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SEASONALITY IN FUNGISTASIS OF PETROLEUM CONTAMINATED NIGERIAN COASTAL SOIL

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ABSTRACT

Research was carried out to aid in understanding the seasonality in the fungistatic effect (a fertility attribute) of petroleum contaminated coastal soil of Nigeria. Using the agar disc technique and *Mucor hiemalis* as test organism, a high level of fungistatic effect was observed in the soil samples tested for both (dry and rainy) seasons. The effect however varied between the seasons. A stronger fungistatic power of the contaminated soil was observed during the dry season, (which recorded a mean spore germination percentage; MGP of 8.8%) than in the rainy season (with a MGP of 17%). The highest level of soil fungistasis was observed in February during the dry season when a total loss in spore germinatability was noticed. On the other hand the least fungistatic effect of the soil was encountered in June (MGP = 20%) during the rainy season. The low moisture level and concentrated effect of soil oil content (due to high soil temperature) were identified as the probable fungistatic factors responsible for low spore germination rate during the dry season. The inhibited spores were significantly (at 95% probability level) transformed by treatment with 1.0% (w/v) glucose solution. The mean transformed germination percentages (MTGP) of 5% and 7% were observed respectively in the dry and rainy seasons. This is an indication that soil fungistatisis inhibit spores germination rather than destroy the viability of the spores and could be annulled by the provision of an organic nutrient supplement. Its implication to bioremediation of oil polluted soils is discussed.

Keywords: Seasonality, Petroleum, Fungistasis, Soil, Bioremediation.

INTRODUCTION:

Drastic changes in soil biophysicochemical properties that would affect soil fertility are common in petroleum contaminated soils (Jones 1969, Odu 1978 and Roscoe et al 1998, Holliday and Deuel 1994). Microbial hydrocarbon metabolism usually lead to the release of nutrients in oil polluted soils (Odu 1978 b, Dragun 1993, Holliday and Deuel 1994). However, their ability to metabolise hydrocarbons in natural environments is determined by the prevailing environmental stresses.

Microbial decomposition of hydrocarbons has been demonstrated at temperature as low as 5° C and as high as 70° C (Zobell 1969). Petroleum hydrocarbons are oxidized ten to twenty times more rapidly at 25° C than at 50° C (Odu 1977). Holliday and Deuel (1994) observed a much higher level of hydrocarbon degradation in soils amended with organic nutrient supplements than in soil with low nutrients level.

Two seasons are basically observed in Nigeria. These are the dry season which occurs between November and March and the rainy seasons between April and October. The soil temperature is generally higher during the dry season ranging between 30 and 45°C while the soil temperature during the rainy season ranges from 20 to 30°C (Antai and Mgbomo 1989). This investigation was conducted to examine the effects which the two seasons have on the fungistatic properties of petroleum contaminated soil obtained from Rumuekpe oil field in Emohua Local Government Area of Rivers State, Nigeria.

MATERIALS AND METHOD

STUDY AREA

The oil spill sites investigated are located at Rumuekpe, a village in Emohua local government area of Rivers State, Nigeria. The spill which occurred in 1994 resulted in hundreds of barrels of crude oil being introduced into the soil. Three sampling sites designated A, B, and C were chosen at the Rumuekpe oil spill area (Fig. 1).

COLLECTION AND ANALYSIS OF SAMPLES

Sampling started in November 1994 and ended in August 1995. Three (3) sampling quadrants measuring 5m² were mapped out at each site along the pipeline. Each quadrants was located 10m from the other. Composite soil sample from three quadrants of each study site were obtained from the surface soil (0-15cm soil depth) with a sterile spatula and placed in sterile 100ml glass containers. Samples were collected

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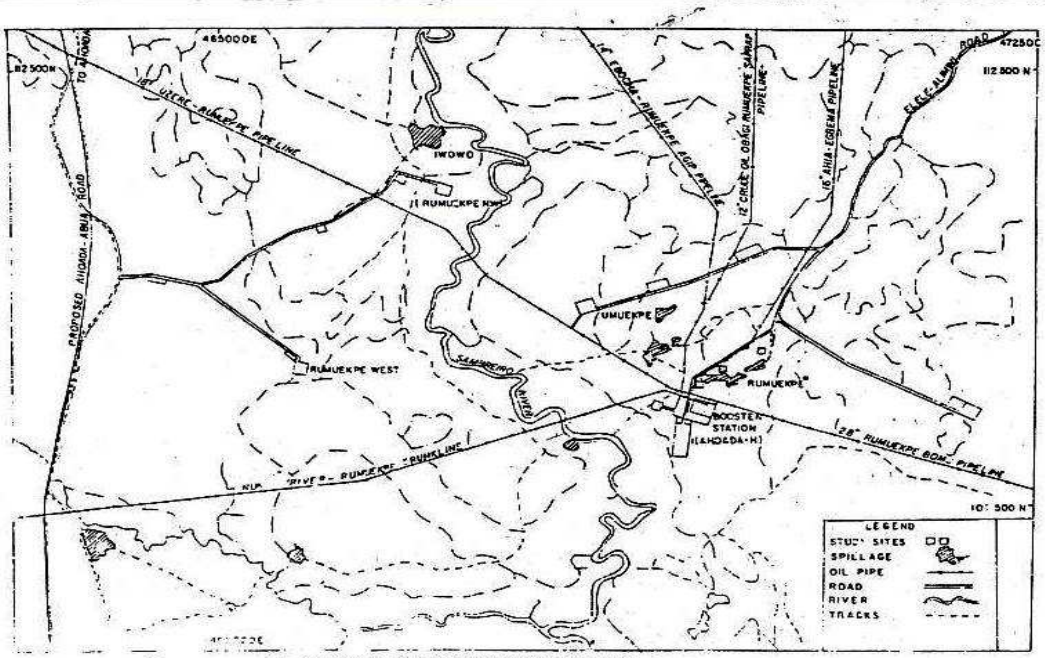

FIG. 1: SKETCH OF FIELD SHOWING THE THREE STUDY SITES

Table 1:
Physicochemical Properties of oil contaminated and uncontaminated (garden) soils.

Soil Properties	Oil Contaminated Soil	garden soils (Uncontaminated)
Sand (%)	83.6	75.1
Silt (%)	6.5	12.1
Clay (%)	10.6	17.0
pH	5.7	6.7
Organic carbon (%)	4.74	1.10
Total nitrogen (%)	0.07	0.12
C/N ratio	67.71	8.8
Exchangeable cations (me/100g)		
K	3.3	1.31
Na	6.8	0.04
Mg	6.8	1.01
Ca	3.9	1.90
Oil content (ug/g)		
i Wet season	174,000	20
ii Dry season	180,200	80

Values are mean of duplicate determination

monthly during the dry season (November 1994 to February 1995) and the rainy season (May to August 1995). Every month, 5 samples were collected from each sampling quadrant making a total of 45 samples from the quadrants for each season.

PHYSICOCHEMICAL ANALYSES OF SOIL SAMPLES

These were carried out using standard procedures. Soil extracts were prepared by rotating upside down 100g of the oil contaminated soil in 25ml of deionized water for 30 minutes followed by centrifuging at 600 rpm for 10 minutes. The pH of the soil was measured by Beckmangs glass electrode. From the soil extract the organic carbon was determined by Wakley-Black method and total nitrogen by microkjedahl method (AOAC 1975, Udo and Ogunwale 1986, Jakobsen 1992). Soil particle size distribution was determined by the hydrometer method (Allison 1965 and AOAC 1975).

The oil content of the soil was estimated by spectrophotometric analysis at 260nm using cyclohexane as the extraction solvent (Ewa-Oboho 1994) while changes in the moisture content of the soil during the study periods were estimated by oven -drying to constant weight (Allison 1965).

A comparative analytical study was also carried out with garden soil (uncontaminated) samples collected from a garden situated at the main campus of University of Uyo. The garden soil, which was relatively oil free served as a control experiment to measure the difference in fungistatic power between oil contaminated and uncontaminated soils.

SENSITIVITY TEST AND DETERMINATION OF SOIL FUNGISTASIS

Spores suspension obtained from molds (*Absidia* sp, *Aspergillus carbonarius, Peniciluim* sp and *Mucor hiemalis*) isolated from oil polluted soil samples were screened for their sensitivity to soil fungistasis by the agar-disc method (Sparking 1981) using Czapeks agar (CZ) as the culture medium.

Mucor hiemalis was chosen as the best organism for fungistasis examination on account of its sensitivity and rapid sporulation. Spores suspension prepared from 60h old sporulating culture of *M. hiemalis* was adequately agitated to release the spores from the sporangia before inoculation on agar discs placed on 1cm² filter papers which in turn were positioned on the surface of soil samples contained in sterile petri dishes.

The soil - agar discs plates were incubated at room temperature (28 $\pm$ 2°C) for 3h. to allow the diffusion of inhibitory substances from the contaminated soil into the agar discs before inoculation. The discs were thereafter inoculated with drop of the spores

suspension using a sterile glass rod and incubated at 28 ± 2°C for 16h before microscopic examination of the (Amanns Lactophenol) stained discs.

The germinated and ungerminated spores per microscopic field were counted and express as the mean germination percentage (MGP) of the total number of spores exposed to soil fungistatic factors. The tests were also carried out with the garden soils samples for comparison, while the filter paper - agar disc experiment served as the control.

EXAMINATION OF FUNGISTATIC LEVELS OF THE SOIL SAMPLES

The antimicrobial levels of the soil fungistatic factors were examined with oil polluted and garden soil samples obtained during the dry and rainy seasons. In this test, carried out in triplicates, 4 agar discs which had been exposed to the soil fungistatic factors for 16h were recovered for microscopy and subsequently placed on filter paper moistened with 1.0% (w/v) glucose solution. The agar discs were then incubated for another 24h before re-examination. The number of inhibited spores transformed by the application of glucose solution was counted and expressed as the mean transformed germination percentage (MTGP). Analysis of variance was performed by the one-way classification method of Walpole (1974).

RESULTS AND DISCUSSION

Some properties of the oil contaminated and garden (uncontaminated) soil samples tested are given in Table 1. Apart from its higher level of acidity, the contaminated soil also contained higher amounts of exchangeable cations. However the total nitrogen and available phosphorus contents of the contaminated soil were remarkably lower than the values obtained from the garden soil. There was also slight variation in soil particles between the oil polluted soil and garden soil. Seasonal changes in the oil content of the contaminated soil was not significant. The slightly higher concentrated oil content observed during the dry season may be attributed to the low soil moisture content and usually higher temperatures of tropical soils during the dry season (Antai and Mgbomo 1989).

Table 2. Effect of 1.0% (w/v) glucose solution on the germination ability of inhibited spores of Mucor hiemalis

SPORES TREATMENT		RAINY SEASON					DRY SEASON				
		MAY	JUN	JUL.	AUG.	MEAN	NOV.	DEC.	JAN.	FEB.	MEAN
Oil Spilled soil	1	24.2	26.6	19.4	28.5	24.6	11	9.4	10.4	4.5	8.8
	2	15	20	15	18	17	6	4	6	0	4.0
Garden soil	1	58	52	48	51.1	52.2	32.4	29.3	34.2	31.1	31.7
	2	23	35	37.5	35.5	35.7	17.5	20	18	12	12.7
Filter paper agar disc	1	78	77.3	74	82	77.8	74	78	74	80	76.5
	2	63	66.5	62	70	65.3	57.5	63	60	68	62.1

Means are significant at 95% probability level.

Note: 1 = % spore germination rate after treatment with 1.0% glucose solution.
2 = % spore germination rate before treatment with 1.0% glucose solution.

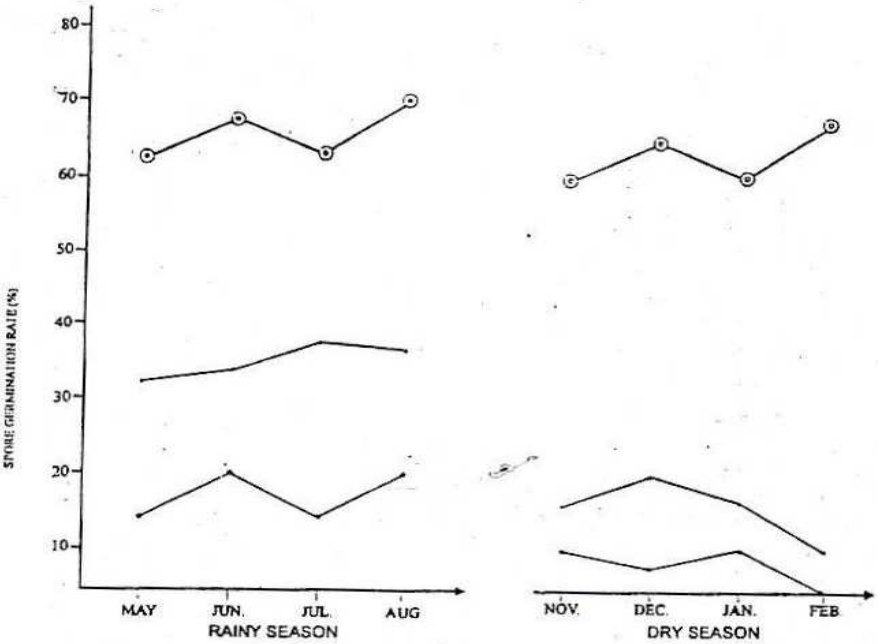

FIG. 2. Seasonal Changes in the fungistatic level of oil spilled (*), and garden (•), soil; θrepresents the rate of spore germination on filter paper-ager disc medium (control experiment)

The result of the fungal spores germinatability study showed the occurrence at a very high level of fungistatic factors in oil polluted soil when compared to the uncontaminated garden soil. This may be attributed to differences in some physicochemical properties of the soil. For instance, nitrogen and phosphorus are known to enhance the growth and proliferation of indigenous microbial population in soil. These elements are in relatively short supply in oil polluted soils and might affect the rate of spores germination (Atlas 1995).

A remarkable difference on the fungistatic power of the soils was observed between seasons (fig. 2). The fungistatic power of the contaminated soil was higher in the dry season than in the rainy season. The highest level of fungistasis was recorded in the month of February (dry season) with a total loss of spore germinatability after 16h exposure to petroleum contaminated soil while the least fungistatic effect was observed in June (rainy season) with a 20% mean spore germination rate.

Variation in the fungistatic power of the soil with seasons is in line with previous reports on similar investigation (Sawada and Nilta 1975, Higashida and Takao 1985, 1986). Atlas et al (1978) on their work on Prudohoe Bay crude oil spill on an Artic Coastal plain soil reported a variation with season, on microbial activities in soil. Higher numbers of bacteria were observed in the summer months than in the winter. Jones (1975) noted a similar response in temperate agricultural soil treated with high concentration of an oil sludge. And most recently Antai and Mgbomo (1989) observed a higher number of aerobic heterotrophs in petroleum contaminated coastal soils of Nigeria during the rainy season than in the dry season. The work of Antai and Mgbomo (1989) seems to agree with the results obtained in the present study, although the total number of hydrocarbon degrading bacteria encountered by them were higher in the dry season than in the rainy season.

The higher spore germination rate recorded (Fig.2) after exposing the spore to oil polluted soil in the rainy season is an indication of reduced fungistatic power of the soil during the rainy period. On the other hand, the spore inhibitory effect of the oiled soil appeared higher in the dry season (Fig. 2). In the dry season, the fungistatic power of the soil increased with decrease in the soil moisture content indicating that soil moisture rather than oil content is the main determining factor of fungistasis in petroleum contaminated soil during the dry season. Its adverse influence is further enhanced by the increased gas volatility and diffusion rates and concentrated effect of the non-nutrient factors that usually characterize soils during dry seasons. The interplay of these factors plus the recalcitrant nature of the major nutrient source (hydrocarbons) in petroleum contaminated soil is responsible for the low spore germination ability in the dry season.

In the rainy season, the determining fungistatic factor of the polluted soil appeared to be the high

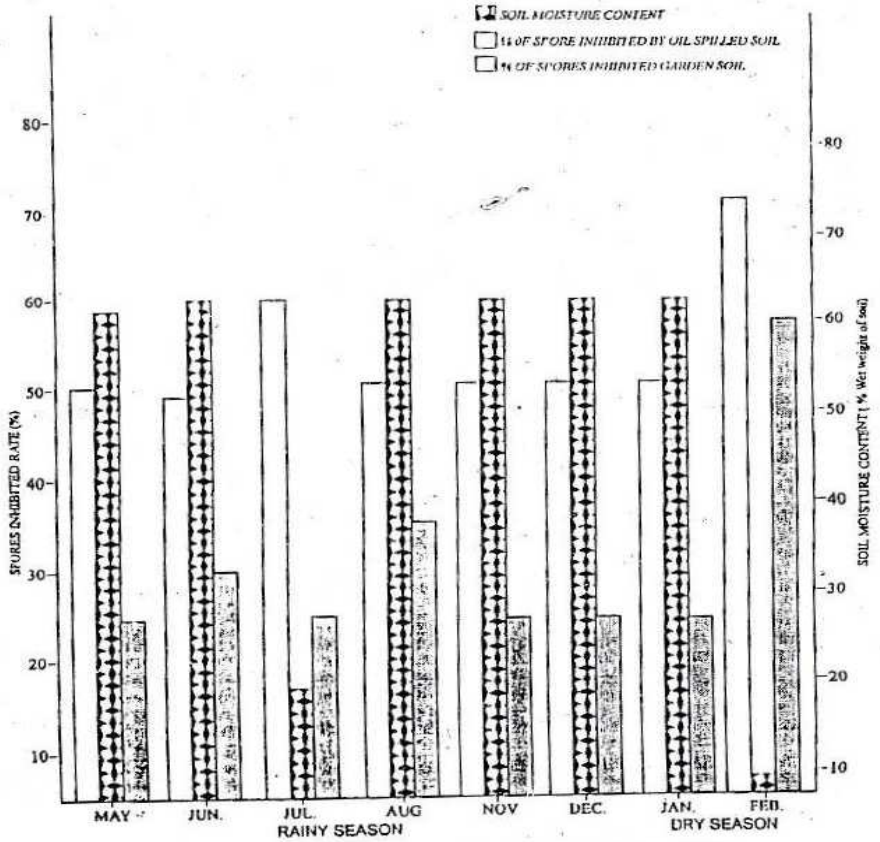

Fig. 3 The relationship between soil moisture content and the fungistatic power of oil spilled and garden soil during rainy and dry seasons.

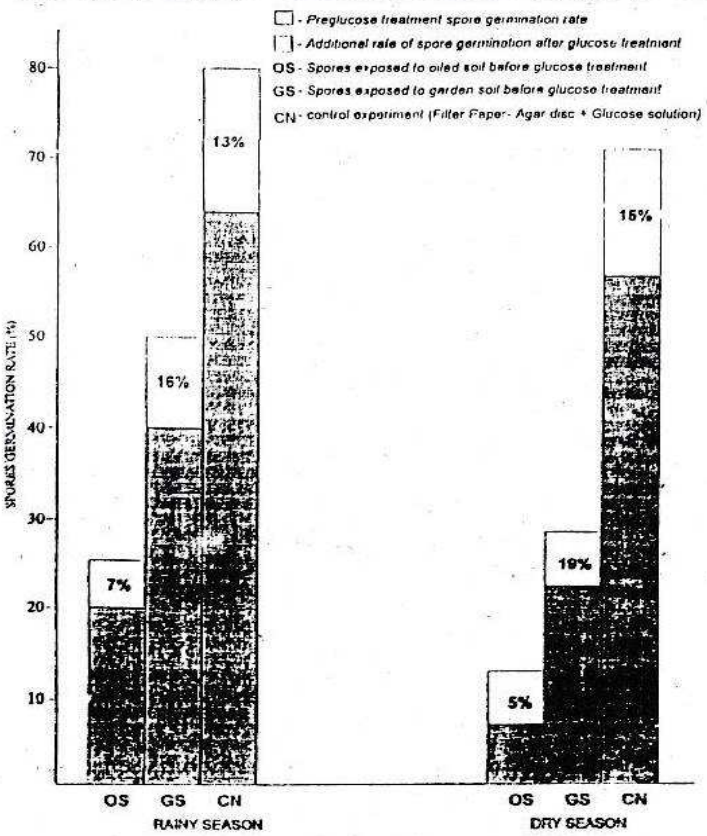

Fig. 4 Mean transformed germination percentage (MTGP) of M hiemalis spores induced by 1.0% glucose

moisture content of the soils. Spore inhibition rate was remarkably reduced by the high moisture content of soil although not at equal level with that observed in the relatively (oil-free) garden soil. The slight increase in the amount of inhibited spores during the month of August (Fig. 3) is an indication of the possible influence of excess water in oil polluted soils. The effect of this on *non insitu* spore germinatability of the mold was indirect as was reflected by the permanent water soaked state of the filter papers that separates the agar discs from the soil samples. With the low water holding capacity of oil contaminated soils, the reducing conditions provided would negatively influence spore germination.

The results of the spore resuscitation experiment shows that the inhibitory factors of oil polluted soil may disappear or be counteracted by the application of readily available source of nutrients to the soil. Treatment of inhibited spores with glucose solution increased the rate of spore germination significantly (at 95% probability level). The mean percent germination rate of spore in oil polluted soil was increased from 17.5% to 24.6% during the rainy season and from 4% to 8.8% in the dry season (Table. 2) A better effect of the replenishment was observed in spores exposed to the uncontaminated garden soil. A mean transformed germination percentage (MTGP) of 7% and 16% were obtained respectively for oil spilled and garden soils during the rainy season compared to the 5% for oil spilled soil and 19% for garden soil obtained during the dry season (Fig. 4) This suggests that the fungistatic effect of petroleum contaminated soil is more persistent in the dry season than in the rainy season.

The resuscitation of inhibited spores by glucose solution indicate that soil fungistasis inhibit rather than destroy the viability of microbial spores. It also shows that organic nutrient supplementation would, provide an easily utilizable nutrient source for microbial activities, annul the inhibitory effect on the soil and consequently promote microbial metabolism for a higher nutrient cycling rate in oil polluted agricultural soils. This reclamation approach would however be better done in the rainy season and more especially in the month of June than in the dry season.

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