International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 928-934**
http://www.ijcmas.com

Original Research Article

Effects of Palm Oil Mill effluent (Pome) on soil bacteria and enzymes at different seasons

I.J.Ibe[1], J.N.Ogbulie[2], J.C.Orji[2], P.I.Nwanze[1], C.Ihejirika[3] and R.N.Okechi[4*]

[1]Department of Microbiology, Federal Polytechnic Nekede, Owerri
[2]Department of Microbiology, Federal University of Technology, Owerri
[3]Department of Environmental Technology, Federal University of Technology, Owerri
[4]Department of Biotechnology, Federal University of Technology, Owerri
**Corresponding author*

Keywords

POME, enzyme activity, total heterotrophic bacteria, nitrifying bacteria, population

A B S T R A C T

Soil samples collected from palm- oil- mill- effluent-polluted soil were studied to determine the effect of POME on soil bacteria and enzymes during different seasons. Standard microbiological and biochemical methods were used to enumerate the population and evaluate the activity of enzymes respectively. POME caused a significant decrease (p=0.05) in the population of the microbial groups studied at the different seasons. Total heterotrophic bacterial population decreased from 6.4 x 10^5 ±0.19 to 6.1 x 10^3±0.01 in the dry season, and 4.1 x 10^6±0.08 to 5.7 x 10^4±0.15 during the rainy season. The population of the other bacterial groups, phosphate-solubilizing, nitrifying, lipolytic, celluloytic and palm-oil-utilizing bacteria followed the same trend. During the dry season, the activity of the enzymes decreased, except that of acid phosphatase which increased from 2.60±0.02 µmol-p-nitrophenol in the control to 2.90±0.09 µmol-p-nitrophenol in the impacted soil. Lipase showed the least activity (1.30± 0.05g^{-1} 30 min) in the impacted soil. In the rainy season, alkaline phosphatase showed the least activity. Here, the activity of lipase increased in the impacted soil while the pollutant caused a decrease in the activity of the other enzymes studied. POME impacted the parameters studied negatively.

Introduction

Soil is the key component of natural ecosystem (Adedokun and Ataga, 2007; Adenipekun, 2008; Onuh *et al.*, 2008), and as such environmental sustainability depends largely on a sustainable soil ecosystem. Kolwan *et al*., (2006) defined soil as the top layer of the earth's lithosphere, formed from withered rock that has been transformed by living organisms. The soil is composed of five components: minerals, water, soil air, organic matter and soil living organisms. Due to its chemical composition and physical properties soil forms a habitat for massive amounts of microorganisms and other living organisms.

Microorganisms found in the soil include bacteria, algae, fungi, actinomycetes, protozoa and viruses (Willey *et al*., 2008),

but bacteria constitute the basic mass of all soil microorganisms. They are characterized by high metabolic activity. Most soil bacteria are characterized by the ability to adhere to surfaces of the mineral molecules and to the soil colloids.

Man's industrial and economic activities in his immediate environment have brought about improved living conditions to him. However, these activities produce wastes which inevitably get disposed on land and negatively shift the ecological balance thus threatening man's life and health (Olorunfemi *et al.,* 2008).

The global production of palm oil is growing at a very high rate and the pollution caused by waste materials from the palm oil mills has become a serious problem (Orji *et al.,* 2006). In Nigeria, the business of palm oil extraction is dominated by the peasant farmers who use mainly the semi-mechanized method of extraction (Orji *et al.;* 2006) and the palm oil mill effluent generated is poured away into available pieces of land near the mill (Orji *et al.,* 2006).

Palm oil is an edible plant oil derived from the pulp (Reeves *et al.*, 1999) of the fruit of the oil palm *Elaeis guineensis*. Oil palm is the most productive oil-producing plant in the world, with one hectare of oil palm producing between 10-35 tonnes of fresh fruit bunch (FFB) per year (Hartley, 2004 and Ma *et al*., 2000). The palm has a life over 20 years, but the economic life is 20-25 years

Palm oil mill effluent (POME) is one of the major wastes from the palm oil industry and it has the most problematic environmental pollution potential among the palm oil mill wastes. It is the residual liquid waste product obtained after extraction of oil from the fruits of the oil palm (Orji *et al.,* 2006). Often palm oil mill effluent (POME) is discharged indiscriminately into the environment, particularly on farmlands (Ogboghodo *et al.*, 2001, 2003, 2006). POME discharged from an oil mill, is objectionable and could pollute streams, rivers or surrounding land (Hartley, 2004, Roslan *et al*., 2009). Orji *et al.,* (2006) showed that soils where palm oil mill effluents were freshly discharged had very scanty microbial population and diversity. The entire ecosystem changes when new materials are added to the soil, as microorganisms die off or move away from contaminants or pollutants. The soil microorganisms have enzymes which make them to show a variety of metabolic activity, that ensures the permanence (continuity) of element cycles in nature. The enumeration of microorganisms and assessment of the activity of soil enzymes provide an integrative measure of the biological status of the soil (Li *et al.,* 2005*).*

This work assessed the effects of POME on some groups of soil bacteria and the activity of soil enzymes.

Materials and Methods

Soil Sample Collection

Soil samples were collected using disinfected trowel from 0-15 cm depth, in soils polluted with POME, and adjacent agricultural land for the control samples; from Mmahu in Ohaji/Egbema LGA noted for palm oil processing in Imo State. A set of soil samples was collected during the dry season (December – March) and another set of soil samples was collected during the rainy season (April – October). The co-ordinates of Ohaji/Egbema Local Government Area are longitude $4^{o}49'$ N and latitude $6^{o}34'$ E.

Samples Preparations for Analysis

Bulked or composite soil samples from impacted and control sites were air-dried, ground, sieved (2mm) and stored at room temperature (28±2^{O}C) for 24h.

Microbiological Analysis

Estimation of Total Heterotrophic Bacteria (THB)

The method described by Okpokwasili and Okorie (1988) was used to estimate the population of heterotrophic bacteria in the impacted and control soil samples.

Estimation of POME-utilizing Bacteria (PUB)

One (1) gram of POME-impacted soil sample was weighed and transferred into a test tube containing 9ml of sterile distilled water, shaken and serial diluted. T

hen 0.1ml aliquot of the appropriate dilution was inoculated on to mineral salt of Mills *et al*., (1978) as modified by Okpokwasili and Okorie (1988); and POME as the sole carbon source (Alias and Tan, 2005). The inoculated medium was incubated at room temperature (28±2^{o}C) for 24h.

Estimation of Nitrifying Bacteria (NB)

An aliquot (0.1ml) of 10^{-3} dilution was inoculated on mineral salt agar medium [0.06 g $CaCl_2$; $MgSO_4.7H_2O$; 0.06 G $FeCl_2$; 0.106 g KH_2PO_4; K_2HPO_4; 0.06 g $Ca(NO3)_2$ and 0.488 g $(NH_4)_2SO_4$] solidified with 1% Difco Noble Agar, using spread plate technique. Bacterial growth was obtained after 48-72h incubation at room temperature (28±2^{o}C) (Nwaugo *et al*., 2004).

Estimation of Lipolytic Bacteria (LB)

This was carried out according to Mohan *et al*., (2008). Aliquots of diluted soil samples were plated on tributyrin agar for 24h, and the formation of halo zones around the colonies on tributyrin agar was considered as positive result for the test.

Enumeration of Phosphate Solubilizing Bacteria (PSB)

About 0.1ml of 10^{-3} dilution of soil sample was inoculated on NBRI-BPB medium using spread plate method, and incubated at 30^{o}C for 3 days. The medium was solidified with 1% Difco Noble Agar. Production of yellow halos around the colonies was taken as positive result (US Patent, 2003).

Enumeration of Cellulolytic Bacteria (CEB)

The organisms were enumerated by plating serially diluted soil samples on cellulose agar which contained carboxy methyl cellulose (CMC), according to the method of Hatami *et al*., (2008).

Enzyme assay

Acid and Alkaline Phosphatases

They were assayed based on the colorimetric estimation of the p-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5 for acid phosphatase activity and pH 11 for alkaline phosphatase activity) sodium p- nitrophenyl phosphate solution and toluene (Alef and Nannipieri, 1995).

Lipase Assay

The method described by Onilude *et al*., (2010) was employed in this assay.

Urease Assay

This was assayed using the method described by Alef and Nannipieri (1995). The method is based on the determination of ammonia released after incubation of soil samples with urea solution for 2h at 37^{o}C.

Cellulase Assay The assay method described by Alef and Nannipieri (1995) was used. The method is based on the determination of released reducing sugars after the incubation of soil samples with carboxy methyl cellulose salt solution (CMC) for 24h at 50^{o}C.

Assay for Dehydrogenases

The assay method as described by Cassida *et al*., (1964) was used. The assay involved colorimetric estimation of 2, 3, 5-triphenyl formazan (TPF) produced by the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) by soil microorganisms.

Statistical Analysis

SPSS was used to carry out a paired sample T-test to analyze the data and make inferences.

Results and Discussion

Seasonal microbial loads: The control had greater numbers of microorganisms than the impacted soil in all the groups of microorganisms in the different seasons. POME had significant (p=0.05) negative impact on the soil. During the dry season, total heterotrophic bacteria (THB) had the highest count in the control while nitrifying bacteria (NB) had the lowest count in the impacted soil. In the rainy season, the population of the different bacterial groups in the impacted soils decreased significantly (p=0.05) when compared to the control. THB had the highest count both in the control and the impacted soil (4.1 x 10^6±0.08 – 5.7 x 10^4±0.15 cfu/g) respectively, while POME-utilizing bacteria (PDB) had the second highest population, having decreased from 3.1 x 10^4 ±0.19– 1.1 x 10^4 ±0.01cfu/g. NB had the least count in the control (3.9 x 10^3 cfu/g). Phosphate-solubilizing bacteria (PSB) decreased from 1.9 x 10^4 ±0.19– 1.6 x 10^3 ±0.02cfu/g, cellulolytic bacteria (CEB) decreased from 3.2 x 10^4±0.25 – 5.2 x 10^3 ±0.08 cfu/g and lipolytic bacteria (LB) decreased from 1.7 x 10^4 ±0.13– 1.9 x 10^3 ±0.15 cfu/g.

Enzymes activity: In the dry season, except for acid phosphatase which increased from 2.60±0.02 µmol-p-nitrophenol in the control to 2.90±0.09 µmol-p-nitrophenol in the impacted, the activities of the enzymes decreased in the impacted soil. Dehydrogenase had the highest activity in the control (22.40±0.22 mg g^{-1} $2h^{-1}$), and lipase showed the least activity (1.30± 0.05g^{-1} 30 min) in the impacted soil. Alkaline phosphatase also showed very low activity (1.40±0.12 µmol-p-nitrophenol).

POME affected the soil enzymes significantly (p=0.05) as well, during the rainy season. They all decreased in activity in the impacted soil except lipase which increased in activity (2.40±0.02 – 2.80±0.28 g^{-1} 30 min). Alkaline phosphatase showed the least activity (1.90±0.19 µmol-p-nitrophenol) in the impacted soil. Dehydrogenase activity decreased from 28.76±0.3 mg g^{-1} $6h^{-1}$ in the control to 24.01±0.00 mg g^{-1} $6h^{-1}$ in the impacted soil (though it had the highest activity in both the control and the impacted soils), cellulase from 4.10±0.19– 3.0±0.17 mg g^{-1} $6h^{-1}$, urease from 3.80±0.05 – 3.50±0.05 g^{-1} 30 min and acid phosphatase from 3.60±0.02 – 3.1±0.01 µmol-p- nitrophenol.

There was significant difference (p=0.05) in the population of the bacterial groups in the POME-impacted soil when compared to the control (Table1). The control had higher numbers of microorganisms than the impacted soil in all the bacterial groups, indicating that POME had negative effect on the microorganisms. Although POME contains metabolizable nutrients, the high concentration of POME at the dump site, together with excess water suppressed the growth of the organisms. The excessive moisture may have created anaerobic conditions (Nwaugo *et al*., 2008). Additionally, the decomposition of POME by soil microbes could have induced O_2-depletion in the surface soil, thereby inhibiting aerobic microbial activity (Nwoko and Ogunyemi, 2010). The toxicity of POME may also be due to the presence of phenols and other organic acids which are responsible for its phytotoxic effect and antibacterial activity (Capasso *et al*., 1992, Pascual *et al*., 2007). Total heterotrophic bacteria (THB) had the highest count in the control. Palm oil-utilizing bacteria (PUB) had the highest population in the impacted soil. This is expected because the pollutant may have provided a good environment for them to flourish, since it contains utilizable components for the organisms. Zaliha *et al*., (2007) and Nwaugo *et al*., (2009) obtained similar results with lipolytic bacteria. In this work, phosphate-solubilizing bacteria (PSB) and nitrifying bacteria (NB) had very low densities in the impacted soil, which is indicative of the adverse effect of POME when it is in high concentration (Nwaugo *et al*., 2008). Okwute and Isu (2007) observed that ammonium oxidizers (nitrifiers) were not present in POME-impacted soil but grew in non-POME-impacted soil. POME, thus, had adverse effect on nitrification, which is very sensitive to environmental changes like pH change. The POME increased the acidity of the soil which affected nitrification.

The activities of the enzymes decreased or increased significantly (p=0.05) in the impacted soil (Table 2). The activities of the enzymes decreased, except that of acid phosphatase which increased. This may be due to the high concentration of phenols and other organic acids at the dump site (Pascual *et al*., 2007). Dehydrogenase (DHA) showed the highest activity, which is in accordance with the population of the organisms. But when compared to its activity in the control, DHA activity reduced, which is indicative of the stress on the organisms by the pollutant. This agrees with Li *et al*., (2005), Walsh *et al*., (1994) and Huang *et al*., (2009) that DHA should form part of bio-indicators of soil pollution assessment.

Table.1 Effect of POME on Microbial Load of Soil (cfu/g) in Different Seasons

	Dry Season		**Rainy Season**	
Microbial Group	**Control**	**Impacted Soil**	**Control**	**Impacted Soil**
Nitrifying Bacteria	$3.4 \times 10^3 \pm 0.19$	$1.2 \times 10^3 \pm 0.15$	$3.9 \times 10^3 \pm 0.19$	$1.6 \times 10^3 \pm 0.08$
Phosphate Solubilizing Bacteria	$1.1 \times 10^4 \pm 0.01$	$1.3 \times 10^3 \pm 0.04$	$1.9 \times 10^4 \pm 0.19$	$1.6 \times 10^3 \pm 0.02$
Total Heterotrophic Bacteria	$6.4 \times 10^5 \pm 0.19$	$6.1 \times 10^3 \pm 0.01$	$4.1 \times 10^6 \pm 0.08$	$5.7 \times 10^4 \pm 0.15$
Cellulolytic Bacteria	$2.1 \times 10^4 \pm 0.19$	$4.7 \times 10^3 \pm 0.13$	$3.2 \times 10^4 \pm 0.25$	$5.2 \times 10^3 \pm 0.08$
Lipolytic Bacteria	$1.4 \times 10^4 \pm 0.19$	$1.6 \times 10^3 \pm 0.02$	$1.7 \times 10^4 \pm 0.13$	$1.9 \times 10^3 \pm 0.15$
Palm Oil Degrading Bacteria	$2.3 \times 10^4 \pm 0.26$	$7.3 \times 10^3 \pm 0.13$	$3.1 \times 10^4 \pm 0.19$	$1.1 \times 10^4 \pm 0.01$

Table.2 Effects of POME on the activity of soil Enzymes Relative to the Control

Enzymes	Dry Season Control	Impacted Soil	Rainy Season Control	Impacted Soil
Dehydrogenase (mg g^{-1} $6h^{-1}$)	22.140±0.22	16.21±0.19	28.76±0.30	24.01±0.00
Acid Phosphatase (µmol-p-nitrophenol)	2.60±0.02	2.90±0.09	3.60±0.02	3.10±0.01
Alkaline Phosphatase (µmol-p-nitrophenol)	2.40±0.17	1.40±0.12	3.20±0.03	1.90±0.19
Urease (mg g^{-1} $2h^{-1}$)	3.40±0.09	2.90±0.18	3.80±0.05	3.50±0.05
Cellulase (mg g^{-1} $6h^{-1}$)	3.40±0.02	2.20±0.02	4.10±0.19	3.00±0.17
Lipase (g^{-1} 30 min)	2.10±0.02	1.30±0.05	2.40±0.02	2.80±0.28

References

Adedokun, O. M and Ataga, A. E. 2007. Effects of amendments and Bioaugumentation of Soil polluted with Crude Oil, automotive Gasoline Oil, and spent engine oil on the growth of cowpea *(Vigna unguiculata* L Walp). *Scientific Research and* Essay 2 (5): 147-149.

Adenipekun, C. O. 2008. Bioremediation of Engine Oil polluted soil by *Pleurotus Tuber-regium* Singer, a Nigerian White-rot Fungus. *African Journal* of *Biotechnology* 7(1): 055-058.

Alias, Z and Tan, I. K. P. 2005. Isolation of palm oil-utilizing, polyhdroxyalkanoate (PHA)-producing bacteria by an enrichment technique. *Bioresources Technology* 96(11): 1229-1234.

Cassida, L. E., J. D. Klein and Santoro, D. 1964. Dehydrogenase activity. *Soil Science* 98: 371-374.

Hartley, C. W. S. 2004. Environmental Impact of Oil Palm Plantations in Malaysia. Palm Oil Research Institute of Malaysia (PORIM) Occasional Paper. 33: 1-27.

Hatami, S., H. A. Alikhami, H. Besharati, N. Salehrastin, M. Afrousheh and Yazdani, J. Z. 2008. Investigation of Aerobic Cellulolytic Bacteria in some of North Forest and Farming Soils. *American-Eurasian Journal of Agriculture and Environmental Sciences*, (3)5: 713- 716.

Huang, S., B. Peng, Z. Yang, L. Chai and Zhou, L. 2009. Chromium Accumulation, microorganism population and enzyme activities in soil around chromium-containing slag heap of steel alloy factory. *Trans. Nonferrous Methrological Society of China* 19: 241-248.

Kolwzan, B., W. Adamiak, K. Grabas and Pawleczyk, A. 2006. *Introduction toEnvironmental Microbiology*. Oficyyna Wydawnicza Politechniki Wroclawskiej, Wroclaw.

Li, H., Y. Zhang, C. G. Zhang and Chen, G.. X. 2005. Effects of Petroleum-contaminating waste water irrigation on bacterial diversity and enzymatic activities in a paddy soil irrigation area. *Journal of Environmental Quality* 34: 1073-1080.

Ma, A. N., Y. Tajima, M. Asahi and Hannif, J. 2000. A novel treatment process forPalm Oil Mill Effluent. Palm Oil Research Institute of Malaysia (PORIM) Occasional Paper 33: 1-27.

Nwaugo, V. O., G. C. Chinyere and Inyang, C. U. 2008. Effects of palm oil mill effluent (POME) on Soil Bacterial Flora and enzyme activities

in Egbema. *Plant Products Research Journal,* 12, 10-13.

Nwaugo, V. O., C. A. Etok, G. N. Chima and Ogbonna, C. E. 2009. Environmental impact of cassava mill effluent on physicochemical and microbial community structure and functions. *World Journal on Environmental Research*, 8(3): 19-19.

Ogboghodo, I. A., I. O. Osemewota, S. O. Eke and Iribhogbe, A. E. 2001. Effects of Cassava (*Manihot esculenta* Crantz) grating mill effluent on textural, chemical and biological properties of surrounding soil. *World Journal of Biotechnology*. 2: 292-301.

Okpokwasili, G. C and Okorie, B. B. 1988. Biodegradation potentials of microorganisms isolated from car lubricating oil. *Tribiology International,* 21: 215-222.

Okwute, O. L and Isu, N. R. 2007. Impact analysis of palm oil mill effluent on the aerobic bacterial density and ammonium oxidizers in a dumpsite in Anyigba, Kogi State. *African Journal of Biotechnology* 6 (2): 116-119.

Olorunfemi, D., E. O. Emoefe, and Okieimen, F. E. 2008. Effect of cassava processing effluent on seedling height, biomass and chlorophyll content of some cereals. *Research Journal* of *Environmental Sciences,* 2 (3): 221-227.

Onilude, A. A., R. O. Igbinabolor and Wakil, S. N. 2010. Effect of varying relativehumidity on the rancidity of cashew kernel oil by lipolytic organisms. *African Journal of Biotechnology*, 9(31): 4809-4896.

Onuh, M. O., O. K. Maduike and Ohia, G. U. 2008. Effects of poultry manure and cow dung on the physical and chemical properties of crude oil-polluted soil. *Science World Journal*, 3: 45-50.

Orji, M. U., S. O. Nwokolo and Okoli, I. 2006. Effects of palm oil mill effluent on soil Microflora. *Nigerian Journal* of *Microbiology* 20(2): 1026-1031.

Reeves, J. B and Weihrauch, J. L 1999. Consumer and Food Economics Institute, Composition of foods: fats and oils. Agriculture handbook 8-4. U.S. Dept. of Agriculture, Science and Education Administration. Washington D.C. pp 4.

US Patent. PatentStorm 6638730. Composition for qualitative screening of phosphate solubilizing microorganisms and a qualitative method for screening microorganisms. Issued October 28, 2003.

Walsh, C., J. P. R. Gooderham, M. R. Grace, S. Sdraulig, M. I. Rosyidi and Lelono, A. 2002. The relative influence of diffuse and point-source disturbances on a small upland stream in East Java Indonesia: a preliminary investigation. *Hydrobologia Journal* 487: 183-192.

Willey, J. M, L. M. Sherwood and Woolverton, C.J. 2008. Prescott, Harley, and Klein's Microbiolog,*y* 7th Edition. McGraw -Hill, New York, 1-1088.