BIOLOADS AND ENZYME ACTIVITIES OF PETROLEUM POLLUTED EFE RIVER WETLAND, UMULOLO, OKIGWE, IMO STATE. NIGERIA.

Ike, C. C.
Department of Biological Sciences, Rhema University, Nigeria. P.M.B. 7021 Aba, Abia State.

ABSTRACT

The study investigated bioloads and enzyme activities of petroleum polluted Efe River wetland. The bioloads and trend of enzymatic activities in petroleum polluted and unpolluted wetlands were evaluated using standard methods. Bioloads for all microbial groups enumerated, had highest values during rainy season when compared with dry season counterpart. Total viable count (TVC) had highest counts in the lightly polluted wetlands – rainy/ dry seasons {(3.9 ±0.19) x 10^5, (3.2 ±0.21) x 10^5}, followed by control "unpolluted wetlands" {(3.4 ±0.17) x 10^5, (2.8 ±0.16) x 10^5} and lowest values in the heavily polluted wetlands {(3.3 ±0.23) x 10^3, (2.8 ±0.29) x 10^3} respectively. The same trend was observed with other microbial groups assessed. Bacillus species was the most prevalent for both seasons - lightly polluted wetlands – rainy/ dry season (87.5% ±0.11/ 75% ±0.16), heavily polluted wetlands (50% ±0.32/ 25% ±0.15), and control "unpolluted wetlands" (75% ±0.14/ 62.5% ±0.24). The values of soil enzymatic activities followed the same trend with bioloads. Dehydrogenase enzyme (µg TPF g^{-1} hr^{-1}) activities recorded very high values (4658 ±57) in the assessed wetlands, unlike alkaline phosphatase enzyme that had the least values (82.67 ±1.12). High values of enzymatic activities were observed in lightly polluted wetland, followed by control (unpolluted wetland) and heavily polluted wetland in that order. All values obtained in bioloads, prevalence and enzymatic activities, when compared were statistically significant ($p<0.05$). This study has shown that petroleum on heavy impaction could cause negative effects on wetlands bioloads and enzyme activities while light impaction encourages bioloads replication and increased enzyme activities resulting in improved indicator values.

Key words: Petroleum, Bioloads**,** Enzymes**,** Wetland, Efe River
*Correspondence author: E-mail: chrismacaug@yahoo.com

1.0 Introduction

Wetlands are areas where water covers the soil, or is present either at or near the surface of the soil all year or for varying periods of time during the year, including during the growing season. Water saturation (hydrology) largely determines how the soil develops and the types of plant and animal communities living in and on the soil. Wetlands support both aquatic and terrestrial species. The prolonged presence of water creates conditions that favor the growth of specially adapted plants (hydrophytes) and promote the development of characteristic wetland (hydric) soils (Kotze *et al*., 1994).

Wetlands vary widely because of regional and local differences in soils, topography, climate, hydrology, water chemistry, vegetation and other factors, including human disturbances. Indeed, wetlands are found from the tundra to the tropics and on every continent except Antarctica. Two general categories of wetlands are recognized: coastal or tidal wetlands and inland or non-tidal wetlands. Tidal wetlands are found along the Atlantic, Pacific, Alaskan and Gulf coasts. They are closely linked to estuaries where sea water mixes with fresh water to form an environment of varying salinities. Non-tidal wetlands are most common on floodplains along rivers and streams (riparian wetlands), in isolated depressions surrounded by dry land (playas, basins and "potholes"), along the margins of lakes and ponds, and in other low-lying areas where the groundwater intercepts the soil surface or where precipitation sufficiently saturates the soil (vernal pools and bogs). Many of these wetlands are seasonal (they are dry one or more seasons every year), particularly in the arid and semiarid regions, and may be wet periodically. The quantity of water present and the timing of its presence in part determine the functions of a wetland and its role in the environment (Kotze *et al*., 1994).

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, 2011). 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 *et al*., 2008; Achuba and Peretiemo-Clarke, 2008), as well as inhibitory effect on the nitrate and phosphate reductase activities of plants (Odjegba and Atebe, 2007).

Petroleum is an oily, thick, flammable, usually dark-colored liquid, a mixture of various hydrocarbons, occurring naturally in various parts of the world and commonly obtained by drilling. It can used in natural or refined state as fuel, or separated by distillation into gasoline, naphtha, benzene, kerosene, paraffin, etc. Literally, "rock oil" is a general term for crude oil and natural gas. Petroleum is the primary source of automotive fuels and lubricant oils. Petroleum is a complex mixture of hydrocarbons and paraffins in some areas, and aromatics and cyclo-paraffins in other areas. It was discovered to have formed from decomposition of animal and vegetable life under heat and pressure during geologic periods. It occurs usually in deep rock strata but sometimes near the surface. When 'cracked' and refined, it produces hundreds of petrochemicals that are converted into tens of thousands of products, with new ones appearing continually (Kotze *et al*., 1994).

Efe river wetland in Umulolo runs along both shores of the river, connecting to Okigwe Mechanic village where spent oils and petroleum disposals are continually indiscriminate, affecting wetland quality and yields of the teaming farmers from Umulolo. Recently, yields of some crops like vegetables and other cash crops harvested are in shortage following these petroleum pollutions around the wetland. This has caused scarcity in the supply of these crops to the teeming populace in the metropolis. Therefore, this study is aimed at investigating the bioloads and enzyme activities of petroleum polluted Efe River wetland during the seasons.

2.0 Materials and Methods.

2.1 Study area.

The study area is Umulolo in Okigwe Local Government Area, Imo State, in the South-East Geopolitical zone of Nigeria. The people of Umulolo are known as predominant farmers with rich soil and cultural history. They are of the Igbo tribe and are located within the following geographical coordinates; 5.1167^{0}N, and 7.3667^{0}E. The area is of tropical climatic conditions with rain forest features. The soil type is silt-clay with rocky bedrock for a purified aquifer and the weather is typical of rain forest, with an average annual temperature ranging between 26 - 35°C as lowest and highest values respectively. They are known as major producers of yam, cassava, maize, palm oil, stone escavation and many others.

2.2 Experimental design.

The investigation was done on site on a plot of wetland, measuring twelve (12) feet by six (6) feet (12ftx12ft), and partitioned into three equal parts with internal gap of one and half ($1^{1/2}$) foot. The partitioned plots were spiked with graded volumes (either 5 or 20 Liters) of petroleum to represent lightly and heavily polluted wetlands respectively. Control samples were not spiked with petroleum.

The experimental plots of wetland were exposed to climatic elements (rain and sunlight) throughout the period of the study. This study was carried out on site in rainy and dry season

2.3 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 enzyme analysis after one week of soil contamination with spilled petroleum. A total of twelve (12) samples were collected for the study with control samples inclusive. Samples were taken to the laboratory in sealed ice packs for microbiological and enzyme analysis within twenty four (24) hours of collection and analysed immediately.

2.4 Microbiological analysis of samples:

Serial dilutions of the soil samples were done in the appropriate folds. Spread plate and streaking culturing techniques of Capuccino and Sherman (2010) were used to enumerate and isolate bacteria and fungi in the samples. The bacterial bioloads enumerated include total count (TC), 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, wet mount and lactophenol staining. The schemes of Barnett and Hunter (2000), and Watanabe, (2010) were used for identification.

2.5 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.

2.6 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.

3.0 Results

Bioloads of Efe river wetland (unpolluted and petroleum polluted) according to seasons was shown in Table 1. The highest bioloads was observed with total viable count (3.9±0.19) x 10^5 of rainy season in the lightly polluted wetland, while the least bioloads was observed with nitrifying bacteria count (0.4±0.31) x 10^1 of dry season in the heavily polluted wetland.

Table 2 showed the prevalence of bacterial and fungal species in Efe river wetland (unpolluted and petroleum polluted) according to seasons. The highest percentage prevalence was recorded with *Bacillus* species in both rainy and dry seasons of lightly polluted wetland (75±0.14/75±0.16). The least percentage prevalence was observed with *Rhizopus* species in both rainy and dry seasons of heavily polluted wetland (zero values).

Table 3 showed soil enzyme activities of Efe river wetland (unpolluted and petroleum polluted) according to seasons. The highest enzymatic value was recorded with dehydrogenase in both rainy and dry seasons of lightly polluted wetland (4658±57/3849±75). The least enzymatic value was observed with urease in both rainy and dry seasons of heavily polluted wetland (14.28±11/11.14±13).

The values obtained between the various samples, when compared were statistically significant ($p<0.05$).

4.0 Discussion

Petroleum means "rock oil" or "oil from the rock", a term which could also be used to describe crude oil. Petroleum has been discovered to impose negative effects on microbial bioloads and enzyme activities at heavy impaction as indicated in the results. The heavy impacted wetlands creates non-conducive environment for the microbes, which leads to reduced microbial activities, that transforms into reduced bioloads. The light impacted wetlands lead to increase in bioloads and enzyme activities. 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 petroleum in light impaction in wetlands triggers off decomposition activities and other soil activities (Amadi *et al*., 1996).

Most organic degrading microorganisms in wetlands are aerobic, and under light impaction, the wetland becomes rich with these organic substances in the quantity that can trigger replication activities. It was observed that lightly impacted wetlands encouraged microbial activities through replication and degradation of petroleum and other organic matters. This was possible as light impaction of petroleum on wetlands encourages the decomposition activities of petroleum degrading bacteria (PDB), which are aerobic in nature, and are capable of utilizing petroleum as a source of carbon and nitrogen, hence leaving its by-products as substrates for other microorganisms. The shift in high microbial bioloads at the lightly polluted wetlands, 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. Also in agreement with higher bioloads in lightly impacted samples were the works of Ike *et al*., 2014 on impact of crude oil pollution on the physicochemical and microbiological parameters of Orashi river wetland and Nwaugo *et al*., 2007 on soil enzymatic activities of petroleum produced (formation) water and the induced changes in bacterial quality. Heavy impaction of the wetland poses threat to microbial lives as the oil part of the rock (petroleum) seals wetland pores, thereby heats up the environment and creates unconducive environment for the microbes to survive, This leads to the poor bioloads observed in Table 1 as the suffocation do not support the degrading activities of petroleum degrading bacteria (PDB), which are mostly aerobic microorganisms (Ike *et al*., 2014).

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 five (5) bacteria species; *Bacillus, Pseudomonas, Staphylococcus, Escherichia coli, Flavobacterium,* and three (3) fungal species; *Aspergillus, Penicillium, Rhizopus* 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, except *Rhizopus* species that was absent in heavily polluted wetlands. The values obtained in the bacterial prevalence were similar in trend to the report of Ike *et al*., 2014 and Nwaugo *et al*., 2007.

Enzymatic activities of wetlands play an important role in nutrient cycles with regard to wetland fertility and quality. Hence, it is one of the critical indexes in determining wetland quality. This however reiterates the parallel and direct relationship between bioloads, and enzyme activities in determining wetland quality Nwaugo *et al*., 2007. That means high bioloads transform into release of soil nutrient elements giving rise to increased enzyme activities. A close observation at the results indicated that, the most sensitive enzyme was urease, followed by dehydrogenase.

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 enzyme 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 heavy petroleum impact on wetland microbial spectrum. The results obtained in this study agreed with reports of Ike *et al*., 2014 on impact of crude oil pollution on the physicochemical and microbiological parameters of crude oil polluted Orashi river wetland. and Nwaugo *et al*., 2007 on soil enzymatic activities of petroleum produced (formation) water and the induced changes in bacterial quality. There is statistical significance among different values obtained in the results ($p<0.05$).

4.1 Conclusion

It was deduced from this work that petroleum at light impaction would trigger increase in bioloads and enzyme activities, while on heavy impaction could adversely affect bioloads and enzyme activities.

5.0 References

Achuba, F. I., and Peretiemo-Clarke, B. O. (2008)**.** Effect of spent engine oil on soil catalase and dehydrogenase activities. *International Journal of Agrophysics,* **22**: 1–4.

Amadi, A., Samuel, D. A., 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.

Barnet, H. L., and Hunter, B. B. (2000). Illustrated Genera of Imperfect Fungi. 3rd Edition. Burgess Publishing Company. Minnesota, USA. pp 41-111**.**

Boone, D. R., Castenholz, R. W., Garrity, G. M., Brenner, D. J., Krieg, N. R., and Staley, J. R. (Eds.). (2005). Bergey's Manual of Systematic Bacteriology. Second Edition. New York: SpringerVerlag.

Cappucino, G. J. R., and Sherman, B. (2010). Microbiology: A Laboratory Manual, 9th Edition. The Benjamin Publishing Coy. California.

Gogoi, B. K., Dutta, N. N., and Krishnamohn, T. R. (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.

Ike, C. C., Nwaugo, V. O., Anochie, C. C., and Nweke, C. O. (2014). Impact of crude oil pollution on the physicochemical and microbiological properties of orashi river wetland in Egbema. Nigeria. *Journal of Biodiversity and Environmental Sciences (JBES)*, **5(4)**: 89-99.

Kotze, D .C., Breen, C. M., and Klug, J. R. (1994). WETLAND-USE, a wetland management decision support system for the KwaZulu-Natal Midlands. WRC Report No 501/2/94. Water Research Commission, Pretoria.

Nwaugo, V. O., Onyeagba, R. A., Akabugwo, E. I., and Ugbogu, O. C. (2008). Soil Bacteria Flora and Enzymatic Activities in Zinc and Lead Contaminated Soil. *Journal of Biokemistri*, **20 (2)**: 77-84.

Nwaugo, V. O., Onyeagba, R. A., 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, V. J., and Atebe, J. O. (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, P. I., Effiong, G. S., Obi, J. C., and Ibia, T. O. (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

Sathiya-Moorthi, P., Deecaraman, M., and Kalaichelvan, P. T. (2008). Bioremediation of automobile oil effluent by *Pseudomonas* species. *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**. (**2010). Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species, Third Edition. CRC Press. pp 211 – 376.

Table 1: Bioloads of Efe River wetland (unpolluted and petroleum polluted) according to seasons.

Organisms	Rainy Season			Dry Season		
	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted	Control (Unpolluted Wetland)	Lightly Polluted	Heavily Polluted
TVC (CFU/g soil)	(3.4 ± 0.17[b]) x 10^5	(3.9 ± 0.19[a]) x 10^5	(3.3± 0.23[c]) x 10^3	(2.8 ± 0.16[b]) x 10^5	(3.2 ± 0.21[a]) x 10^5	(2.8± 0.29[c]) x 10^3
PDB (CFU/g soil)	(2.7 ± 0.15[b]) x 10^4	(3.1 ± 0.33[a]) x 10^4	(2.6± 0.21[c]) x 10^2	(2.5 ± 0.23[b]) x 10^4	(2.9 ± 0.29[a]) x 10^4	(2.4± 0.17[c]) x 10^2
PSB (CFU/g soil)	(3.1 ± 0.22[b]) x 10^2	(3.3 ± 0.19[a]) x 10^3	(1.5± 0.25[c]) x 10^1	(1.2 ± 0.39[b]) x 10^2	(1.0 ± 0.55[a]) x 10^3	(0.5± 0.27[c]) x 10^1
NB (CFU/g soil)	(1.6 ± 0.20[b]) x 10^2	(1.8 ± 0.16[a]) x 10^3	(0.6± 0.21[c]) x 10^1	(1.3 ± 0.24[b]) x 10^2	(1.4 ± 0.17[a]) x 10^3	(0.4± 0.31[c]) x 10^1
Fungi (CFU/g soil)	(1.0 ± 0.11[b]) x 10^2	(1.3 ± 0.27[a]) x 10^2	(1.4± 0.17[c]) x 10^1	(0.7 ± 0.38[b]) x 10^2	(1.0 ± 0.47[a]) x 10^2	(1.0± 0.15[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: TVC - Total Viable Count, PDB - Petroleum Degrading Bacteria, PSB - Phosphate Solubilizing Bacteria, NB - Nitrifying Bacteria.

Table 2: Prevalence of bacterial and fungal species in Efe River wetland (unpolluted and petroleum polluted) according to seasons.

Organisms	Rainy Season: Control (Unpolluted Wetland)			Rainy Season: Lightly Polluted			Rainy Season: Heavily Polluted			Dry Season: Control (Unpolluted Wetland)			Dry Season: Lightly Polluted			Dry Season: Heavily Polluted		
	NE	NO	%	NE	NO	%	NE	NO	%	NE	NO	%	NE	NO	%	NE	NO	%
Bacillus species	8	6	75 $\pm0.14^{b}$	8	7	87.5 $\pm0.11^{a}$	8	4	50 $\pm0.32^{c}$	8	5	62.5 $\pm0.24^{b}$	8	6	75 $\pm0.16^{a}$	8	2	25 $\pm0.15^{c}$
Pseudomonas species	8	5	62.5 $\pm0.27^{b}$	8	6	75 $\pm0.46^{a}$	8	3	37.5 $\pm0.44^{c}$	8	4	50 $\pm0.28^{b}$	8	5	62.5 $\pm0.17^{a}$	8	2	25 $\pm0.11^{c}$
Staphylococcus species	8	4	50 $\pm0.31^{a}$	8	3	37.5 $\pm0.28^{b}$	8	0	0	8	4	50 $\pm0.16^{a}$	8	2	25 $\pm0.08^{b}$	8	0	0
Escherichia coli	8	4	50 $\pm0.47^{a}$	8	3	37.5 $\pm0.36^{b}$	8	0	0	8	3	37.5 $\pm0.09^{a}$	8	3	37.5 $\pm0.17^{b}$	8	0	0
Flavobacterium species	8	3	37.5 $\pm0.15^{b}$	8	4	50 $\pm0.30^{a}$	8	2	25 $\pm0.22^{c}$	8	3	37.5 $\pm0.21^{b}$	8	4	50 $\pm0.19^{a}$	8	1	12.5 $\pm0.15^{c}$
Penicillium species	8	3	37.5 $\pm0.12^{a}$	8	3	37.5 $\pm0.23^{a}$	8	1	12.5 $\pm0.09^{b}$	8	2	25 $\pm0.27^{b}$	8	3	37.5 $\pm0.12^{a}$	8	1	12.5 $\pm0.42^{c}$
Aspergillus species	8	2	25 $\pm0.31^{b}$	8	3	37.5 $\pm0.30^{a}$	8	1	12.5 $\pm0.12^{c}$	8	2	25 $\pm0.14^{b}$	8	3	37.5 $\pm0.19^{a}$	8	1	12.5 $\pm0.41^{c}$
Rhizopus species	8	1	12.5 $\pm0.32^{b}$	8	2	25 $\pm0.46^{a}$	8	0	0	8	1	12.5 $\pm0.42^{a}$	8	2	25 $\pm0.07^{a}$	8	0	0

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

Table 3: Soil enzyme activities of Efe River wetland (unpolluted and petroleum 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})	3026 ± 35[b]	4658 ± 57[a]	1341 ± 119[c]	2506 ± 143[b]	3849 ± 75[a]	1134 ± 107[c]
Cellulase (µg g^{-1} hr^{-1})	2089 ± 41[b]	2789± 69[a]	980 ± 14[c]	1989 ± 20[b]	2600 ± 19[a]	820 ± 55[c]
Urease (µg g^{-1} hr^{-1})	80.12 ± 13[b]	191.37 ± 36[a]	14.28 ± 11[c]	68.22 ± 18[b]	85.15 ± 21[a]	11.14 ± 13[c]
Acid Phosphatase (µg -p- nitrophenol)	274.11 ± 0.84[b]	326.63 ± 0.39[a]	129.76 ± 0.77[c]	310.45 ± 0.41[b]	360.23 ± 0.80[a]	156.37 ± 0.59[c]
Alkaline Phosphatase (µg -p- nitrophenol)	271.29 ± 0.39[b]	282.21 ± 0.45[a]	146.19 ± 0.65[c]	239.43 ± 0.43[b]	250.33 ± 0.54[a]	82.67 ± 1.12[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.