocarbon degraders, for each month at Rumuekpe (R) and Upenekang(U)

2 Results and discussion

An average actinomycetes count of 10^3 cfu/g of soil was obtained from the two study sites (Rumuekpe and Upenekang). Total count of actinomycetes were higher during the dry season than during the rainy season(Fig. 2) indicating seasonal variation in the number of actinomycetes present in oil spilled soil. In soil samples collected from Rumuekpe(R) spill site, the highest mean total count of 8.4×10^3 cfu/g of soil was obtained in January(mid-dry season). The lowest mean total count of 3.2×10^3 cfu/g for actinomycetes was obtained in August during the peal of the rainy season (Fig. 2). At Upenekang(U) spill site, the highest mean total count actinomycetes was 9×10^3 cfu/g and this was also recorded in January during the dry season; while the lowest count of 4.6×10^3 cfu/g was obtained in the month of July during the wet season (Fig. 2). The counts of oil degrading actinomycetes were positively correlated with the total count of actinomycetes obtained from the soils.

The lower counts of actinomycetes obtained during the rainy season may be attributed to the adverse weather condition in the tropics(heavy rainfall) during the winter. During the rainy season oil polluted soils are waterlogged producing reducing conditions in areas of poor drainage(Odu, 1977; McGill, 1977; Roscoe, 1989). This condition does not favor the growth of filamentous bacteria which are mostly obligate aerobes. There is a possible decrease in the oxygen level of oil contaminated ultisols during the rainy season. The lesser number of actinomycetes obtained from Rumuekpe may be attributed to the slight higher oil content of the soil and other oil induced changes in soil properties that was not investigated.

A total of seven genera of actinomycetes were isolated from the two sites. Five of the isolate were present in soils from Rumuekpe and six in Upenekang soil samples (Table 3). Microellobosporia sp. (R_7) was isolated only from Rumuekpe soil, while *Streptosporangium* (U_5)

and *Microbispora* (U_6) species were obtained only in Upenekang soil.

The population of actinomycetes in soil decreased markedly with soil depth, which is in agreement with the findings of other researchers (Harpal, 1982; Highashida, 1985). The low number of actinomycetes in the lower segments of the soil may be ascribed to the combined effect of decreasing amount of organic substrate and poor aeration of the deeper soil layers (Higashida, 1986). More actinomycetes were observed in the upper segment (0—10 cm soil depth) of the soil columns in both sites investigated (Fig. 3). However, a deeper distribution (40 cm soil depth) was noticed in the dry season. At 40 cm soil depth, actinomycetes genera isolated were Microbispora and Norcadia species. The species high level of fragmentation into small round hyphal spores (Alexander, 1977) may have contributed to their widespread distribution.

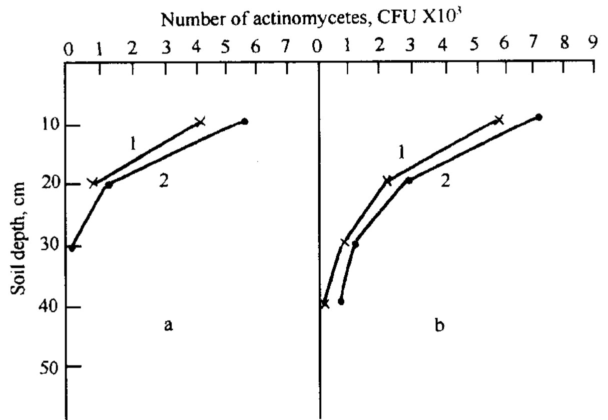

Fig. 3 Comparison of distribution of actinomycetes population in soil columns during the (a) wet and (b) dry season (values represents the mean of monthly counts recorded for 4 months per season)
1. actinomycetes in Rumuekpe soil; 2. actinomycetes in Upenekang soil

The quantitative variation in the distribution of actinomycetes with changes in season and between study sites were not sufficient to significantly alter the species diversity indices (Table 3). While ecologist have noted that a decrease in diversity often accompanies environmental stress (Hood, 1975), this is by no means a univerisal observation, particularly where microbial communities are concerned. Wassel and Mills (Wassel, 1983) found a significantly lower diversity at sites affected by acid mine drainage than at control sites. On the other hand, Hood *et al*. (Hood, 1975) reported an increase in the species diversity of resistant bacterial enrichment effect. In this investigation only four (*Actinoplanes*, *Norcadia*, *Streptomyces* and *Streptosporangium* species) out of the seven species of actinomycetes isolated from the oil polluted soil showed a remarkable ability (78% weight loss of bonny light crude oil within 35 days of incubation) to oxidize hydrocarbon (Table 4).

Table 3 Actinomycetes genera in hydrocarbon contaminated Nigerian ultisol and their oil degradability

Isolate	Code	Study site		Oil degradability
		Rumuekpe(R)	Upenekang(U)	
Actinoplanes sp.	UR_1	+	+	+
Micromonspora sp.	UR_2	+	+	–
Nocardia sp.	UR_3	+	+	+
Streptomyces sp.	UR_4	+	+	+
Stretosporangium	U_5	–	+	+
Microbispora sp.	U_6	–	+	–
Microellobospora sp.	R_7	+	–	–
Total		5	6	4

Notes: + : present or ability to degrade oil; – : absent or inability to degrade oil. Oil degradability of isolates was confermed by their growth on oil agar.

Table 4 Weight losses from bonny light crude oil resulting from the growth of actinomycets

Incubation period(weeks)	*Norcadia* sp.	*Streptomyces* sp.	*Streptosporangium* sp.	*Actinoplanes* sp.
1	18 ± 1.2	20.3 ± 1.4	16 ± 1.2	10 ± 2.2
2	48 ± 0.9	51 ± 1.2	21 ± 1.4	14 ± 2.3
3	56 ± 1.2	58 ± 2.2	25 ± 2.2	16 ± 2.2
4	67 ± 2.4	68 ± 2.4	34 ± 1.4	18 ± 1.0
5	73 ± 0.1	78 ± 1.1	41 ± 1.2	22 ± 1.4

Notes: significant at 95% probability level. Standard deviation are based values obtained from three replications

It is obvious from the results of this study that although actinomycetes are present in very low numbers in oil polluted soils, they play active role in the degradation of crude oil in tropical ultisols.

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(Received for review March 23, 1999. Accepted May 15, 1999)