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Impact of crude oil pollution on the physicochemical and microbiological properties of orashi river wetland in Egbema, Nigeria

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Abstract

The study investigated impact of crude oil pollution on the physicochemical and microbiological parameters of crude oil polluted Orashi River wetland. The physicochemical, microbiological parameters and trend of enzymatic activities in crude oil polluted and unpolluted wetlands were evaluated. Bioloads had their highest values in the lightly polluted wetlands with values of rainy season leading, followed by control (unpolluted wetlands) and lowest values in the heavily polluted wetlands. Bacillus species was the most prevalent in all wetlands for both seasons - lightly polluted wetlands – rainy/ dry season (100% ±0.08/ 85.7% ±0.17), heavily polluted wetlands – rainy/ dry season (42.9% ±0.23/ 28.6 ±0.12%), control (unpolluted wetlands) – rainy/ dry season (85.7% ±0.11/ 71.4% ±0.33). Most of the physicochemical parameters measured had highest values in lightly polluted wetlands - rainy/ dry season (total nitrogen - 2.98 ± 0.15 µg/g / 2.74 ± 0.72 µg/g, available phosphorus - 12.15 ± 0.19 µg/g / 11.43 ± 0.57 µg/g and conductivity - 6.75 ± 0.30µm/s / 6.43 ± 0.27µm/s), followed by control (unpolluted wetlands) and with lowest values in the heavily polluted wetlands. Soil pH recorded low values in the polluted wetlands with the least value in the heavily polluted wetlands, while soil organic carbon and temperature values showed a negative trend to the above with lowest values in the control (unpolluted wetlands), followed by a higher values in the polluted wetlands. All physicochemical parameters have some pattern of trend except for exchangeable cations that were not definite in trend. Soil enzymatic activities values follow the same trend with bioloads and physicochemical parameters. All values obtained in bioloads, prevalence, physicochemical and enzymatic activities when compared between control (unpolluted wetlands), lightly polluted and heavily polluted wetlands were statistically significant ($p<0.05$). This study has shown that crude oil on heavy impaction could

cause adverse effects on wetlands quality parameters while light impaction encourages different wetlands quality/ fertility indexes.

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Introduction

Wetlands are lands between terrestrial and aquatic ecosystems and are periodically inundated or saturated with water (Mitsch and Gosselink, 2007). They are rich in soil nutrients producing good soil conditions that favour the growth of various vegetations. Wetlands rank among the most productive and valuable ecosystems in the world and perform numerous important functions like farming, groundwater recharge etc. They are generally rich in mineral salts due to water supply from the surroundings via runoff and/or ground water (Ogban *et al.*, 2011).

Wetlands are ecologically sensitive and vulnerable to human disturbances. When wetland is polluted, the ecosystem is altered, and agricultural activities are affected. Wetlands contaminated with heavy crude oil impaction can create unconducive life conditions in the soil, due to some inherent factors like poor aeration, immobilization of soil nutrients, loss of water-holding capacity, lowering of soil pH, and reduction in soil enzyme activities (Sathiya-Moorthi, 2008; Achuba and Peretiemo-Clarke, 2008) as well as inhibitory effect on the nitrate and phosphate reductase activities of plants (Odjegba and Atebe, 2007).

Crude oil is naturally occurring, unrefined/ unprocessed oil composed of hydrocarbon deposits found deep beneath the earth's surface. Crude oil has ranging viscosity and can vary in colour to various shades of black and yellow depending on its hydrocarbon composition. Crude oil can be refined to produce usable products such as gasoline, diesel and various forms of petrochemicals. Crude oil pollution is a threat to the environment and the remediation is a major challenge to environmental research (Chorom *et al.*, 2010). Contamination of soil by crude oil could lead to a reduced microbial density and activities (Amadi *et al.*, 1996). Apart from its phytotoxicity, excess oil in soil may also limit the availability of nitrogen (John *et al.*, 2010). In the case of relatively light crude oil contamination, it stimulates the soil biochemical processes such as organic matter decomposition, ammonification, nitrification, symbiotic and non-symbiotic nitrogen fixation and geochemical cycling of elements, which thereafter increases the number and activities of microorganisms (Amadi *et al.*, 1996).

Crude oil end-products have been used for many decades for illumination, energy generation and as lubricant. The invention of the internal combustion engine and its fast adoption in all transport forms enlarged the employment of this natural resource, thus increasing its exploration and production demands. These activities involve pollution risks that can be minimized, but not totally eliminated, hence causing several problems for the environment (Pala *et al.*, 2006).

Crude oil exploration and production (E&P) activities occur frequently in the natural wetlands of South-South Nigeria such as the Orashi wetlands in Egbema, Rivers State. Oil exploration and production (E&P) processes can contribute to the localized loadings of total petroleum hydrocarbons (TPH) in the environment through accidental spillage or oil leaks from producing wells, gathering lines, transportation lines and pits. Release of hydrocarbons into the environment is a major cause of soil pollution (Hollinger *et al.*, 1997). Oil exploration and production (E&P) activities have multiple deleterious impacts on the wetland ecosystem. The adverse effect of crude oil on wetlands ranges from loss of vegetation to addition of toxic materials. Thus, wetland degradation in the South-South Nigeria resulting from oil exploration and production (E&P) activities has drawn national and regional attentions.

Orashi wetlands in Egbema are prone to crude oil and associated end-products contamination due to the exploration and production (E&P) activities in the area by major oil companies, leading to distortion in microbial dynamics and imbalance in soil health parameters. However, most studies on the effect of crude oil pollution focus on uplands, while that of wetlands have received less attention. This investigation was conducted with the major objective of assessing the impact of crude oil pollution on the physicochemical and microbiological properties of Orashi River wetland in Egbema, Rivers State, Nigeria.

Materials and methods

Study Area

The study area is Orashi River Wetland at Egbema in Ogba/Egbema/Ndoni Local Government Area (ONELGA) of Rivers State, in the South-Southern Nigeria. Egbema community has vast fertile land including wetlands for agriculture and wildlife, and most of their people are great farmers, hunters and fishermen with rich cultural history. The rainy season begins from April and lasts until October with annual rainfall varying from 1,500mm to 2,200mm (60 to 80 inches). The dry season begins from November and runs through March with two months of Harmattan from late December to late February. The hottest months are between January and March. The community is 80.5km away from Port Harcourt city, the capital of Rivers State. The geographical coordinates are Latitude 4.7572222°, and Longitude 6.7502778°. The area is of tropical climatic conditions with rain forest features and an average annual temperature ranging between 25 - 35°C as lowest and highest values respectively. The soil type is clay mixed with silt.

Experimental Design

The study was carried out on site in rainy and dry season. The crude oil used was Bonny Light Crude Oil, collected from Ebocha Oil Centre, in Egbema, Rivers State, Nigeria. The investigation was done on site on two separate plots of the wetland, measuring eighteen feet squared (18ftx18ft) each, and twelve (12) feet apart from each other. Each of the two separate wetland plots measuring eighteen feet squared (18ftx18ft), were spilled with graded volumes (either 5 or 20 Liters) of crude oil to represent lightly and heavily polluted wetlands respectively. Control samples were not spilled with crude oil and were situated at adjacent extremes of the two wetland plots. The two experimental plots of wetland were exposed to climatic elements (rain and sunlight) throughout the period of the study.

Sample Collections

Soil samples for analysis were collected on site from the surface (0-15cm depth) using alcohol-disinfected trowels, into sterile nylon bags (Ziploc) for both microbiological and physicochemical analysis after one week of soil contamination with spilled crude oil. Three samples were collected from a sampling point and pooled together to give a composite sample. Samples were collected and analyzed, 2 weeks after contamination. A total of fifty six (56) samples were collected for the study with control samples inclusive, twenty eight (28) samples out of these numbers were for rainy season, while the other twenty eight (28) samples were for dry season. Samples were taken to the laboratory in ice packs for both microbiological and physicochemical analysis within twenty four (24) hours of collection.

All soil samples for microbiological analysis were analyzed within twenty four (24) hours after collection. The soil samples for physicochemical analysis were oven-dried at a regulated temperature of 40°C and sieved using a two millimeter (2mm) sieve. The soil samples were stored in air tight glass containers and analyzed within one (1) week of collection.

Microbiological Analysis of Samples

Ten fold serial dilutions of the soil samples were done. Spread plate and streaking culturing techniques (Capuccino and Sherman (2010) were used to enumerate and isolate bacteria and fungi in the wetland samples. The bacterial bioloads enumerated include total heterotrophic count (THC), petroleum degrading bacteria (PDB), phosphate solubilizing bacteria (PSB) and nitrifying bacteria (NB). Pure cultures of bacterial isolates were identified using cultural, morphological and biochemical characterization. Identification of the bacteria to genera level was based on the schemes of Boone *et al.*, (2005). The purified fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture technique, lactophenol staining The schemes of Barnett and Hunter (2000), and Watanabe, (2010) were used for identification.

Physico-chemical Studies

The physicochemical parameters measured include soil particulate matters, pH, temperature, Organic Carbon, Total Nitrogen, Total Phosphorus, Magnesium, Calcium, Sodium, Potassium, and Conductivity. Soil particulate matters were measured using methods of (Kettler *et al.*, 2001). The pH and conductivity were determined using methods of (David *et al.*, 2013). Other physicochemical parameters such as exchangeable cations, total nitrogen, available phosphorus and organic carbon content were analyzed using methods of (Black, 2000).

Soil Enzymatic Activities

The enzyme activities analyzed include Dehydrogenase, Urease, Cellulase and the Phosphatases. Cellulase activity was determined using methods of Vancov and Ken, 2009. Other soil enzymatic activities were determined as described and adopted by Nwaugo *et al.*, 2008.

Data Analysis

Data obtained from this research work were analysed using ANOVA. Descriptive statistics in form of means and standard deviation and Duncan post hoc were also used to assess the data. The analyses were done using SPSS 16.

Results

The microbial bioloads of Orashi river wetland (unpolluted and crude oil polluted) according to seasons are shown in Table 1. Although, bioloads were high during the rainy season, but during the seasons, high bioloads were recorded in the lightly polluted wetlands. This is followed by control (unpolluted wetlands) while the least bioloads were recorded in the heavily polluted wetlands (Table 1). The petroleum degrading bacteria (PDB) showed a significant increase in the lightly polluted wetlands (rainy/ dry seasons) (1.6 ± 0.13) x 10^5 cfu/g soil (1.4 ± 0.37) x 10^5 cfu/g soil than in heavily polluted wetlands (rainy/ dry seasons) (1.1 ± 0.15) x 10^3 cfu/g soil /(0.9 ± 0.19) x 10^3 cfu/g soil (Table 1).

Table 1. Microbial bioloads of Orashi wetland (unpolluted and crude oil polluted) according to seasons.

Organisms	Rainy Season			Dry Season		
	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted
THB (CFU/g soil)	(4.7 ± 0.23^b) x 10^6	(5.1 ± 0.21^a) x 10^6	(4.5± 0.31^c) x 10^4	(4.0 ± 0.12^b) x 10^6	(4.7 ± 0.18^a) x 10^6	(4.0± 0.34^c) x 10^4
PDB (CFU/g soil)	(1.2 ± 0.19^b) x 10^5	(1.6 ± 0.13^a) x 10^5	(1.1± 0.15^c) x 10^3	(1.0 ± 0.25^b) x 10^5	(1.4 ± 0.37^a) x 10^5	(0.9± 0.19^c) x 10^3
PSB (CFU/g soil)	(3.5 ± 0.20^b) x 10^3	(3.6 ± 0.16^a) x 10^4	(1.8± 0.27^c) x 10^2	(1.5 ± 0.47^b) x 10^2	(1.3 ± 0.62^a) x 10^3	(0.8± 0.17^c) x 10^1
NB (CFU/g soil)	(2.0 ± 0.20^b) x 10^2	(2.2 ± 0.17^a) x 10^3	(1.0± 0.14^c) x 10^1	(1.7 ± 0.18^b) x 10^2	(1.8 ± 0.13^a) x 10^3	(1.0± 0.25^c) x 10^1
Fungi (CFU/g soil)	(1.3 ± 0.15^b) x 10^2	(1.6 ± 0.21^a) x 10^2	(1.7± 0.21^c) x 10^1	(1.0 ± 0.41^b) x 10^2	(1.3 ± 0.56^a) x 10^2	(1.3± 0.18^c) x 10^1

Values are given as mean ± SD. Within rows, values followed by the same alphabets are not significantly different but those followed by different alphabets are significantly different.

Legend: THB - Total Heterotrophic Bacteria, PDB - Petroleum Degrading Bacteria, PSB - Phosphate Solubilizing Bacteria, NB - Nitrifying Bacteria.

It was noted that Nitrifying Bacteria (NB) was both the most sensitive and affected group of microorganisms with (1.0 ± 0.14) x 10^1 cfu/g soil /(1.0 ± 0.25) x 10^1 cfu/g soil in heavily polluted wetlands (rainy/ dry seasons), and (2.2 ± 0.17) x 10^3 cfu/g soil /(1.8 ± 0.13) x 10^3 cfu/g soil in lightly polluted wetlands (rainy/ dry seasons), with (2.0 ± 0.20) x 10^2 cfu/g soil /(1.7 ± 0.18) x 10^2 cfu/g soil in control (unpolluted wetlands) (rainy/ dry seasons). Other affected groups of microorganisms were PSB and PDB in that order. However, the least affected was THB with (4.5 ± 0.31) x 10^4 cfu/g soil /(4.0 ± 0.34) x 10^4 cfu/g soil in the heavily polluted wetlands (rainy/ dry seasons), (5.1 ± 0.21) x 10^6 cfu/g soil /(4.7 ± 0.18) x 10^6 cfu/g soil in the lightly polluted wetlands (rainy/ dry seasons) and (4.7 ± 0.23) x 10^6 cfu/g soil /(4.0 ± 0.12) x 10^6 cfu/g soil in the control (unpolluted wetlands) (rainy/ dry seasons). There was significant difference in both seasons between bioloads of lightly polluted wetlands, control (unpolluted wetlands) and that of heavily polluted wetlands, when compared ($p<0.05$). Also, the bioload values showed statistical significance ($p<0.05$) among the different seasons (rainy season bioloads greater than dry season bioloads).

Table 2 shows the prevalence of bacterial and fungal species in the Orashi river wetland (unpolluted and crude oil polluted) according to seasons. Lightly polluted wetlands of both rainy and dry seasons had the highest spread in prevalence of microbial species above the control (unpolluted wetlands) and heavily polluted wetlands.

Table 2. Prevalence of bacterial and fungal species in Orashi wetland (unpolluted and crude oil polluted) according to seasons.

Organisms	Rainy Season									Dry Season								
	Control (Unpolluted Wetland)			Lightly Polluted			Heavily Polluted			Control (Unpolluted Wetland)			Lightly Polluted			Heavily Polluted		
	NE	NO	%	NE	NO	%	NE	NO	%	NE	NO	%	NE	NO	%	NE	NO	%
Pseudomonas species	7	5	71.4 ±0.21^{b}	7	6	85.7 ±0.44^{a}	7	2	28.6 ±0.55^{c}	7	4	57.1 ±0.26^{b}	7	5	71.4 ±0.19^{a}	7	2	28.6 ±0.06^{c}
Bacillus species	7	6	85.7 ±0.11^{b}	7	7	100 ±0.08^{a}	7	3	42.9 ±0.23^{c}	7	5	71.4 ±0.33^{b}	7	6	85.7 ±0.17^{a}	7	2	28.6 ±0.12^{c}
Staphylococcus species	7	5	71.4 ±0.27^{a}	7	3	42.9 ±0.25^{b}	7	0	0	7	5	71.4 ±0.05^{a}	7	2	28.6 ±0.09^{b}	7	0	0
Escherichia coli	7	5	71.4 ±0.53^{a}	7	3	42.9 ±0.17^{b}	7	0	0	7	4	57.1 ±0.06^{a}	7	3	42.9 ±0.12^{b}	7	0	0
Flavobacterium species	7	4	57.1 ±0.04^{b}	7	5	71.4 ±0.32^{a}	7	2	28.6 ±0.13^{c}	7	4	57.1 ±0.19^{b}	7	5	71.4 ±0.16^{a}	7	1	14.3 ±0.15^{c}
Azotobacter species	7	4	57.1 ±0.16^{a}	7	4	57.1 ±0.14^{a}	7	1	14.3 ±0.12^{b}	7	3	42.9 ±0.11^{b}	7	4	57.1 ±0.20^{a}	7	1	14.3 ±0.05^{c}
Aspergillus species	7	2	28.6 ±0.33^{b}	7	3	42.9 ±0.22^{a}	7	1	14.3 ±0.08^{c}	7	2	28.6 ±0.13^{b}	7	3	42.9 ±0.14^{a}	7	1	14.3 ±0.45^{c}
Penicillium species	7	3	42.9 ±0.01^{a}	7	3	42.9 ±0.20^{a}	7	1	14.3 ±0.06^{b}	7	2	28.6 ±0.31^{b}	7	3	42.9 ±0.02^{a}	7	1	14.3 ±0.51^{c}
Acremonium species	7	1	14.3 ±0.44^{b}	7	2	28.6 ±0.42^{a}	7	1	14.3 ±0.04^{b}	7	2	28.6 ±0.44^{a}	7	2	28.6 ±0.05^{a}	7	1	14.3 ±0.27^{b}
Rhodotorula species	7	3	42.9 ±0.09^{b}	7	4	57.1 ±0.10^{a}	7	1	14.3 ±0.15^{c}	7	2	28.6 ±0.52^{b}	7	3	42.9 ±0.31^{a}	7	1	14.3 ±0.50^{c}

NE = Number of samples examined; NO = Number of isolates observed; % = Percentage

Bacillus species was the most prevalent in all wetlands for both seasons {lightly polluted wetlands – rainy/ dry season (100% ±0.08/ 85.7% ±0.17), heavily polluted wetland – rainy/ dry season (42.9% ±0.23/ 28.6% ±0.12), control (unpolluted wetlands) – rainy/ dry season (85.7% ±0.11/ 71.4% ±0.33), while *Pseudomonas*, *Flavobacterium* and *Azotobacter* species were more prevalent in the lightly polluted wetlands - rainy/ dry season {*Pseudomonas* – (85.7% ±0.44/ 71.4% ±0.19), *Flavobacterium* species – (71.4% ±0.32/ 71.4% ±0.16) and *Azotobacter* species (57.1% ±0.14/ 57.1% ±0.20) }. *Staphylococcus* species and *Escherichia coli* were not observed in the heavily polluted wetlands of both seasons. Fungal species (*Aspergillus, Penicillium, Acremonium and Rhodotorula* species) were observed with low prevalence in all wetland samples with little higher values in the lightly polluted wetlands (Tables 2). The values obtained in each of the seasons between lightly polluted wetlands, control (unpolluted wetlands) and that of heavily polluted wetlands, when compared were statistically significant ($p<0.05$).

The results of physico-chemical parameters of Orashi River Wetland (unpolluted and crude oil polluted) according to seasons are shown in Table 3. The wetland parameters such as temperature, organic carbon, total nitrogen, available phosphorus and conductivity among the various seasons, maintained some trends of values. Total nitrogen, available phosphorus and conductivity values were highest in the lightly polluted wetlands – rainy/ dry season (total nitrogen – 2.98 ± 0.15/ 2.74 ± 0.72, available phosphorus - 12.15 ± 0.19/ 11.43 ± 0.57, and conductivity - 6.75 ± 0.30 / 6.43 ± 0.27) and with lowest values in the heavily polluted wetlands – rainy/ dry season (total nitrogen - 0.14 ± 0.34/ 0.13 ± 0.09, available phosphorus – 7.65 ± 0.41/ 6.49 ± 0.10, and conductivity - 6.30 ± 0.34/ 5.90 ± 0.18).

Temperature and organic carbon values showed lowest values in the control (unpolluted wetlands) - rainy/ dry seasons, and higher values in the polluted wetlands with heavily polluted wetlands recording the highest values (Table 3).

Table 3. Soil characteristics of Orashi wetland (unpolluted and crude oil polluted) according to seasons.

Soil Characteristics	Rainy Season			Dry Season		
	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted
Sand (%)	92.20 ± 0.42[a]	91.40 ± 0.32[b]	90.00 ± 0.15[c]	92.00 ± 1.07[a]	91.08 ± 0.27[b]	89.80 ±0.43[c]
Silt (%)	4.40 ± 0.51[a]	4.15 ± 0.15[b]	4.00 ± 0.22[c]	4.38 ± 0.30[a]	4.20 ± 0.58[a]	4.08 ± 0.07[b]
Clay (%)	3.40 ± 0.76[b]	4.45 ± 0.14[b]	6.00 ± 0.26[a]	3.62 ± 0.21[b]	4.72 ± 0.44[b]	6.12 ± 0.03[a]
pH	6.90 ± 0.20[a]	5.50 ± 0.08[b]	3.50 ± 0.09[c]	6.80 ± 0.12[a]	5.45 ± 0.48[b]	3.00 ± 0.11[c]
Temperature (°C)	31.10 ± 0.26[c]	33.30 ± 0.57[b]	36.40 ± 0.35[a]	32.98 ± 0.93[c]	35.20 ± 0.64[b]	38.40 ± 0.18[a]
Organic Carbon (µg/g)	8.53 ± 0.05[c]	8.90 ± 0.21[b]	9.16 ± 1.06[a]	8.31 ± 1.00[c]	8.67 ± 1.07[b]	9.01 ± 0.61[a]
Total Nitrogen (µg/g)	2.66 ± 0.17[b]	2.98 ± 0.15[a]	0.14 ± 0.34[c]	2.45 ± 0.50[b]	2.74 ± 0.72[a]	0.13 ± 0.09[c]
Total Phosphorus (µg/g)	11.11 ± 0.60[b]	12.15 ± 0.19[a]	7.65 ± 0.41[c]	10.41 ± 0.64[b]	11.43 ± 0.57[a]	6.49 ± 0.10[c]
Magnesium (Mg) (meq/100g)	1.40 ± 0.11[a]	1.40 ± 0.18[a]	1.28 ± 0.27[b]	1.32 ± 0.09[b]	1.45 ± 0.05[a]	1.27 ± 0.29[c]
Calcium (Ca) (meq/100g)	2.34 ± 0.13[a]	2.35 ± 0.35[a]	2.20 ± 0.06[b]	2.28 ± 0.14[a]	2.28 ± 0.09[a]	2.23 ± 0.21[b]
Sodium (Na) (meq/100g)	0.05 ± 0.28[a]	0.05 ± 0.21[a]	0.07 ± 1.02[b]	0.06 ± 1.11[b]	0.05 ± 1.43[a]	0.05 ± 0.56[c]
Potassium (K) (meq/100g)	0.10 ± 0.09[b]	0.13 ± 0.30[a]	0.10 ± 0.15[b]	0.11 ± 0.22[a]	0.11 ± 0.55[a]	0.09 ± 0.14[c]
Conductivity (µm/s)	6.70 ± 0.21[b]	6.75 ± 0.30[a]	6.30 ± 0.34[c]	6.40 ± 0.67[b]	6.43 ± 0.27[a]	5.90 ± 0.18[c]

Values are given as mean ± SD. Within rows, values followed by the same alphabets are not significantly different but those followed by different alphabets are significantly different.

Conversely, soil pH values showed highest values in the control (unpolluted wetlands) – rainy/ dry season 6.90 ± 0.20/ 6.80 ± 0.12, followed by lightly polluted wetlands – rainy/dry season 5.50 ± 0.08/ 5.45 ± 0.48, and with lowest values in the heavily polluted wetlands – rainy/ dry season 3.50 ± 0.09/ 3.00 ± 0.11 (Table 3). All parameters showed some pattern of trend except for exchangeable cations (magnesium, calcium, sodium and potassium) that were not consistent and definite in trend. The soil particulate values showed highest values in the rainy season among sand and silt percentages of control (unpolluted wetlands) - 92.20 ± 0.42/ 4.40 ± 0.51), followed by lightly polluted wetlands - 91.40 ± 0.32/ 4.15 ± 0.15 and low values in the heavily polluted wetlands - (90.00 ± 0.15/ 4.00 ± 0.22). On the contrary, clay percentages were highest in the heavily polluted wetlands, followed by lightly polluted wetlands, and control (unpolluted wetlands) Table 3. There is significant difference between values obtained in the lightly polluted wetlands, heavily polluted wetlands, and control (unpolluted wetlands), when compared ($p<0.05$).

The results of enzymatic activities of Orashi River Wetland (unpolluted and crude oil polluted) according to seasons are shown in Table 4. Soil enzymatic activity values followed a similar pattern with the soil microbial bioloads. The highest enzymatic values were recorded in the lightly polluted wetlands while the lowest enzymatic values were observed in the heavily polluted wetlands. Differences in values for both seasons between lightly polluted wetlands, heavily polluted wetlands, and control (unpolluted wetlands) were statistically significant ($p<0.05$) Table 4.

Table 4. Soil enzymatic activities of Orashi wetland (unpolluted and crude oil polluted) according to seasons.

Enzymes	Rainy Season			Dry Season		
	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted
Dehydrogenase (μg TPF g^{-1} hr^{-1})	4037 ± 46^b	5769 ± 68^a	1452 ± 130^c	3617 ± 155^b	4960 ± 86^a	1245 ± 118^c
Cellulase (μg g^{-1} hr^{-1})	3200 ± 62^b	3900 ± 90^a	1130 ± 30^c	3100 ± 37^b	3700 ± 27^a	940 ± 91^c
Urease (μg g^{-1} hr^{-1})	91.67 ± 20^b	102.92 ± 43^a	25.83 ± 8^c	79.58 ± 27^b	96.25 ± 34^a	23 ± 18^c
Acid Phosphatase (μg -p- nitrophenol)	395.04 ± 0.64^b	447.90 ± 0.47^a	250.38 ± 0.78^c	431.21 ± 0.58^b	481.28 ± 0.78^a	275.42 ± 0.89^c
Alkaline Phosphatase (μg -p- nitrophenol)	392.26 ± 0.45^b	403.39 ± 0.38^a	267.07 ± 0.17^c	360.27 ± 0.56^b	371.40 ± 0.98^a	173.88 ± 1.20^c

Values are given as mean ± SD. Within rows, values followed by the same alphabets are not significantly different but those followed by different alphabets are significantly different.

The most sensitive enzyme was urease which had 102.92 ± 43 μg g^{-1} hr^{-1}/ 96.25 ± 34 μg g^{-1} hr^{-1} in lightly polluted wetlands for rainy/ dry seasons (which are 3.98/ 4.18 times higher than the heavily polluted wetland), 91.67 ± 20 μg g^{-1} hr^{-1}/ 79.58 ± 27 μg g^{-1} hr^{-1} in control (unpolluted wetlands) – rainy/ dry seasons and 25.83 ± 8 μg g^{-1} hr^{-1}/ 23 ± 18 μg g^{-1} hr^{-1} in the heavily polluted wetland – rainy/ dry seasons respectively (Table 4). The dehydrogenase closely followed the urease in sensitivity. In the lightly polluted wetlands, dehydrogenase had 3.97/ 3.98 times more activity than in the heavily polluted wetland – rainy/ dry seasons respectively. Values for the cellulase are as shown in Table 4 and it showed similar trend though not as sensitive as the urease and dehydrogenase. The least sensitive enzymes were the acid and alkaline phosphatase. However, the activities of these enzymes were significant ($p<0.05$) when compared with values obtained in the lightly polluted wetlands, control (unpolluted wetlands) and heavily polluted wetlands of both seasons.

Four (4) fungal species were isolated in both polluted and unpolluted wetlands for both seasons (*Aspergillus* species, *Penicillium* species, *Acremonium* species, and *Rhodotorula* species) and six (6) bacterial species were also isolated in both polluted and unpolluted wetlands for both seasons (*Pseudomonas* species, *Bacillus* species, *Staphylococcus* species, *Escherichia coli*, *Flavobacterium* species and *Azotobacter* species). *Staphylococcus* species and *Escherichia coli* were observed as species that could not utilize crude oil in cultures.

Discussion

Crude oil as a natural oil has been discovered to adversely affect microbial bioloads at heavy impaction as indicated in the results. The heavy impact of wetlands with crude oil creates non-conducive environment for the microbes, which leads to reduced microbial activities, reduced bioloads, and immobilization of essential wetland nutrients. The impact of wetland with crude oil, at a considerable low volume, often known as light impaction will lead to increased microbial activities and biochemical processes such as decomposition of organic matter, which invariably transforms to increased number and activities of microorganisms. Microorganisms have been known to be involved in soil nutrient releases and degradation of organic matter, and most of these microorganisms are aerobic microorganisms. The presence of crude oil in light impaction in wetlands triggers off the much needed decomposition activities. nitrification processes (fixing of nitrogen), and other geochemical cycling of elements (Amadi *et al*., 1996).

A critical look at the microbial bioloads results of Orashi wetland (unpolluted and crude oil polluted) according to seasons showed that heavily crude oil polluted wetlands were negatively affected with low microbial bioloads recorded for all the groups of microorganisms. This was as a result of different negative effects, the heavy crude oil impaction could cause, ranging from non-conducive environment, reduced microbial activities, and distortion of available wetland nutrients, hence the low microbial bioloads recorded. The heavy crude oil impaction on the wetlands creates non-conducive environment by sealing up the soil pores, thereby creating an anaerobic environment that suffocates, kills and do not support the degrading activities of petroleum degrading bacteria (PDB), which are mostly aerobic microorganisms. On the lightly polluted wetlands, it was observed that even in the absence of amendment agent, it encouraged microbial activities through replication and degradation of crude oil and other organic matters. This was possible as light impaction of crude oil on wetlands encourages the decomposition activities of petroleum degrading bacteria (PDB), which are aerobic in nature, and are capable of utilizing crude oil as a source of carbon and nitrogen, hence leaving its by-products as substrates for other microorganisms. This gave rise to the high microbial bioloads recorded, which affirms soil fertility. This shift in high microbial bioloads at the lightly polluted wetlands, even better than that of unpolluted wetlands could not only be by replication, but by chemotactic response as most bacteria are mobile and can move towards a sensed nutrient source. This assertion agreed with report of Gogoi *et al*., 2003, that bacteria are motile and exhibit a chemotactic response, by sensing a contaminant and moving towards it. In a similar report, Nwaugo *et al*., 2007 made same observation of highest bioloads in the lightly impacted soil when they studied the effect of petroleum produced (formation) water and the induced changes in bacterial quality and soil enzymatic activities in a farmland.

The most sensitive and affected group of microorganisms was the nitrifying bacteria. Others that were affected by the change in wetland qualities through crude oil impaction were phosphate solubilizing bacteria and petroleum degrading bacteria. The fungal bioloads were low in the different wetland groups because they are not motile like bacteria, instead the engage in filamentous growth.

In wetland prevalence, the highest spread was observed in the lightly polluted wetlands, far better than what was obtained in the unpolluted wetland. The least prevalence was in the heavily polluted wetlands. The microorganisms isolated include six (6) bacteria species; *Pseudomonas, Bacillus, Staphylococcus*, *Escherichia coli, Flavobacterium, Azotobacter*, and four (4) fungal species; *Aspergillus, Penicillium, Acremonium and Rhodotorula* species. *Bacillus* species was the most prevalent in all wetlands. *Staphylococcus* species and *Escherichia coli* were not observed in the heavily polluted wetlands of both seasons. Fungal species were observed in low prevalence in all the wetlands. Also, the rainy season wetlands prevalence spreads were better than that of dry season counterpart. The values obtained in the bacterial prevalence were similar in trend to the report of Nwaugo *et al*., 2007.

The impaction of Orashi River Wetland with different grading of crude oil strikes a change in the physicochemical parameters of the wetlands. The highest values of physicochemical parameters were recorded in the lightly polluted wetlands. This was followed by the unpolluted wetlands and with the least in the heavily polluted wetlands. The contamination of soil by crude oil with heavy impaction could lead to a depression of microbial density and activities, which affects adversely the physicochemical parameters of the polluted wetlands, while that of light impaction increases microbial density and activities, with increased physicochemical parameters of the polluted wetlands (Amadi *et al*., 1996). Nitrogen together with phosphorus is considered the most limiting nutrient (Nnamchi, 2010). However, bioloads, physicochemical parameters and enzymatic activities are critical index in determining soil quality/ fertility (Keddy, 2002). These critical indexes of soil quality are directly related. This means, high bioloads transform into release of soil nutrient elements giving rise to high physicochemical values with increased enzymatic activities. Hence, the observed high physicochemical values in the lightly polluted wetlands and the low physicochemical values observed in the heavily polluted wetlands in the results are in agreement with report of Nwaugo *et al*., 2007.

The pH had its highest values in the control (unpolluted wetlands), slightly lower values in the lightly polluted wetlands and lowest values in the heavily polluted wetlands. This confirms the report of Sathiya-Moorthi, 2008, that impaction of wetland with crude oil lowers the soil pH. This is because crude oil is a complex mixture of hydrocarbons, composed of aliphatic, aromatic and asphaltene fractions along with nitrogen, sulfur and oxygen-containing compounds, and constituents of these hydrocarbon compounds reacts with soil components to release hydrogen ions into the wetlands, hence reduction of soil pH value. Organic carbon and temperature values were higher in the polluted wetlands than in the control (unpolluted wetlands). This was as a result of simulation of wetland samples with graded volumes of crude oil to effect different levels of pollution. The highest value for organic carbon was recorded in the heavily polluted wetlands, because they were the highest simulated with twenty (20) litres crude oil to make them heavily polluted, The high carbon value recorded was attributed to high volume crude oil simulation as it is a complex mixture of hydrocarbons, composed of aliphatic, aromatic and asphaltene fractions along with nitrogen, sulfur and oxygen-containing compounds, and constituents of these hydrocarbon compounds reacts with soil components to release carbon ions into the wetlands, hence increase in organic carbon value.. The highest temperature values were recorded in the heavily polluted wetlands. This increase in temperature was attributed to high volume crude oil simulation which creates non-conducive environment by sealing up the soil pores, thereby creating an anaerobic environment that heats up, hence an increase in temperature values. This may be attributed to the fact that oil acted as trap for heat and reduced the release of heat into the air (John *et al*., 2010). Other soil parameters like nitrogen, phosphorus and conductivity had its highest values in the lightly polluted wetlands, with lower values in the control (unpolluted wetlands) and lowest values in the heavily polluted wetlands. The result of low physicochemical values obtained in the heavily polluted wetlands confirms the report of nutrient elements immobilization by heavy crude oil impaction on wetlands (Nwaugo *et al.*, 2007; Amadi *et al.*, 1996; Atuanya, 1987).

Enzymatic activities of wetlands play an important role in nutrient cycles with regard to wetland fertility. Hence, it is one of the critical indexes in determining wetland quality. This however reiterates the parallel relationship between bioloads, physicochemical parameters and enzymatic activities in determining wetland quality Nwaugo *et al.*, 2007. That means high bioloads transform into release of soil nutrient elements giving rise to high physicochemical values with increased enzymatic activities. A close observation at the results indicated that, the most sensitive enzyme was urease, followed by dehydrogenase. I n all, the least sensitive enzymes were the acid and alkaline phosphatase. However, the activities of these enzymes were significant ($p<0.05$) in the heavily polluted wetlands – rainy/ dry seasons when compared with values obtained in the lightly polluted wetlands/ control (unpolluted wetlands). Most enzymatic activities had the highest values in the lightly polluted wetlands, followed by control (unpolluted wetlands) and heavily polluted wetlands. It was noted that the lowest enzymatic values were recorded in the heavily polluted wetlands. This is not far from the adverse effect caused by heavily crude oil impact on wetland microbial spectrum. Nwaugo *et al.*, 2007 and Sathiya-Moorthi, 2008 reported that heavy impaction of soil with crude oil will always create non-conducive environment for microorganisms. The results obtained in this study agreed with reports of Nwaugo *et al.*, 2007 on soil enzymatic activities of petroleum produced (formation) water and the induced changes in bacterial quality.

Conclusion

In conclusion, the adverse effects caused by heavily crude oil impaction on wetlands like distortion of soil microbial dynamics, reduced physicochemical parametric values and soil enzymatic activities, and also the encouragement of microbial replication and degradation activities, with improved wetlands quality/ fertility indexes by light crude oil impaction were all observed and confirmed in this study, hence the need to remediate the former which is heavily crude oil impacted wetlands to an improved quality state.

References

Achuba FI, and Peretiemo-Clarke BO. 2008**.** Effect of spent engine oil on soil catalase and dehydrogenase activities. International Journal of Agrophysics **22**, 1–4.

Amadi A, Samuel DA, and Anthony N. 1996**.** Chronic effects of oil spill on soil properties and microflora of a rainforest ecosystem in Nigeria. Journal of Earth, Environmental Science, Water, Air and Soil Pollution **86**(**4**), 1-11.

Atuanya EJ. 1987. Effect of oil pollution on physical and chemical properties of soil: A case study of waste oil contaminated delta soil in Bendel State Nigeria. Journal of Applied Science **55**, 155–176.

Barnet HL, and Hunter BB. 2000. Illustrated Genera of Imperfect Fungi. 3rd Edition. Burgess Publishing Company. Minnesota, USA. pp 41-111**.**

Black CA. 2000. Method of Soil Analysis II. American Society of Agronomy, Madison. pp. 573-590.

Boone DR, Castenholz RW, Garrity GM, Brenner DJ, Krieg NR, and Staley JR. (Eds.). 2005. Bergey's Manual of Systematic Bacteriology. Second Edition. New York: SpringerVerlag.

Cappucino GJR, and Sherman B. 2010. Microbiology: A Laboratory Manual, 9th Edition. The Benjamin Publishing Coy. California.

Chorom M, Sharifi HS, and Motamedi H. 2010. Bioremediation of a Crude Oil - Polluted Soil by Application of Fertilizers. Iran Journal of Environmental Health Science and Engineering **7 (4)**, 319-326.

David DL, Daniel AB, and Wahedi JA. 2013. A Study of Water Quality of Two Lacustrine Wetlands of the Upper Benue Basin, Adamawa State, Nigeria. International Journal of Science and Knowledge **2(1)**, 34-41.

Gogoi BK, Dutta NN, and Krishnamohn TR. 2003. "A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site". Journal of Advances in Environmental Research **7 (4)**, 767-782.

Holliger S, Gaspard G, Glod C, Heijman W, Schumacher RP, Schwarzenbach, and Vazquez F. 1997. "Contaminated environments in the subsurface and bioremediation: organic contaminants," Journal of Federation of European Microbiological Societies (FEMS) Microbiology Review **20 (3-4)**, 517–523.

John RC, Akpan MM, and Essien JP, and Ikpe DI. 2010. Impact of crude oil pollution on the densities of nitrifying and denitrifying bacteria in the rhizosphere of tropical legumes grown on wetland soil. Nigerian Journal of Microbiology **24 (1)**, 2088-2094.

Keddy PA. 2002. Wetland Ecology: Principles and Conservation. Cambridge University Press. London.

Kettler TA, Doran JW, and Gilber TL. 2001. Simplified Method for Soil Particle-Size Determination to Accompany Soil-Quality Analysis. Soil Science Society of American Journal **65 (3)**, 849-852.

Mitsch WJ, and Gosselink JG. 2000. Wetlands: Third Edition. John Wiley & Sons, Inc. New York.

Nnamchi CI. 2010. Effects of nutrient supplementation, pH and salinity on the growth of some PAH-degrading bacteria isolated from Nsukka soils in Nigeria. Nigerian Journal of Microbiology **24 (1)**, 2068-2080.

Nwaugo VO, Onyeagba RA, Akabugwo EI, and Ugbogu OC. 2008. Soil Bacteria Flora and Enzymatic Activities in Zinc and Lead Contaminated Soil. Journal of Biokemistri **20 (2)**, 77-84.

Nwaugo VO, Onyeagba RA, Azu N, and Nworie O. 2007. Petroleum Produced (Formation) Water Induced Changes in Bacterial Quality and Soil Enzymatic Activities in a Farmland in Egbema, Southern Nigeria. Journal of Revista Estudos de Biologia **29** (**66**), 89-97.

Odjegba VJ, and Atebe JO. 2007. The effect of used engine oil on carbohydrate, mineral content and nitrate reductase activity of leafy vegetable (*Amaranthus hybridus*). Journal of Applied Science & Environmental Management **11(2)**, 191–196.

Ogban PI, Effiong GS, Obi JC, and Ibia TO. 2011 Characteristics, Potentials, and Constraints of Wetland Soils for Agricultural Development in Akwa Ibom State, South-Eastern Nigeria. Nigerian Journal of Agriculture, Food and Environment **7(2)**,80-87

Pala M, De Carvalho D, Pinto JC, and Sant Anna Jr GL. 2006. "A suitable model to describe bioremediation of a petroleum-contaminated soil". Journal of International Biodetermination & Biodegradation **58(6)**, 254-260.

Sathiya-Moorthi P, Deecaraman M, and Kalaichelvan PT. 2008. Bioremediation of automobile oil effluent by *Pseudomonas sp*. Journal of Advanced Biotechnology **31**, 34–37.

Vancov T, and Keen B. 2009. Rapid isolation and high-throughput determination of cellulase and laminarinase activity in soils. Journal of Microbiological Method **79**, 174-177.

Watanabe T. 2000. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species, Third Edition. CRC Press. pp 211 – 376.