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Seasonal Effect on the Bacterial and Fungal Population of an Oilfield Wastewater-Polluted Soil in Nigeria

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ABSTRACT: Seasonal effect on the bacterial and fungal populations of an oilfield wastewater - polluted soil was investigated for a period of 12 months. Many bacterial and fungal species were present in the soil and the wastewater of the area investigated. Some of the organisms occurred in both the soil and oilfield wastewater while others occurred only in the soil. Eighteen (18) organisms were isolated, 4 species (2 bacteria and 2 fungi) occurred in all the seasons whereas the other organisms occurred in at least one season. This showed that different seasons selectively favour the growth of certain microbial types. The chemical analysis of the oilfield wastewater revealed the presence of inorganic components and oil residues in the wastewater. The microbial counts of the polluted soil when compared to the control samples in all the locations did not reveal a clear seasonal-pollutant effect on the microbial densities. However, fungal counts showed somewhat seasonal-pollutant effect in two locations. The chemical constituents of the wastewater are relatively of low toxicity and hence had no toxic effects on the soil microbial population. Seasonal variations showed that the drier seasons supported large active microbial populations and the wetter season had smaller populations. However, seasonal influence was more pronounced on the fungi than on the bacteria. *@JASEM*

A major discharge from crude oil production platforms is oilfield wastewater otherwise called produced formation water. This is water that is finally discharged into the environments after oil-water separation process (Wilkinson, 1982; Somerville et. al; 1987). The wastewater is subjected to some forms of treatment. The treatment is achieved by addition of chemicals such as water clarifier and biocides as well as passing the wastewater through stages that ensure removal of oil from the water (Read and Blackman, 1980; Girling, 1989). Part of the treated wastewater is injected into the formation to enhance oil recovery while the remaining water is discharged into concrete pits or pond, where the operation is on shore (DPR, 1991). While in the pit, the wastewater could contaminate the soil environment as a result of overflow or seepage from faulty waste pits. The introduction of a pollutant into the environment could result in a shift in microbial numbers. Jones (1977); Clark and Patrick (1987); Obire (1988), reported a reduction in number of microbes in polluted soil when compared to non-polluted soil. Also, a combination of pollutant effect and climatic conditions can result in significant changes in the microbial population of soils. Studies by Marshall and Devinny (1988) revealed strong seasonal influence on soil microbial population in the presence of a pollutant. The hotter, drier months had increased viable microbial numbers with relatively smaller and less viable population occurring during cooler, wetter seasons (Marshall and Devinny, 1988). There has been no information on the combined effect of seasons and oilfield effluent water on the soil microbial population. This paper therefore, assessed the effect of seasons on the population of bacteria and fungi in oilfield effluent water-polluted soil in the Niger Delta area of Nigeria. The aim of this study is to draw a relationship between seasonal influence and ecological impact of the wastewater on soil bacteria and fungi.

MATERIALS AND METHODS

Soil samples were collected from Obagi oilfield in Ogba-Egbema-Ndoni Local Government Area of Rivers State, Nigeria (see fig. 1). Duplicate soil samples (surface soils, 0 to 20cm depth) were obtained from four locations using auger borer and bulked together into small sterile polythene bags labelled according to the locations, 1, 2, 3 and 4. These were taken to the laboratory for further treatment.

Water samples were collected from Obagi flow station at the outlet of the separation/treatment plant using sterile sample bottles 200ml capacity. The sample bottles were filled from gentle stream of the wastewater after flushing the interior of the nozzle of the valve with a flow of the water for 2 – 3 minutes. All the samples were taken to the laboratory for analysis within 2hrs of collection. Samples (soil and water) were collected twice in a month for 12 months. During the sampling period, four seasons (peak of dry season, dry season, peak of rainy season and rainy season) were considered based on the data obtained from the Ahoada rainfall zone.

The oilfield wastewater was analysed for chemical constituents such as pH, sodium, calcium, chloride, magnessuim, carbonate, bicarbonate, nitrate, sulphate, iron and oil-in-water. Iron, nitrate, sulphate and oil-in-water were analysed using spectrophotometer (Model No. HACH-DREL 2000)

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in accordance with the manufacturer's instructions. The analysis of bicarbonate, carbonate, chloride, sodium, calcium and magnessium ions were carried out in accordance with titration procedures specified by APHA (1985). The pH of the wastewaters was determined using a pH meter (Model No. 7 Corning) in accordance with the manufacturer's instructions. The bulked soil samples were air – dried and passed through a 2cm sieve to remove the coarse fragments. Three kilograms (3 kg) of the fine earth was transferred into 5 litre size sterile plastic planting buckets perforated at the bottom to allow excess water to drain off. The buckets were labelled according to locations. All the buckets were each watered for 2 days with 500ml of the wastewater and incubated for 5 days. These were taken as polluted soil samples. Corresponding duplicated soil samples were treated in similar way with sterilized distilled water to serve as control.

Serial ten – fold dilution as described by Ofunne (1999) was followed for isolation and enumeration of bacteria and fungi in soils and oilfield wastewater. Appropriate decimal dilutions of the samples were plated onto Mannitol – Soil-Extract agar (Obire and Wemedo, 1996); Nutrient agar (Oxoid CM 13) for bacteria; and onto Potato Dextrose agar (Laboratory preparation) for fungi. Mannitol-soil-Extract agar medium was used for soil samples and Nutrient agar medium was used for water samples. 0.1 ml of cycloheximide was incorporated into Mannitol – Soil – Extract agar to suppress fungal growth while 0.1ml of streptomycin solution was incorporated into Potato Dextrose agar to suppress bacterial growth. The spread plate technique was used for all inoculations. The inoculated plates were incubated at 28°C for 3 – 5 days; and discrete colonies that developed were counted as the total viable counts for heterotrophic bacteria and viable counts for fungi in the soil. Subculturing purified all isolates. The difference in the microbial counts of the control and polluted soils at the locations and the seasons was taken as the effect of the pollutant and seasonal changes on the microbial population of the soil. Pure bacterial isolates were identified on the basis of their cultural, morphological characteristics and by reference to Buchanan and Gibbons, (1974); Cowan, 1974. Fungal pure isolates were identified on the basis of their cultural and morphological characteristics and by reference to Alexopoulos and Sun, (1962); Barnett and Hunter (1972).

RESULTS AND DISCUSSION

The bacterial and fungal types isolated from soils and oilfield wastewaters in the two seasons are shown in table 1. Of the eighteen organisms isolated, only *Microcococcus*, *Streptomyces*, *Penicilluim* and *Rhizopus* occurred in all the seasons. *Aerococcus*, *Enterobacter* and *Fusairum* occurred in the other seasons but not in the peak of dry season. *Aspergillus* occurred in all the seasons except the peak of rainy season. *Corynebacterium* and *Streptococcus* occurred in all the seasons except the dry season. Bacillus did not occur in the rainy season only; *Acinetobacter* and *Clostridium* occurred in the peak of dry season and dry season only. *Alcaligenes*, *Klebsiella* and Staphylococcus occurred in the rainy season and peak of rainy season only. *Escherichia* occurred in the peak dry season and *Pseudomonas* occurred in the peak of rainy season only.

Table 2: Chemical constituents of the oilfield wastewater*

	Chemical constituents										
Season	pH	Na^+ (mg/L)	Cl^- (mg/L)	Ca^{2+} (mg/L)	Mg^{2x} (mg/L)	CO_3^- (mg/L)	HCO_3^- (mg/L)	NO_3^- (mg/L)	SO_4^{2-} (mg/L)	Fe^{2+} (mg/L)	Oil (ppm)
A	7.7	2879	4438	52.1	23.8	140	4118	26.6	19.0	1.09	26.9
B	7.2	2718	4189	58.1	26.8	185	3813	27.6	28.0	1.40	33.2
C	7.4	2530	3904	46.8	25.9	160	3586	16.1	18.0	1.32	57.2
D	7.7	2116	3266	44.1	18.4	158	4148	22.6	32.6	1.73	71.4

*A = Peak of dry season ; B = Dry Season ; C = Rainy Season ; D = Peak of rainy season * Mean seasonal values*

Table 1. Bacteria and fungi isolated from soil and oilfield wastewater during the four seasons

	Seasons			
	PDS	DS	RS	PRS
BACTERIAL SPECIES				
*Acinetobacter**	+	+	-	-
*Aerococcus**	-	+	+	+
*Alcaligenes**	-	-	+	+
*Bacillus***	+	+	-	+
*Clostridium***	+	+	-	-
*Corynebacterium***	+	-	+	+
*Enterobacter***	-	+	+	+
*Escherichia***	+	-	-	-
*Klebsiella***	-	-	+	+
*Micrococcus**	+	+	+	+
*Pseudomonas**	-	-	-	+
*Staphylococcus**	-	-	+	+
*Streptococcus**	+	+	-	+
*Streptomyces**	+	+	+	+
FUNGI SPECIES				
*Aspergillus**	+	+	+	-
*Fusarium***	-	+	+	+
*Penicillium**	+	+	+	+
*Rhizopus**	+	+	+	+

+ =Species occurred - =Species did not occur
*Organisms isolated from both soil and oilfield wastewater samples.
** Organisms isolated from soil samples only.

PDS =Peak of Dry season DS = Dry season
RS = Rainy season PRS = Peak of Rainy season.

The chemical constituents of the oilfield wastewater are shown in table 2. The mean seasonal values ranged as follows: pH 7.2-7.7; Sodium, 2116 – 2879; Chloride, 3266-4438; Calcium, 44.1-58.1; Magnessium, 18.4-26.8; Carbonate, 140-185; Bicarbonate, 3586-4148; Nitrate, 16.1-27.6; Sulphate, 18.0-32.6; Iron, 1.09-1.73 and oil-in-water, 26.9-71.4. Figure 2 & 3 showed the mean total colony counts (cfu/g of soil) for heterotrophic bacteria and fungi respectively in the four locations. The counts at the different seasons ranged as follows: Peak of dry season, $78.0x10^5$ to $85.5x10^5$ in control samples and $62.2x10^5$ to $74.5x10^5$ in polluted samples for bacteria; $24.7x10^4$ to $35.8x10^4$ in control samples and $28.0x10^4$ to $33.3x10^4$ in polluted samples for fungi; dry season, $74.8x10^5$ to $96.0x10^5$ in control samples and $76.2x10^5$ to $93.0x10^5$ in polluted samples for bacteria; $23.9x10^4$ to $38.3x10^4$ in control soil and $16.1x10^4$ to $35.0x10^4$ in polluted samples for fungi; rainy season, $65.7x10^5$ to $81.8x10^5$ in control samples and $65.2x10^5$ to $88.8x10^5$ in polluted samples for bacteria; $16.7x10^4$ to $31.7x10^4$ in control samples and $10.0x10^4$ to $28.3x10^4$ in polluted samples for fungi; peak of rainy, season $46.0x10^5$ to $67.3x10^5$ in control samples and $59.1x10^5$ to $75.2x10^5$ in polluted samples for bacteria and $10.0x10^4$ to $15.0x10^4$ in control samples and $5.3x10^4$ to $14.3x10^4$ in polluted samples.

The mean seasonal colony counts (cfu/g of soil) for heterotrophic bacteria and fungi are shown in figure 4. The counts for bacteria and fungi are as follows: peak of dry season, $82.5x10^5$ in control samples and $70.7x10^5$ in polluted samples, for bacteria; and $30.0x10^4$ in control samples and $30.5x10^4$ in polluted samples for fungi; $30.1x10^4$ in control samples and $30.5x10^4$ in pulluted samples for fungi; dry season, $80.7x10^5$ in control soils and $84.1x10^5$ in polluted samples for bacteria; $30.6x10^4$ in control samples and $24.5x10^4$ in polluted samples for fungi; rainy season, $71.3x10^5$ in control samples and $76.3x10^5$ in polluted samples for bacteria; $21.3x10^4$ in control samples and $19.8x10^4$ in polluted samples for fungi; peak of rainy season, $55.7x10^5$ in control samples and $64.7x10^5$ in polluted samples for bacteria; $12.5x10^4$ in control and $10.5x10^4$ in polluted samples for fungi.

The present investigation revealed that many species of bacteria and fungi inhabit the Obagi soil and can survive in the oilfield wastewater. All the organisms occurred in the soil. Most of the indigenous soil organisms, as described by Alexander (1977), occurred in the soil only but not in the oilfield

wastewater (see Table 1). Of the eighteen organisms observed only 2 species of bacteria and 2 fungi species occurred in all the seasons while the other organisms occurred in some seasons and not in other seasons (see table 1). This means that different seasons encouraged the growth and proliferation of certain organisms and not the other organisms. The reponse of different microbial types to seasonal changes may be due to their physiological potentials, which could be altered at the different seasons. Marshall and Devinny (1988) reported that variations of climatic conditions such as distinct wet and dry seasons selectively favour the growth and proliferation of different physiological types of microorganisms.

The chemical constituents' analysis of the oilfield wastewater showed that the wastewater of Obagi oilfield is composed of hydrocarbon and non-hydrocarbon residues. The inorganic components of the Obagi oilfield wastewater were similar to those of U.K. North sea oilfield produced water observed by Somerville et al; 1987. The component materials identified in oilfield produced water by Somerville et. al., (1987) were found to be of low acute toxicity hence are unlikely to lead to ecotoxicological problems. The data obtained for bacteria and fungi in the four locations showed that microbial counts increased in the control samples and decreased in the polluted samples in some seasons and reversed in other seasons. There was no clear-cut pattern of decreee in microbial counts in the polluted samples when compared with the control samples as to deduce real pollutant effect on the microbial numbers. The decrease was observed in some seasons while in the other seasons the reverse was the case. Thus the combined seasonal-pollutant effect on the totality of the bacterial and fungal counts in all the locations cannot be deduced from the results obtained. However, fungi somewhat decreased in the polluted samples in two locations.

The data obtained for chemical parameters in this study are relatively of lower values than their corresponding concentrations identified by Somerville *et al.*, 1987. They are also unlikely to impact toxic effects on the soil microbial populations. The effect of season on the cumulative counts of bacteria and fungi in the soil was shown in fig. 4. The results showed that microbial numbers were generally higher during the peak of dry season and dry season than the peak of rainy season and rainy season. Marshall and Devinny (1988), observed strong seasonal influence an microbial population of a petroleum wasteland treatment; the microbial populations being smaller during the cooler, wetter seasons and the drier months supported large active populations. In this study, microbial numbers were higher during the drier seasons than the wetter seasons. The reason adduced was that during the wetter seasons, lower temperatures inhibited microbial activity; also saturation of the soil by rain limited activity by reducing aeration (Marshall and Devinny, 1988). However, seasonal influence on microbial numbers was more pronounced on the fungi population than those of bacteria. Marshall and Devinny (1988) observed that fungal population fall to very low numbers in late fall and winter, and are sometimes undetectable. Fungi are likely more susceptible to seasonal changes than bacteria especially when exposed to additional stress such as toxic effect of the wastewater.

In conclusion, some of the bacterial and fungal types isolated from the soil also occurred in the oilfield wastewater. Climatic changes such as distinct wet and dry seasons selectively favoured certain microbial types. While this study revealed the presence of certain inorganic constituents in the Obagi produced waters; it affirmed that the oil content as well as the inorganic constituents of the oilfield wastewater have not been shown to impact any toxic effects on the soil micro-organisms. The difference in the microbial counts between the control and polluted samples in all the locations and in most of the seasons was not significant enough as to deduce real combined seasonal – pollutant effect on microbial populations. The study revealed that the microbial populations were affected by the seasons with the wetter seasons having smaller population and the drier seasons supporting large active population. However, fungi were more severely affected by seasonal changes than bacteria.

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REFERECES

Alexander, M. (1977) Introduction to soil microbiology. 2nd Edition John Wiley and sons. New York, U.S.A.

Alexopoulos, C.J., Sun, S.S. (1962). Introductory mycology. 2nd Edition. John wiley and sons, New York, U.S.A.

American Public Health Association (1985). Standard Methods for the Examination of water and wastewater. 16th Edition. Washington, D.C.

Barnett, H.L., Hunter, B.B (1972). Illustrated Genera of Imperfert fungi. 3rd Edition, Burgess publishing company, U.S.A.

Buchanan, R.E., Gibbons, N.E. (Eds). Bergey''s Manual of Determinative Bacteriology. 8th Edition. Williams and Wilkins Co., Baltimore.

Clark, J.R., Partrick, J.M., Jr. (1987). Toxicity of sediment incorporated drilling fluids. Mar. Poll. Bull. 18(11). 600-603.

Cowan, S.T. (1974). Cowan & Steel''s Manual for the Identification of Medical Bacteria. Cambidge University press, Cambridge.

Department Of Petroleum Resources (DPR) (1991). Environmental Guideline and Standards for the Petroleum Industry in Nigeria. Ministry of Petroleum Resources, Lagos pp 30-37.

Girling, A.E. (1989). Acute and chronic toxicity of produced water from a North Sea oil production platform to the Calanoid Copepod, *Acartia tonsa.* Bull. Environ. Contam. Toxicol. 43:286-286.

Jones, J.G. (1977). The Long-term effects of kerosene pollution on the microflora of a moorland soil. J. Appl. Bacteriol. 43:123-128.

Marshall, T.R., Devinny, J.S. (1988). The microbial ecosystem in petroleum wasteland treatment. Wat. Sci. Tech. 20(11/12): 285-291.

Obire, O. (1988). Studies on the biodegradation potential of some microorganisms isolated from water systems of two petroleum-producing areas in Nigeria. Nig. J. BOT. 1:81-90.

Obire, O. (1998). The suitability of various Nigerian petroleum fractions as substrate for bacterial growth. Discovery and Innovation. 5:45-49.

Obire, O., Wemedo, S.A. (1996). The effect of oilfield wastewater on the microbial population of a soil in Nigeria. Niger Delta Biologia. 1 (1): 77-85.

Odu, C. T.I., Nwoboshi, L.C., Esuruoso, O.F., Ogunwale, T.A. (1985). Environmental study of the Nigerian Agip Oil, Company Operational Areas. In: The Petroleum Industry and the Nigerian Environment. Proceedings of FMW&H and NNPC conference, pp 274-283.

Ofunne, J.I. (1999). Bacteriological Examination of Clinical specimens. Achugo publications, Ama J.K. Recreation park, Owerri, Nigeria.

Read A.O., Blackman, R.A.A. (1980). Oily water discharges from off shore North Sea installations: A perspective. Mar. Pollut. Bull. 11:44-47.

Somerville, H.J., Benneth, D., Davenport, J.N., Holt, M.S., Lynes, A., Mahieu, A., Mcourt, B., Parker, J.G., Stephenson, R.R., Watkinson, R.J., Wilkinson, T.G. (1987). Environmental effects of produced water from North sea oil operations. Mar. Poll. Bull. 18(10): 549 – 558.

Wilkinson, T.G. (1982). An environmental programme for offshare oil operations. Chemistry and Industry, 20 the February, pp 115 – 123.