

# Ecotoxicological Dynamics of the Coastal Soil Ecosystem of Oil Producing Regions of Ondo State, Nigeria

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How to cite this paper: Akinsemolu, A.A., Akinyosoye, F.A. and Arotupin, D.J. (2018) Ecotoxicological Dynamics of the Coastal Soil Ecosystem of Oil Producing Regions of Ondo State, Nigeria. *Open Journal of Ecology*, **8**, 250-269.

https://doi.org/10.4236/oje.2018.84016

**Received:** February 4, 2018 **Accepted:** April 24, 2018 **Published:** April 27, 2018

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The industrial revolution marked the beginning of unprecedented anthropogenic growth and technological advancement that also inadvertently led to acute environmental degradation. This technological advancement was driven by the use fossil fuels such as crude oil. Crude oil extraction through drilling has resulted in widespread environmental pollution and deterioration of natural habitats. The Ondo State region in Nigeria presents one such expanse where large scale crude extraction operations have caused hazardous environmental pollution and toxic substance contamination. This study is a comprehensive and holistic study of the terrestrial soil ecosystem aimed towards elucidating the potential ecotoxicity that may have adversely affected the area. The results indicated that the terrestrial soil ecosystem was largely acidic (~pH6) and the organic matter content ranged from 6% to 12% indicating the soil was hydric. The results also indicated that the terrestrial soil environment was contaminated with toxic heavy metals including cadmium (Cd), chromium (Cr), lead (Pb) and arsenic (As). The toxic heavy metal concentration of the soil ecosystem was higher during the dry season. The Cr concentration in the soil samples was >3 ppm in most of the sampling sites, which exceeded WHO maximum permissible limit. Mean concentrations of the heavy metals in the soil samples in both seasons were of the order: Cr > Pb > Cd > As. The soil ecosystem was also characterized by a diverse and large population of microorganisms including bacteria like Enterobacter, Escherichia coli, and several species of fungi.

# **Keywords**

Anthropogenic Growth, Crude Oil Extraction, Ecotoxicity, Toxic Heavy Metals, Ilaje

## **1. Introduction**

Over the past several decades, uncontrolled anthropogenic growth has led to overutilization and exploitation of natural resources as well as widespread environmental pollution and degradation. One of the more significant damaging effects of this unrestrained growth has been the uncontrolled assembly of excess waste materials, which is contaminated with a wide range of noxious substances as well as toxic heavy metals and various detrimental materials [1] [2] [3]. Reckless discharge practices for such waste products added an additional environmental burden to natural ecosystems and had resulted in hazardous consequences [4] [5]. This waste disposal has mostly affected terrestrial soil ecosystems, turning the useful soil systems into wastelands [6]. According to a report published by the United Nations Environment Program [7] on the Environmental Assessment of Ogoniland, such soil contamination not only affects the socio-economic life of the inhabitants of the affected region but also it has an adverse effect on the drinking water quality. Therefore, regulation and reversal of this colossal degradation of natural ecosystems necessitate an appropriate socioeconomic valuation of natural resources, along with an efficient and sustainable utilization of these natural resources and employment of responsible waste treatment technologies [8].

The extraction of crude oil and natural gases has had hazardous consequences on natural environments. Kvenvolden and Cooper [9] reported that crude-oil seepage is about 600,000 metric tons per year. Crude oil extraction through drilling in terrestrial, marine or coastal environments has been a source of significant concern. This drilling often leads to industrial accidents such as spillage and acute environmental degradation due to irresponsible waste expulsion practices.

This study has focused on the terrestrial soil ecosystem of the Ondo state region in Nigeria. This region is a major site for offshore and mainland crude oil drilling operations that are carried out by several multinational oil corporations [10]. Furthermore, due to the lack of adequate wastewater treatment facilities in the region, a substantial amount of the wastewater produced in the region flows through the network of rivers into the area under investigation and frequently contaminates the surrounding natural environment [11].

Soil samples were collected and studied to examine the nature and degree of potential environmental pollution in the natural environment of the region. To this end, several standard soil quality parameters, as well as physicochemical parameters, were analyzed in samples that were collected from several different sampling regions. Toxic heavy metals are generally defined as metals or metalloids that have relatively high density, occur in multiple oxidation states, and cause extreme toxic effects on living organisms even upon exposure to low concentrations [12] [13]. The toxic heavy metals are found either naturally in a given area or can accumulate in the region as a result of anthropogenic activities. They have the ability to interact and bind to cellular components and can inhibit

metabolic functions and activities of living cells [14].

There have been several studies which sought to determine the extent and causes of toxic heavy metal distribution in various parts of the world [15] [16] [17] [18]. The results of Manta, Angelone, Bellanca, Neri and Sprovieri [15] demonstrated that in parts of Italy, the source of Pb, Zn, and Hg in topsoil could be traced to anthropogenic pollution, while other metals like Mn and Ni among were thought to be primarily naturally occurring metals. Lin, Teng and Chang [16] demonstrated that in Taiwan, urbanization and industrialization had led to the contamination of natural soil environments with toxic heavy metals. Arora *et al.* [18], demonstrated that in parts of India, use of irrigation water contaminated with toxic heavy metals led to bio-accumulation within vegetables that were being consumed by the general population. Li, Ma, van der Kuijp, Yuan and Huang [17] summarized that mining activities and irresponsible mining waste discharge practices across several provinces of China led to toxic heavy metal pollution in the region.

Microorganisms are the keystone of any natural ecosystem as they regulate vital nutrient cycles in a natural environment and hence the microbial population dynamics of the terrestrial soil environment were also analyzed. Therefore, this study presents a comprehensive picture of the terrestrial soil ecosystem and reveals several facets of the natural environment that can lead to widespread pollution and environmental degradation with devastatingly hazardous consequences.

#### 2. Materials and Methods

#### 2.1. Soil Ecosystem Study Area

The study area under investigation is located in the oil producing coastal region of the Ilaje community, Ondo State, Nigeria (Lat.  $5^{\circ}50'N - 6^{\circ}09'N$  and Long.  $4^{\circ}45'E - 5^{\circ}05'E$ ) (**Table 1**). It has an area of 1318 km<sup>2</sup> and a population of 336,740 [19]. Soil samples were obtained from the ten (10) major kingdoms which

Tab	ole 1	. Location	of the	study	y area.
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	LOCATIONS	GPS RE	ADING
1	Mahin	N06°10'151	E004°48'688
2	Ugbo-Nla	N06°08'541	E004°47'617
3	Idi-Ogba	N06°08'544	E004°47'692
4	Ilowo	N06°02'851	E004°50'574
5	Obenla	N06°00'939	E004°52'596
6	Odo Nla	N05°56'453	E004°56'917
7	Awoye	N05°55'019	E004°58'243
8	Ikuyinminu	N05°55'409	E004°57'660
9	Ayetoro	N06°06'340	E004°46'625
10	Igbokoda	N06°21'086	E004°48'101

make up the Ilaje community: Igbokoda, Mahin, Ugbo-Nla, Idi-Ogba, Ayetoro, Ilowo, Obenla, Odo Nla, Ikuyinminu, and Awoye during the dry and wet seasons. Igbokoda serves as the control because it is farthest from the oil drilling activities. The sites were chosen for accessibility, and they represent different geological groups, land usage, sub catchment and soil types of the Ilaje community. The meteorological conditions of the study area are characteristically hot and humid. The mean annual rainfall is around 1500 - 2000 mm [20]. The wet season in this region lasts from March through October while November to February constitutes the dry season. The mean maximum temperature is ~34.33°C, minimum ~26.02°C, and the relative humidity is 68.00% [20].

#### 2.2. Field Sampling and Collection of Soil Ecosystem

Soil samples were collected from ten (10) different sample sites following standard protocols from horizontal top soil surface (depth of 70 cm) as described by [21]. The non-conservative parameters were pH and temperature; these were determined *in situ*. The samples were immediately cooled ( $2^{\circ}C - 4^{\circ}C$ ) and transported to the laboratory in insulated containers for further analysis.

## 2.3. Analysis of Physicochemical Parameters

The soil conservative and non-conservative parameters were analyzed following standard protocols as described previously [21]. The non-conservative parameters (temperature and pH) were measured using portable instruments on site. Conservative parameters: ammonium, total chlorides, nitrogen, sulfate and phosphate content were measured following standard protocols as described previously [21] [22] [23]. The organic matter content of the soil was measured by a modified Walkley and Black method [21] [24].

# 2.4. Analysis of Heavy Metal and Metallic Nutrients

Pre-treatment of samples including acid digestion were performed according to standard methods as described previously [21]. A Perkin- Elmer ASS-280 Flame Atomic Absorption Spectrophotometer from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria was used to quantify the following metals: calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), arsenic (As), lead (Pb), copper (Cu), chromium (Cr), cadmium (Cd), according to standard operating procedures. Different calibration curves were utilized for each metal. The mean value of three repetitions was considered as the actual measurement.

# 2.5. Microbiological Analysis of Soil Ecosystem

#### Determination of bacterial and fungal populations

The pour plate method was used for the microbiological analysis of samples. Serial dilution and estimation of microbial counts were done by following the protocols of Ben-David and Davidson [25]. Briefly, a 0.1 mL aliquot of serially diluted samples was inoculated into plates containing Nutrient Agar (NA) for the cultivation of heterotrophic bacteria. Eosin Methylene Blue (EMB) was used for the cultivation of gram-negative bacteria. These plates were inoculated at 37°C for 24 hours. A 0.1 mL aliquot of serially diluted samples was seeded into prepared plates of Potato Dextrose Agar (PDA) containing chloramphenicol (30 mg/L) for the isolation of fungi. The plates were incubated for seven days at ambient room temperature (25°C - 30°C). Bacterial and fungal colonies were recorded in colony forming unit (CFU/mL) and spore forming units (SFU/mL) per mL respectively.

#### Identification and characterization of bacterial population

Bacterial characterization was based on the Bergey's Manual of Determinative Bacteriology [26], which demonstrated the use of various biochemical experiments such as carbohydrate fermentation, methyl red and Voges-Proskauer test, starch hydrolysis, lactose utilization, motility test, catalase test, citrate test and Gram staining. Bacteria were identified by Gideon Informatics Database, which compares samples utilizing biochemical characteristics.

#### Microscopic examination and identification of fungal isolates

Fungal identification was based on the characteristics of the colonies viz. surface appearance, shape, texture, and the color. Further characterizations were made according to the cultural attributes of sexual and asexual reproductive structures such as arthrospores, conidial head, sporangia, septate/non-septate mycelia [27] [28]. Microscopic observations were performed according to the Wet Mount Method using a needle [29]. The morphological and cultural attributes served as the basis of all identifications.

#### 2.6. Molecular Analysis: Polymerase Chain Reaction

#### **DNA** extraction

Total DNA was extracted from fungal and bacterial cells utilizing Zymo Research (ZR) bacterial DNA kit following the manufacturer's protocol (Zymo Research, CA, USA).

## Polymerase Chain Reaction (PCR) Amplification of Extracted DNA

PCR was done to amplify the 16S rRNA genes using primer set 1525r (5'-AAG GAG GTG WTC CAR CCG CA-3') and 27f (5'-AGA GTT TGA TCM TGG CTC AG-3') for bacteria and PTS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3') for fungi [30] [31]. The PCR cycles and subsequent purification of the product was done following the method described earlier.

#### Sequence Analysis

The PCR product purified by adding 70 percent ethanol and centrifuging the mix at 9000 rpm. An ABI 3130xL Genetic Analyzer (Applied Biosystems, California, USA) was utilized for subsequent nucleotide sequencing. The sequence was compared to the National Center for Biotechnology Information (NCBI) GenBank database using BLAST (Basic Alignment Search Tool) program [30].

The phylogenetic tree was constructed using phyloT (http://phylot.biobyte.de/).

# 2.7. Statistical Analysis

Statistical analysis was performed using the SPSS 22.0 statistical package (IBM, California, United States).

# 3. Results

# 3.1. Physicochemical Analysis of Soil Ecosystem

Several standard soil quality parameters were analyzed to examine the nature and extent of pollution within marine and terrestrial soil ecosystems. The pH values indicated that the soil ecosystem was acidic during the dry as well as the wet season (**Figure 1(a)**). In all the sampling sites, the temperature of the soil environment was approximately 30°C during the dry season and approximately



**Figure 1.** Physicochemical parameters of the soil ecosystem at different sampling sites during dry and wet seasons. (a) pH of the soil ecosystem of at different sampling sites during dry and wet seasons. (b) Temperature of the soil ecosystem of at different sampling sites during dry and wet seasons. (c) Total chlorinity of the soil ecosystem of at different sampling sites during dry and wet seasons. (d) Organic matter of the soil ecosystem of at different sampling sites during dry and wet seasons. Error bars indicate standard deviation.

28°C during the wet season (Figure 1(b)). Additionally, the total chloride ion concentration of the soil samples also exhibited seasonal variance; the total chlorine concentration of the soil samples was higher during the wet season than in the dry season (Figure 1(c)). The organic matter content of the soil was higher during the dry season compared to the wet season (Figure 1(d)) at sampling sites 2 and 9; the other sampling sites had higher organic matter concentrations during the wet season (Figure 1(d)).

#### 3.2. Non-Metallic and Metallic Nutrient Content of Soil Ecosystem

The capacity of any given natural ecosystem to respond to external perturbations relies partially on the accessibility as well as the availability of several nutrients. Moreover, the excess availability or loading of some nutrients such as phosphorus and sulfur can have undesirable consequences such as eutrophication. Hence, the concentrations of several nutrients have been surveyed in this study. The results established that ammonia was present in excess (ammonia content  $> \sim 20$  ppm) at many of the sampling sites (sampling sites 1 to 6; Figure 2(a)). The FEPA permissible limit for ammonia in soil is 0.6 ppm. Furthermore, the total Kjeldahl nitrogen content was also high during both the wet and dry seasons in the soil environment (Figure 2(b)). The terrestrial soil ecosystem also had an excessive concentration of sulfur and phosphorus content during both wet and dry seasons (Figure 2(c) and Figure 2(d)). Levels of calcium, potassium and magnesium were higher during the dry season than during the wet season. This behavior was in contrast to sodium, which was higher during the wet season (Figure 3). On the other hand, the sodium concentration of the soil ecosystem was higher during the wet season compared to the dry season (Figure 3).

#### 3.3. Toxic Heavy Metal Content of the Soil Ecosystem

Toxic heavy metals cadmium, chromium, lead and arsenic in the soil samples were analyzed to estimate the extent of pollution in the region. The results demonstrated that all sampling sites except sampling site 10 (a control site situated farthest from drilling operation sites) were contaminated with high levels of cadmium metal, particularly during the dry season (**Figure 4(a)**). Additionally, the wet season featured slightly lower cadmium amounts in the soil samples (**Figure 4(a)**). The chromium content of the soil ecosystem also exhibited a similar pattern of seasonal variations (**Figure 4(b)**). The chromium concentration in the soil samples was >3 ppm in most of the sampling sites. However, the chromium concentration at site 10 was almost negligible at 0.01 ppm compared to the other sampling sites. Concentrations of lead were relatively low during the wet seasons but spiked considerably in dry season (**Figure 4(c)**). Arsenic also exhibited this type of behavior (**Figure 4(d)**). However, it is imperative to note that the heavy metal contamination for all metals was significantly lower at the control site (site 10).



**Figure 2.** Non-metallic nutrient content of the soil ecosystem of at different sampling sites during dry and wet seasons. (a) Ammonia content of the soil ecosystem of at different sampling sites during dry and wet seasons. (b) Total Kjeldahl nitrogen content of the soil ecosystem of at different sampling sites during dry and wet seasons. (c) Sulphur content of the soil ecosystem of at different sampling sites during dry and wet seasons. (d) Total phosphorus content of the soil ecosystem of at different sampling sites during dry and wet seasons. (d) Total phosphorus content of the soil ecosystem of at different sampling sites during dry and wet seasons. (d) Total phosphorus content of the soil ecosystem of at different sampling sites during dry and wet seasons. Error bars indicate standard deviation.

# 3.4. Microbial Population Dynamics of the Soil Ecosystem

In the present study, the microbial community structure of the terrestrial soil environment at different sampling sites was investigated to understand the microbial population dynamics of the ecosystem during the dry and wet seasons. The heterotrophic count study was conducted to estimate the bacterial population size present in the soil. The results indicated that during the dry season, the bacterial population size in the soil was larger compared to the wet season (**Figure 5(a)**). The fungal population size also exhibited a similar pattern of seasonal variation (**Figure 5(b**)). The results showed the presence of a different bacterial population in all the soil samples collected during the study. The presence of several anaerobic microorganisms that belong to *Enterobacter* were identified during the study (**Figure 6; Table 2(a)**, **Table 2(b)**). Many organisms



**Figure 3.** Metallic nutrient content of the soil ecosystem of at different sampling sites during dry and wet seasons. (a) Calcium content of the soil ecosystem of at different sampling sites during dry and wet seasons. (b) Sodium content of the soil ecosystem of at different sampling sites during dry and wet seasons. (c) Potassium content of the soil ecosystem of at different sampling sites during dry and wet seasons. (d) Magnesium content of the soil ecosystem at different sampling sites during dry and wet seasons. Error bars indicate standard deviation.

belonging to the genera *Azospirillum*, *Aeromonas*, *Pseudomonas* and *Bacillus*, were abundantly present in the soil ecosystem during the different seasons (Figure 6; Table 2(a), Table 2(b)). The presence of several enteric bacteria belonging to the genera *Enterobacter* and a strain of *E.coli* also indicated the presence of disease causing pathogenic bacteria in the soil environment (Figure 6; Table 2(a), Table 2(b)). Most of the fungi that were identified during the study belong to *Ascomycota* (example *Penicillium chrysogenum; Penicillium oxalicum; Aspergillus terreus*) (Figure 7; Table 3).

# 4. Discussion

This study investigated a variety of aspects of the terrestrial soil ecosystem within



**Figure 4.** Level of toxic heavy metal contamination of the soil ecosystem at different sampling sites during dry and wet seasons. (a) Level of cadmium contamination of the soil ecosystem at different sampling sites during dry and wet seasons. (b) Level of chromium contamination of the soil ecosystem at different sampling sites during dry and wet seasons. (c) Level of lead contamination of the soil ecosystem at different sampling dry and wet seasons. (d) Level of arsenic contamination of the soil ecosystem at different sampling sites during dry and wet seasons. (d) Level of arsenic contamination of the soil ecosystem at different sampling sites during dry and wet seasons. (d) Level of arsenic contamination of the soil ecosystem at different sampling sites during dry and wet seasons. Error bars indicate standard deviation.



**Figure 5.** Microbial load of soil samples in different locations during dry and wet season. (a) Bacterial load of soil samples in different locations during dry and wet season. (b) Fungal load of soil samples in different locations during dry and wet season.



Figure 6. Phylogenetic tree of isolated bacterial population of the soil ecosystem of the study area.

the Ondo state, Nigeria. Several international oil corporations are engaged in extracting oil from this site [10] which has led to substantial and significant environmental degradation in the region [11]. Ten locations were selected near and around the crude oil drilling and extraction sites. Igbokoda (site 10) was selected as a control site as it was located farthest from the drilling site and was least affected by contamination of toxic pollutants and substances.

Initially, standard soil quality parameters were checked to explore the various facets of this sensitive ecosystem. Federal Environmental Protection Agency (FEPA) National Guidelines and Standards for Environmental Pollution Control in Nigeria were used to compare concentrations of contaminants in soil with established guidelines [32]. Where possible, the lowest standard was used for comparison to provide a conservative estimate of risk. Guidelines for the marine environment were used in preference of freshwater. Additionally, measured data were compared with previous work in the region to establish trends. Physicochemical parameters of soil samples were higher during the dry season and similar to previous reports for Ondo State and areas of the Ilaje community [33] [34] [35] [36].

Table 2. (a) Frequency occurrence of bacteria in soil locations during dry and wet season; (b) Frequency occurrence of bacteria in soil locations during dry and wet season.

(a)																				
Organisms			I	Locat	ions (	dry s	eason	Locations (wet season)												
		2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Acinetobacter baumannii ACICU	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Aeromonas hydrophila subsp. hydrophilia ATCC 7966	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	-
Alcaligenes sp.BN3	-	-	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Azospirillum lipoferum 4B	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus altitudinis	-	-					-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus cereus ATCC 10987		+	+	+		+	-	-	+	+	-	+	-	+		+			+	+
Bacillus megaterum QM B1551		+	-	-	-	+	+	+	-	-	-	+	-	-	-	+	+	+	-	-
Bacillus mycoides	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Bacillus subtilis	-	+	+	-	-	+	-	-	-	+	-	+	+	-	-	+	-	-	-	+
Bacillus NLAE-zl-256	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus NLAE-zl-C302	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacterium NLAE-zl-H271	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrobacter freundii	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
Clostridium perfringens ATCC 13124	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-
Clostridium ventriculi	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Corynebacterium xerosis	-	-	-	-		-	-	-	-	-	-	-		-	-	-	-	-	-	-

\*\*\*Key: + =present; - = absent.

#### (b)

Organisms			I	locat	ions (	dry s	easor	1)	Locations (wet season)											
		2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Enterobacter aerogenes KCTC 2190	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Enterobacter ludwigii strain SWA1	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Enterobacter sp UIWRF0285	-	-	+	-	+	+			+	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli K-12	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-
Klebsiella oxytoca	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-
Lysinibacillus fusiformis ZCI	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
Lysinibacillus varians	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus flavus	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-
Proteus mirabilis ATCC 29906	-	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	-	-	+	+
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa PA01	-	+	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-	+	-
Rhodobacter sphaeroides 2.4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus subsp. aureus</i> NCTC 8325	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vibrio fluvialis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vibrio metschnikovii	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Vibro mimicus VM573	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-

\*\*\*Key: + =present; - = absent.



Figure 7. Phylogenetic tree of isolated bacterial population of the soil ecosystem of the study area.

Organisms –			L	ocati	ons (	dry s	easor	n)		Locations (wet season)										
		2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Aspergillus fumigatus	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+
Aspergillus niger ATCC1015	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Aspergillus flavus NRRL3357	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+
Aspergillus pseudoglaucus	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	+	
Aspergillus sp ASR-161	+	+	-	-	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-
Aspergillus terreus NIH2624	+	+	+	-	+	+	-	+	-	-	+	+	+	-	+	+	-	+	-	-
Bjerkandera adusta		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Bjerkandera sp.B33/3	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Chrysosporium tropicum	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
Coriolopsis sp arf5	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Curvularia lunata</i> m118	-	+	-	-	-	+	-	-	-	-	-	+	-	-		+	-	-	-	-
Mucor circinelloides f circinelloides 1006PhL	-	-	-	+	-	-	+	-	-	-	-	-	-	+	+		+	+	-	+
Nemania serpens var. serpens	-	-	-	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-
Penicillium chrysogenum	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-
Penicillum oxalicum	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	-	-	+	-
Pichia kudriavzevii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pichia sporocuriosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus oryzeae	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Scedosporium apiospermum	-	+	-	-	+	+	-	-	-	-	-	+	-	-	+	+	-	-	-	-
Talaromyces verruculosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trametes polyzona	-	+	+	+	+		-	-	-	+	-	-	-	-	-	-	-	-	-	-
Trichodema reesei RUT C-30	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+
Trichoderma asperellum		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Frequency occurrence of fungi in soil locations during dry and wet season.

\*\*\*Key: + = present; - = absent.

The pH of the soil ecosystem was around pH 6 during both dry and wet seasons in most of the sampling sites, indicating the soil was consistently acidic; this acidic environment in turn significantly influenced the indigenous microorganisms within the soil environment.

The presence of high quantities of phosphorus, soil ammonia as well as the total Kjeldahl nitrogen indicates that the soil environment is rich in nutrients that may be accessible to the different organisms in the ecosystem. Soil organic matter is closely associated with soil inorganic constituents and is vital for the maintenance of soil particle aggregate integrity [37]. The organic matter present in the soil ecosystems also increases the water retention capacity, affects various thermal properties of soils and plays a major role in regulating soil pH [37]. The average organic matter content observed in this study was approximately 6% with a high of 12% at site 2 and a low of 5% at site 10. These results indicate that at several sampling locations soil was not humic [37] [38]. The soil organic content in all the sampling sites was less than 20% - 25% which demonstrates that the soil environment in the region was largely hydric [37] [39]. The sodium content of the soil environment in most sampling sites was particularly low in comparison with [32]; low sodium levels can potentially have adverse effects on the growth and survival of different organisms in the soil environment.

Toxic heavy metals have the ability to exert detrimental effects on human beings as well as other living organisms in a wide array of ways even at low concentrations [40]. The key human organ systems that might be potentially affected include the skeletal system, digestive system, nervous system as well as reproductive system [14]. Due to their widespread incidence within the lithosphere and high toxicity on living systems in low quantities [41], these toxic heavy metals are widely considered to be enormously hazardous and perilous constituents of the natural environment.

Accumulation of an excess of amount of Cd in human beings can cause a plethora of diseases such as renal disturbance and osteomalacia, and it is also highly poisonous [14] [42]. The results indicated the presence of Cd in the ecosystem (3 ppm); these levels of Cd exceed environmental guidelines established by FEPA (0.2 ppm) and represent a significant public concern [18] [32].

Chromium occurs in multiple oxidation states. However, naturally occurring chromium exists primarily as Cr(III) and hexavalent chromium, among which Cr (VI) is much more toxic to living cells than Cr (III) [14]. Hexavalent chromium ions can enter living cells via phosphate and sulfate transport pathways. Inside the living cells, Cr (VI) ions induce oxidative stress as a result of its acutely high redox potential and induces several deleterious events including DNA damage, DNA strand breakage and disruption of cell signaling pathways [43]. Furthermore, DNA damage via Cr (VI) induced oxidative stress can also cause cancer. Since Cr was present in a relatively high concentration in the soil ecosystem, this Cr has the potential to enter living systems through biological accumulation. The most detrimental toxic effects of Pb are on the central nervous system of human beings and higher mammals where this toxic metal has the capability to disrupts brain development [44]. Lead was also detected within the natural soil ecosystem during this study which indicated potential lead contamination in the region. However, lead was within permissible limits established by FEPA [32].

Within the natural environment, arsenic (As) is found mostly in trivalent arsenate (As III) as well as pentavalent arsenite (As V) oxidation states. In contrast to most other major toxic heavy metals including Pb and Cr, which exert higher toxicity at a higher oxidation state, trivalent arsenic is more toxic than the higher pentavalent state of arsenic. As (III) has the potential to act as an analogue of phosphate ions, and thus it enters living cells through phosphate accumulation pathways [45]. Consequently, it restricts oxidative phosphorylation and ATP synthesis [14]. The results indicated the presence of arsenic in the soil environment. Since arsenic exerts a wide array of toxic effects on living systems, the presence of arsenic in the natural environment might have monumentally harmful effects on the ecosystem health. These results also indicated that during the wet season there was a dilution of these pollutants in the ecosystem due to higher rainfall. It is important to note that the control site at Igbokoda, which was least affected by the crude oil extraction operations, was also least affected by toxic heavy metal contamination. All heavy metal levels at this site did not exceed FEPA guidelines [32].

The results indicated that there is a diverse population of bacteria and fungus present in the water column as well as the soil ecosystem. The microorganisms represented diverse taxonomic and functional groups that are capable of performing a wide array of diverse ecosystem functions within the terrestrial soil ecosystem [46]. Most of the fungi belonged to the Ascomycota which are capable of producing microscopic spores inside specialized, elongated sac like structures, also known as "asci" [47]. It can be hypothesized that the prevailing acidic pH of the environment prominently favored the growth of these diverse population of fungi. Within the soil environment, the heterotrophic population of microorganisms relied on the presence of organic compounds present in the natural ecosystem. Ros, Rodríguez and Hernández [48] observed that Ascomycota is one of the principal phyla observed in bioremediation treatments. Acinetobacter sp., one of the predominant bacteria identified in the samples have been reported to oxidize As (III) rapidly within 48 - 72 h of incubation [49]. In addition, the most widespread microbes from this study belonged to the Bacillus genus. Their presence in the study area is easily accounted for, given their ability to form microfilms that give them the ability to survive in multiple environments [50]. Bacillus species thrive in almost every environment, some living as free bacteria and others as pathogens to various organisms, including humans. They have been isolated from the air, ocean, soil, silt, lake, fresh water, and other surfaces before [51] [52]. However, Bacillus cereus and Bacillus subtilis are predominantly soil-dwelling bacteria that are known for their benefits to rhizospheres and are therefore not uncommon in such locations as those in the Ilaje community. Members of this genus present themselves as both useful and outright pathogens. They are recognized as plant growth promoting bacteria, decontamination agents, and generators of industrial enzymes for food, textile, and chemical industries [52]. Most of the microorganisms isolated from the soil samples are very much adaptable to toxic environments and can decrease the load of toxic substances by their successful transformation and remediation [53].

## **5.** Conclusion

This study was a comprehensive analysis of various diverse aspects of this multifaceted yet susceptible natural ecosystem of a region which is the site for extensive offshore as well as onshore crude oil drilling operations by several multinational corporations. In general, concentrations of metals and cations were higher during the dry season than wet season and were similar to previous reports. Concentrations of contaminants in soil were compared to FEPA standards for the protection of environmental and human health. Concentrations of As, Pb, Cr, and Cd in soil were lesser than FEPA guidelines. Elevated concentrations of heavy metals and cations are likely anthropogenic in nature and might be from oil and gas activity in the area and due to the release of untreated urban wastes. The findings of this study demonstrated that the region was contaminated by a plethora of toxic pollutants including toxic heavy metals like cadmium, chromium, lead and arsenic. There was a clear pattern of seasonal variation in most of the contaminants and other parameters as they were evidently higher during the dry season in comparison to the wet season. Therefore, this study also explored the potential ecotoxicity which poses a grave menace to the socioeconomic well-being and health risk for the local people. This study provides a baseline for future studies: future work will examine changes in these toxic components of the soil using this data.

#### Acknowledgements

The authors would like to thank the academic staff of the Department of Microbiology, Federal University of Technology, Akure for their guidance and unwavering support.

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