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Biological and Chemical Activity in Soils from Two Contrasting Parent Materials Contaminated with Agro-Industrial Effluents

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Abstract: *Soil biological and chemical activity in two texturally contrasting soils (Amaoba and Ajata soils) exposed to agro industrial effluents were analyzed to ascertain the effects of these effluents on soil microbial distribution and soil chemical properties (soil pH, organic carbon, total nitrogen available P and exchangeable basics) across three soil depths. The agro-industrial effluents were palm oil mill (POME) and cassava mill (CME) collected from industries in Abia state. The bacterial and fungal distribution in the effluent soils and a control soil were observed at three depths, the variations in the chemical properties at the three sampling depths were statistically analyzed using a 2 X 3 X 3 factorial analysis of variance, where factor A represents the type of effluent, factor B represent the depth of sampling and factor C represents the treatment replicates and significant treatment means were separated using F_{LSD} at five percent level of probability. The results indicate a significant ($P < 0.05$) effect of the effluents on the soil chemical properties. The values for chemical properties decreased with depth of sampling. The microbial diversity was higher in soils exposed to POME in the Ajata soil than those exposed to CME, The bacterial populations identified include Klebsiella Spp, staphylococcus spp, E. Coli, Psuedomonas spp and Staph aureus for the different depths in both sample sites. Fungal populations identified include Penicillum spp, Rhizopus Spp, Trichoderma sppp, Fusarium spp and mucor. There were generally higher populations of these organisms at the 0-20cm depth. However the most frequent occurring bacteria for the POME and CME affected areas were Eschericha coli, pseudomonas spp, and staphylococcus spp. While for fungi the most occurring spp were Penicillum spp, Rhzopus spp, and Aspergillus spp. There were significant differences in the soil chemical properties with respect to the parent material, type of industrial effluent and depth of soil sampling. The microorganisms with the highest occurring frequency were Penicillium spp, Rhizopus spp, Aspergillus spp, Escherishia coli Pseudomonas spp and Staphylococcus spp. The microbial populations in the soil from different parent material responded to palm oil mill effluent (POM) and cassava mill effluent (CM) as they differed from those obtained in the control soil. Soil chemical properties of the effluent soils also differed from the control soil.*

Keywords: *Agro-industrial effluents, Ajata soil, Carbon, Microbial activity, Escherishia coli*.

1. INTRODUCTION

The number and kinds of microorganisms present in a soil depend on many environmental factors such as amount and type of nutrient, available moisture, level of aeration, pH and temperature (Blume *et al* 2002). Soil microbial populations are very diverse and play fundamental roles in the decomposition of organic matter; however these microorganisms and their habitat face degradation or extinction mainly because of pollutant as well as improper soil management. (Amakiri and Onefeghara,1983). The increased production of wastes by agriculture and other industrial sector is of great concern since these wastes produce chemicals and other bye products that eventually find their way into the soil. Reports by Enwezor *et al*,. (1989), and Okafor, (2007) suggest that the effects of effluents on receiving soil range from introduction of clean water into the soil to the introduction of salinity or sodicity into the soil or even the clogging of soil micro pores with solids. The soil thus polluted results in the alteration of the ecosystem, reduction in agricultural activity and loss in soil fertility. Among the pollutants that degrade the soil, cassava and palm oil effluent are the most common in tropical rural communities, because cassava is one of the most common staple foods and palm oil is a common cooking ingredient in tropical Africa (Ogboghodo et al., 2003).

Research reports by Ogboghodo *et al.,* (2003 and 2006) suggested that effluents from cassava cause soil degradation such as decreases in soil acidity, while effluents from palm oil mill can result in some beneficial soil chemical and physical characteristics, such as increases in organic matter and water

holding capacity, but results in undesirable changes such as decrease in soil pH, increase in salinity (Kittikun *et al*., 2000). There is the need to ascertain the type and level of perturbation caused by these effluents especially with respect to the microbial population of the soil. The objective of this paper was to identify distinct bacterial and fungal populations in soils originating from two contrasting parent materials exposed to agro industrial effluents from cassava and oil palm as well as ascertain if the effluents had varied effects on soil chemical properties with depth.

2. MATERIALS AND METHODS

2.1. Study Sites

Two study sites namely Ajata Umuahia Ibeku and Amaoba–ime Ikwuano were used for this study, both are located on latitude 5 °321'51' N and 5°27'N and longitude 7°33'34'E and 7° 32'E respectively in Abia State in South Eastern Nigeria. The Ajata Ibeku were under lain by Bende-Ameki formation (coarse sand stones interrelations of shales and thin shelly lime stones), the Amaoba –ime soils formed from Benin formation (Coastal plain sands) The study area falls within the Humid tropical climate with marked wet and dry seasons of nine and three months duration respectively, annual temperature varies between a minimum of 22^{o}C and a maximum of 32^{o}C. (NRCRI, 2005)

2.2. Field Sampling

Soil samples were collected in triplicate at three depths; 0-20cm, 20-40cm and 40-60cm from fields contaminated with cassava and palm oil effluents, and a field used as the control site located 100 meters away from the contaminated site. The samples were collected using auger samplers at the selected depths into properly labeled poly bags for laboratory analysis. In the laboratory the soils were air dried and passed through a 2mm mesh and the samples for microbial analysis weighed out into open-ended perforated containers and kept in the fridge while those for chemical analysis were stored the poly bags in the laboratory cupboard pending analysis.

2.3. Laboratory Analysis

The air dried and sieved soil samples were used for the determination of the soils physicochemical properties as follows; Particle size using hydrometer method (Bouyoucos 1951), soil pH was determined in KCl using a soil: liquid suspension ratio of 1:2.5 soil to water ratio (Thomas, 1996). Soil organic carbon was determined by wet oxidation method as modified in Nelson and Sommer (1982). The exchangeable cations in the soils were extracted with neutral ammonium acetate (Feech, 1965), exchangeable sodium was measured with the flame photometer, while Mg was determined by the EDTA titration method (Mehlich, 1978). Exchangeable acidity was determined by the method of Mclean (1965). The effective cation exchange capacity (ECEC) was estimated as the sum of exchangeable metallic cations and the exchangeable acidity was computed using the equation:

$$ECEC = TEB + EA \quad \text{…… …….. eqn. 1}$$

2.4. Soil Microbial Analysis

Total heterotrophic bacterial and fungal loads were determined on nutrient agar and saboraund dextrose agar respectively following the method described by Okereke *et al* (2007). Tenfold serial dilution of each soil samples was prepared and appropriate dilutions were inoculated on the various media using spread plate techniques. For spore bacterial count the serially diluted samples were kept in water bath at 80^{o}C for 10 minutes before plating on a meat peptone agar and after incubation at 37^{o}C for 48hrs emerging colonies were counted and expressed as spores (Angerer *et al*.,1998). While the saboraund dextrose agar plates were incubated at room temperature (25-32^{o}C for 2-5 days).

2.5. Microbial Characterization

The isolation of bacteria and fungi followed the methods described by Adarsh *et al* (2007). From the serially diluted samples 0.1ml of the appropriate dilutions were transferred to the nutrient agar plates and spread evenly using the flamed glass hockey. After incubation (37^{0}C for 24hrs) the culture plates were examined closely for the presence of distinct colonies. Inoculates were collected from such distinct colonies and inculcated into sterile agar plates following strict asepsis to obtain pure cultures. After incubation the resulting sub-cultures were examined for uniformity and used for microbial characterization and subsequent identification.

2.6. Statistical Analysis

Soil data on chemical properties such as exchangeable bases, available phosphorus, effective cation exchange capacity, percent base saturation, organic carbon and soil pH were subjected to analysis of

variance using a 2 X 3X 3 factorial in RCBD, where factor A represents the type of effluent, factor B represent the depth of sampling and factor C represents the treatment replicates and significant treatment means were separated using F_{LSD} at five percent level of probability.

3. RESULTS AND DISCUSSIONS

The textural class of soils used in the study was sandy loam for soils collected from Amaoba and clay loam for soils collected from Ajata site. The soil properties varied with depth and the statistical analysis showed the effluents had significant effect on soil chemical properties studied (Tables 1 and 2). The effect of the effluents on microbial composition (Table 3) indicated that the microbial diversity of the effluent contaminated soils were higher than that of the unaffected soil. The bacterial classes identified include *Klebsiella Spp, staphylococcus spp, E. Coli, Psuedomonas spp* and *Staph aureus* for the different depths in both sample sites. Fungal populations identified include *Penicillum spp, Rhizopus Spp, Trichoderma sppp, Fusarium spp and mucor.* There were generally higher populations of these organisms at the 0-20cm depth. However the most frequent occurring bacteria for the POME and CME affected areas were *Eschericha coli*, pseudomonas spp, and staphylococcus spp. While for fungi the most occurring spp were *Penicillum spp, Rhzopus spp,* and *Aspergillus spp*.

Generally the effluents increase in the composition of the identified organism in the soil ecosystem could be associated with increases in soil chemical properties such as pH, organic carbon and total nitrogen. This observation agrees with the research results of Ogboghodo *et al* 2003 and 2006, and confirms the report of this study that the studied effluents increased the growth of the microorganisms. The population of the microorganism was higher in soils contaminated with POME and higher for the Ajata soil. This is attributable to the fact that bacterial diversity and microbial biomass are significantly affected by particle size, as indicated in the report of Sessitchi *et al* (2001), thus the clay loam soil (Ajata) resulted in higher diversity and biomass than the silt or clay soil. The population of microorganisms for both sites and effluents decreased with increasing depth which is expected as more microbial species occupy the first 0-15cm depth of agricultural soils, the decrease in microbial composition and population can also be associated with decrease in soil pH, organic carbon and total nitrogen. This observation agree with research reports (Sylvia *et al*,. 2005) that indicate that higher acidic conditions down the profile results in microbial population decreased down the depth. For both sites under study, most of the soil microbes could not survive high acidic conditions obtained with deeper depth of sampling. The reports Ogboghodo *et al* (2003 and 2005) also supports the observation that soil pH influenced soil nutrient availability and biological activity such that soils with larger quantity of free H+ ions reduces bacterial activity as well organic matter decomposition and nutrient content of soils, because these nutrient elements boast biological activities in the soil. An assessment of the type of bacteria and fungi present in the different site (Table 3) indicated that both study soil had similar content of microbial species, however the species differed for the two sites with differences in effluent type, when compared with the control sites. This is expected as the effluents introduced diverse carbon source which would encourage microbial growth and inoculation, as indicated by favorable and significant effects of the effluents on soil chemical properties studied. Both effluents had significant effects on soil pH with sampling depths (Tables 1 and 2). The pH values decreases with depth for both contaminated soil. This increase in soil acidity with effluents is attributable to probably the release of OH –ions or H+ ions by the effluents. Other studied properties such as organic carbon, available phosphorus, total nitrogen and percent base saturation also decreased with depth. It is possible that the heterotrophic bacteria obtained newer sources of energy from the biodegraded effluence as a source of food and thus immobilized the nutrients during fermentation; this also explained the different effects of the effluents on some chemical constitution or content of the soil. The effluents effects varied with depth as indicated by statistical significance of the interaction between soil depth and the treatments applied suggesting that effluents produced more effect on the soil surface than on the subsurface.

The POM gave a significant effect on soil pH, organic matter, and available phosphorus, total nitrogen and exchange able acidity at $p< 0.05$ for both soils. A comparison of the two soil types indicated that there was no significant difference in the effect of the treatment on the soils types that is the effleunts (POM and CM) had similar effect on soil characteristics irrespective of the parent material and soil texture. This observation contradicts the indications that the capacity of a soil to sequester carbon is controlled and influenced solely by the soil texture, which is attributed to the

capacity of clay particles to physically protect recent organic additions to soils and form stabilized organo-mineral complexes with the humus fraction while the sandy soil which are well aerated with low and non-reactive surface areas are not able to stabilize organic matter to the same extent as clays or loams, This observation suggest that the primary factor of carbon sequestration in the soil is the availability of carbon sources while the clay mineralogy come in as a secondary determinant. Therefore it is expected that the effluent soils used in this study would produce different degree of carbon available for microbial activity and this would result in differences in the chemical properties studied. Moreover, the percent clay content for the two soils used for the study was significantly different, with values ranging between 7- 15 for the soils in Amaoba while for the Ajata soils values ranged as high as between 36-75%, exchangeable Ca (Cmol/kg) was on the average high in the Ajata soil ((10-20) but moderate in the Amaoba soil (5-10), however, percent base saturation was higher in the Amaoba soils (83%-90% but low in the Ajata soil (22%-36%). Therefore a combination of these variations could explain the observed differences in microbial compositions with respect to the carbon availability.

4. CONCLUSION

This study indicated that the microbial populations in the soil from different parent material responded to palm oil mill effluent (POM) and cassava mill effluent (CM) as they differed from those obtained in the control soil. Soil chemical properties of the effluent soils also differed from the control soil which could explain the observed variation in soil properties and influence on the composition of the microbial community as well as contribute to the population of the microbial community. The microbial populations were higher in soils contaminated with POM and clay loam soils than CM and the sandy loam soil. We also observed significant interaction between soil depth of sampling and values of selected chemical properties which explained the observed variations in the population of soil organisms. POM gave a significant effect on soil pH, organic matter, available phosphorus, total nitrogen and exchangeable acidity ($p<0.05$) in both soils used in this study, also the population of microorganism decreased for both sites and effluents with increasing depth.

Table1. *Physicochemical properties of soils contaminated with cassava mill effluents*

Depth (cm)	Sand %	Silt %	Clay %	OC %	pH H_2O	Total N	Avail. P mg/kg	Ca Cmol/kg	Mg Cmol/kg	Na Cmol/kg	K Cmol/kg	EA Cmol/kg	ECEC Cmol/kg	BS %
AMAOBA PARENT MATERIALS														
0-20	89.00	3.20	7.80	1.98	6.30	0.10	28.00	8.62	2.88	0.05	0.10	1.28	12.93	90
20-40	80.30	7.20	12.50	1.78	6.26	0.07	14.00	6.33	2.80	0.06	0.10	1.60	10.89	85
40-60	75.40	9.10	15.50	1.24	6.20	0.08	15.00	4.02	2.40	0.006	0.09	1.28	7.79	83
AJATA PARENT MATERIALS														
0-20	40.20	23.80	36.00	4.12	5.20	0.21	4.8	12.35	2.08	0.11	0.27	26.10	40.81	36
20-40	33.50	20.90	45.60	2.89	5.10	0.10	4.6	8.30	1.80	0.09	0.24	25.70	36.13	29
40-60	10.00	14.80	75.20	1.01	4.80	0.14	0.8	5.02	1.60	0.08	0.23	23.90	30.83	22
LSD 0.05						0.12	0.16	0.15	0.59		1.33	0.15	0.07	0.01
0.26		1.41	0.94											

Table2. *Physicochemical properties of soils contaminated with palm oil mill effluents*

Depth (cm)	Sand %	Silt %	Clay %	OC %	pH H_2O	Total N	Avail.P mg/kg	Ca Cmol/kg	Mg Cmol/kg	Na Cmol/kg	K Cmol/kg	EA Cmol/kg	ECEC Cmol/kg	BS %
AMAOBA PARENT MATERIALS														
0-20	88.86	5.28	10.86	2.24	6.36	0.08	36	15.15	5.33	0.07	0.11	1.44	22.1	93
20-40	85.86	7.25	8.86	2.19	6.32	0.05	31	11.42	4.20	0.05	0.12	1.92	17.71	89
40-60	77.86	7.28	14.86	1.88	6.26	0.04	29	9.32	2.50	0.06	0.09	1.60	14.11	84
AJATA PARENT MATERIALS														
0-20	32.6	23.31	43.3	4.31	6.23	0.25	5.14	14.02	2.70	0.17	0.22	24.7	41.81	40
20-40	31.9	22.5	45.60	3.01	6.10	0.17	4.90	10.70	2.40	0.09	0.19	24.6	37.98	35
40-60	10.3	14.80	24,9	1.54	6.08	0.16	1.01	7.40	1.80	0.08	0.16	23.4	32.76	29
LSD 0.05	0.12	0.16	0.15	0.59	1.33	0.15	0.07	0.01	0.26	1.41	0.94			

Table3. *Classification and distribution of microorganism with depth at study site*

Study site	Contamination	Depth of Sampling	Class of microorganism	
			Bacteria	Fungi
Amaoba	Control	0-20	Klebsiella spp, Staffaureus, E. Coli, Pseudomonas spp and Streptomyces	Penicillium Spp, Rhizopus spp, Aspergillus and Trichorderma spp
		20-40	E. Coli, Psueudomonas, spp and Staphylococcus Spp	Penicillium Spp, Aspergillus spp and fusarium spp
		40-60	E. Coli, Psueudomonas, spp and Staphylococcus Spp	Penicillium Spp, Rhizopus spp and Aspergillus spp
	POM	0-20	Psueudomonas spp, Bacillus, E. Coli, and Staphylococcus Spp	Fusarium spp Aspergillus spp and Trichorderma spp
		20-40	Staphylococcus Spp, E. Coli, Psueudomonas, spp and	Penicillium spp Mucor and Aspergillus spp
		40-60	E. Coli and Staphylococcus Spp	Penicillium Spp, Aspergillus spp
	CM	0-20	Psueudomonas spp, Bacillus, E. Coli, and Staphylococcus Spp	Rhizopus spp Penicillium Spp and Aspergillus spp
		20-40	Psueudomonas spp, E. Coli, and Staph aureus	Penicillium Spp and Aspergillus spp
		40-60	Klebsiella spp, Staph aureus Pseudomonas spp aureginos	Penicillium Spp and Trichorderma spp
Ajata soil	Control	0-20	Klebsiella spp , staph aureus Pseudomonas spp aureginos	Rhizopus spp Penicillium Spp and Aspergillus spp
		20-40	E coli, staphylococcus spp Pseudomonas spp, Streptomyces	Rhizopus spp Penicillium Spp and Aspergillus spp
		40-60	Staphylococcus spp, E coli Pseudomonas spp (scanty)	Penicillium Spp
	POM	0-20	E coli, staphylococcus spp Pseudomonas spp, Klebsiella spp	Rhizopus spp, Mucor, Trichoderma spp . Penicillium
		20-40	Staph aureus, E. coli and Klebsiella spp	Aspergillus spp Penicillum spp
		40-60	E coli, staphylococcus spp Streptococcus	Aspergillus spp, Rhizopus spp
	CM	0-20	E coli, staphylococcus spp Pseudomonas spp,	Aspergillus spp, Rhizopus spp Fusarium spp and Penicillium spp
		20-40	Staph aureus, E. coli , Klebsiella spp	Rhizopus spp and Mucor
		40-60	E. coli, Staph aureus Pseudomonas spp	Fusarium spp and Penicillium spp

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