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EVALUATION OF BACTERIA AND PHYSICOCHEMICAL QUALITIES OF TAR SAND SOIL FROM GBELEJULODA, ONDO STATE, NIGERIA

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ABSTRACT
An investigation was carried out to determine the bacterial population and physicochemical characteristics of soil samples collected from the tar sand deposit at Gbelejuloda in Irele Local Government, Ondo State, Nigeria. Results revealed that the soils were clayey to brown in colour and clayey to silty-clay particle sizes. Other characteristics examined were pH (2.68-5.06), moisture (44.20-50.18%), organic matter (0.91-4.55%), organic carbon content (0.52-2.45%). The mineral constituents were magnesium (0.50-0.57ppm), calcium (1.0-1.2ppm), potassium (0.19-0.43ppm), sodium (0.16-0.17ppm), nitrogen (0.46-0.67ppm) and phosphorus (3.25-8.27ppm). Bacterial count ranged from 2.0×10^4 to 2.0×10^7 cfu/g. The isolates were identified as *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas stulzeri*, *Staphylococcus aureus*, *Bacillus marcerans*, *Bacillus polymyxa*, *Listeria monocytogenes* and *Listeria grayi*. The pathogenic nature of the isolates, call for adequate precaution in the course of exploring the tar sand.
Keywords: Tar sand, pathogenic, bacteria, physicochemical, minerals

INTRODUCTION

Soil is referred to the outer, loose materials of earth surface, a layer distinctly different from the underlying bedrocks (Arotupin, 2009). Soil supports a complex ecosystem which supports the plants on the surface and new soils are created from breakdown of rocks and sand (Voroney, 2006). The microscopic ecosystem has eco-evolved with plants to collect and store water and nutrients in a form usable by plants (Micheal, 2003).

Soil contains a wide range of organisms such as bacteria, actinomycetes, fungi, algae and protozoa. Some soils may contain up to one million species of microbes per gramme, most of these species being unknown, making soil the abundant ecosystem on earth (Copley, 2005).

Tar sand (bitumen sand) is composed of sand, heavy oil and clay that are rich in minerals and water. The heavy oil in tar sand is called bitumen. The Nigerian bitumen belt lies on the onshore areas of the Eastern Dahomey (Benin) basin (longitude 4^0S, 6.45^0N; latitude 4^0S, 5.1^0E). The probable reserve of bitumen and heavy oil in the entire Nigerian belt is about 120×4300m (Adegoke and Ibe, 1982). The clay content of the Nigerian deposit is very low averaging about 5% and heavy oil extracted from the deposits has a gravity between 5.00 and 14.60. Physical properties reported include softening point (44-52^{o}C), ductility (0.1-1.3mm), penetration (80-100mm), hydrocarbon content (7.20-18.20% by wt), resins (32.12-34.00% by wt) and sulphur (5.00-10.00ppm). In addition, the Nigerian bitumen posses relatively large quality of naphthenes, aromatics and asphaltenes that are similar to the conventional oil. This makes the Nigerian bitumen a very useful alternative source of petroleum hydrocarbon and a potential feedstock for petrochemical industries (Adegoke *et al*., 1991).

This study focused on the physicochemical characteristics as well as the different types of bacteria associated with the

Nigeria tar sand deposit at Gbeleloda in Irele Local Government Area.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from thirty-two different sites on the bitumen soil located in Gbelejuloda in Irele Local Government Area of Ondo State, Nigeria. A non-bitumen soil in Gbelejuloda served as control. Surface of the soil sample sites was cleared and samples were obtained using soil auger at depths of 10cm intervals till a depth of 30cm. The samples were collected in sterile containers and taken to the laboratory for microbiological analysis.

Determination of physicochemical properties of the soil samples

The physicochemical properties of the soil samples were determined. The colour and particle sizes of the soils were determined using standard methods. The pH was measured using pH meter standardized at pH 7.0 using appropriate buffers (Ibitoye, 2006). The moisture content of each soil sample was determined by drying 10grammes of the soil in an oven at 80^0C until a constant weight was reached and the percentage moisture content was calculated. The organic carbon content was determined using the Walkey-Black wet oxidation method as described by Ibitoye (2006). Available phosphorus, exchangeable magnesium, calcium, sodium and potassium ion concentration were determined using standard methods (AOAC, 1990). Total nitrogen was measured using the Macrokjeldahl digestion method (Heads, 1992).

Enumeration and identification of bacterial population

Nutrient agar (NA) medium was prepared according to manufacturer's instruction, sterilized and poured into Petri dishes. One gramme of each soil sample from different sites on the bitumen soil and non-bitumen soil (control) was diluted serially until fifth dilutions. An aliquot of 0.1ml from the fourth and fifth dilution was inoculated on the freshly prepared media, incubated at 37^0C for 24 hours and observed for growth. Colonies were recorded as colony forming units per gram of soil (cfu/g). Isolates were subcultured repeatedly to obtain pure cultures. The pure cultures were characterised using standard microbiological techniques (Olutiola *et al*., 1991; Cowan and Steel, 1993; Holt *et al*., 1994).

Data obtained were subjected to a single factor analysis of variance (ANOVA) while the significant means were separated with the New Duncan's Multiple Range Test (NDMRT) at 5% confidence level (P = 0.05) using Statistical Package for Social Sciences.

RESULTS

The particle size of the soil samples contained high sand content which ranged from 64.00% to 72.80%, clayey content 21.20% to 30.50% and silt content 5.67% to 10.00%. The colours of the soils were clayey to dark brown. The pH ranged from 2.68 to 5.06 while the moisture content ranged from 44.20% to 50.18%. The organic matter and the organic carbon content ranged from 0.91% to 4.55% and 0.52% to 2.45% respectively (Table 1). The exchangeable monovalent cations ranged from 0.16 to 0.17 cmol/kg and 0.19 to 0.43 cmol/kg for sodium and potassium respectively. Calcium and magnesium content of the soil samples ranged between 1.0 to 1.2 cmol/kg and 0.50 to 0.57 cmol/kg. The available phosphorus content of 3.25 to 8.27 cmol/kg were observed, while the nitrogen content ranged from 0.47 to 0.72 cmol/kg (Table 2). Table 3 shows the counts of bacterial species isolated from the soil samples at different depths from the Nigerian tar sand deposit at Gbeleloda, Local Government Area. The count ranged from 2.0×10^4 to 2.0×10^7 (cfu/g) and the species were identified as *Proteus mirabilis, Pseudomonas putida, Pseudomonas stulzeri, Staphylococcus aureus, Bacillus marcerans, Bacillus polymyxa, Listeria monocytogenes* and *Listeria grayi.*

Bacillus marcerans had the highest frequency of occurrence of 21.43% while *Proteus mirabilis* had the lowest frequency of occurrence of 3.57% (Table 4).

Table 1: Physicochemical properties of tar sand soil samples (%)

Soil samples	Particle sizes (%)			pH	Moisture content	Organic matter	Organic carbon
	Sand	Silt	Clay			(%)	
Plot A	72.80^{c}±2.00	5.67^{a}±2.80	21.20^{a}±2.00	2.87^{b}±0.03	49.22^{b}±4.97	4.55^{c}±0.05	2.45^{c}±0.03
Plot B	70.80^{bc}±2.00	10.00^{b}±2.00	22.00^{a}±6.65	2.68^{a}±0.02	44.20^{a}±5.43	3.87^{b}±0.04	2.25^{b}±0.02
Control	64.00^{a}±2.00	8.66^{ab}±1.20	30.50^{b}±1.20	5.06^{c}±0.02	50.18^{b}±4.75	0.91^{a}±0.02	0.52^{a}±0.01

Values with the same alphabet in a column were not significantly different at P<0.05 and n = 3

Table 2: Mineral content of the tar sand soil samples (cmol/kg^{-1})

Soil samples	Na^{+}	K^{+}	Mg^{2+}	Ca^{2+}	N	P
Plot A	0.16^{a}±0.00	0.43^{b}±0.30	0.57^{a}±0.31	1.10^{b}±0.00	0.67^{b}±0.01	3.25^{a}±0.87
Plot B	0.17^{b}±0.01	0.19^{c}±0.00	0.50^{a}±0.80	1.00^{a}±0.80	0.47^{a}±0.01	5.68^{b}±0.03
Control	0.17^{b}±0.00	0.24^{a}±0.01	0.53^{a}±0.13	1.20^{c}±0.80	0.72^{c}±0.03	8.27^{c}±0.07

Values with the same alphabet in a column were not significantly different at P<0.05 and n = 3

Table 3: Total plate count of bacteria in tar sand soil samples

Soil samples	Bacterial count (cfu/g)
A1	2.0×10^{7}
A2	2.0×10^{4}
A3	2.0×10^{6}
B1	2.0×10^{5}
B2	2.0×10^{5}
B3	1.2×10^{6}
C1	1.6×10^{7}
C2	5.0×10^{6}
C3	2.4×10^{6}

Key: Plot A – A1– (0-10cm); A2 – (11-20cm); A3 – (21-30cm); Plot B – B1– (0-10cm); B2 – (11-20cm); B3 – (21-30cm); and Control – C1– (0-10cm); C2 – (11-20cm); C3 – (21-30cm).

Table 4: Frequency of occurrence of bacterial isolates

Isolates	A1	A2	A3	B1	B2	B3	C1	C2	C3	Total	% Occurrence
Proteus mirabilis	-	+	-	-	-	-	-	-	-	1	3.57
Pseudomonas putida	-	+	+	+	+	-	-	-	+	4	14.29
Staphylococcus aureus	+	-	-	+	-	-	+	-	-	3	10.71
Bacillus marcerans	+	-	+	+	+	+	-	-	+	6	21.43
Listeria monocytogenes	-	+	-	+	+	-	+	+	-	5	17.86
Pseudomonas stulzeri	-	+	-	-	+	-	-	-	-	2	7.14
Listeria grayi	-	-	+	-	-	+	-	-	-	2	7.14
Bacillus polymyxa	+	-	-		-	-	-	+	+	4	14.29

Key: Plot A – A1– (0-10cm); A2 – (11-20cm); A3 – (21-30cm); Plot B – B1– (0-10cm); B2 – (11-20cm); B3 – (21-30cm); and Control – C1– (0-10cm); C2 – (11-20cm); C3 – (21-30cm). % - Percentage.

DISCUSSION

The relative proportion of sand, silt and clay is a determinant of soil texture. This proportion is important in determining water holding capacities of the soil. The particle size analysis of soil samples from the tar sand deposit indicated that clay was a major component of the tar sand whereas, the control

soil sample was grouped as silty-clay when compared with the soil textural chart described by Ibitoye (2006). As a result of this, the tar sand has higher water retentive capacity making it unsuitable for agricultural purposes. This is in agreement with previous report that fine-textured soils hold more water than coarse-textured soils and are not ideal for plant growth (Epstein and Arnold, 2005). Oyedele *et al*. (2008) also reported that high clay proportions in soil affects soil fertility and some physical conditions which include; ability to adsorb cations, retain moisture, influence shrink-swell potential, permeability, plasticity, the ease of soil dispersion and tillage. These parameters tend to have marked effect on agricultural practices.

The low pH values in soil samples from the tar sand could be linked with accumulation of acidic metabolites as well as low mineral content in the parent material of the soil. This finding agrees with Ijah and Abioye (2003) who reported that the decrease in pH value in tar sands may be due to increase in degradation activities by microorganisms. Therefore, these tar sand soil samples are strongly acidic in nature. The high content of organic matter and organic carbon in soil samples from the tar sand compared with those from control plot could be attributed to bitumen which is an integral part of tar sand having similar composition as light crude which is rich in organic carbon (Akinmosin *et al*., 2009). The presence of organic matter in soils determine its water capacity, infiltration rate, tilt, nutrient accumulation which are necessary for crop growth and adequate aeration (Martha, 2003).

The exchangeable bases Na^+, K^+, Ca^{2+} and Mg^{2+} in the tar sand soil samples were low compared with those in dumpsites and cropped farmlands. Reports also showed that low pH (acidic) favours the abundance of exchangeable anions, but reduced cation, while high pH (basic) favours the abundance of exchangeable cations, but reduced anions in soils (Oyedele *et al*., 2008). Hence, the former reason could be responsible for the results of cations in this study.

The morphological and biochemical characterization of the bacteria isolates from the soil samples revealed the following genera: *Proteus, Pseudomonas, Staphylococcus, Bacillus* and *Listeria*. The presence of these isolates in the tar sand soil samples indicates the ability of these isolates to survive in bitumen soil through arrays of complex mechanisms of enzymes for the digestion of the mineral materials in the soil environment (Ojo, 2006). The genus *Pseudomonas* and *Bacillus* had been earlier reported to be predominating genera associated with tar sand deposit in Nigeria and their oil degrading capabilities (Oboh *et al*., 2006). Ijah and Abioye (2003) also reported on the utilization and degradation of petroleum hydrocarbon in soils by species of *Bacillus* and *Pseudomonas*. However, the isolation of human pathogenic bacteria genera *Proteus, Staphylococcus* and *Listeria* from the tar sand soil samples suggests recent human activities and discharge of fecal matters.

In conclusion, the analysis of some of the physiochemical parameters of the tar sand samples indicates that tar sand is not suitable for agriculture due to its low mineral contents and pH values which showed that the soil is acidic. The bacteria isolated from this study indicate that most are normal flora of the soil, however, *Proteus, Staphylococcus* and *Listeria* are human pathogens. Thus, there should be adequate awareness on proper handling, mining, exploitation and exploration of the tar sand deposits so as to reduce the risk of infection.

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