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EFFECTS OF CASSAVA MILL EFFLUENT ON SOME CHEMICAL AND MICRO-BIOLOGICAL PROPERTIES OF SOILS IN CROSS RIVER STATE, NIGERIA

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ABSTRACT

The paper examines the effects of cassava mill effluent on the Physicochemical and biological properties of soils of Obubra and Odukpani Local Areas in Cross River State after long time of pollution by the effluent. The soil samples were collected with an auger at the depths of 0-15cm and 15-30cm in each of the polluted and non-polluted soils. The results showed that the texture of the soil ranged from sandy to loamy sand in polluted soils and sandy loam in non-polluted soils. The pH ranged from 5.1 to 6.6 and organic carbon from 0.86 to 1.69% in polluted soils. The total nitrogen content ranged from 0.08 to 0.53% while the CEC was low in polluted soils and ranged from 2.50 to 5.50 cmol kg^{-1}. Calcium and Sodium ranged from 1.80 to 3.00 $cmolkg^{-1}$ and 0.05 to 0.12 $cmolkg^{-1}$ respectively. The bacteria and fungi isolated in the polluted soils of Obubra and Odukpani were *Lactococcus lactic, Bacillus subtilis, Lactobacillus lactis, Aspergillius Niger* and *rhizopus spp,.Pseudomonas aeruginosa* and *Penicullum spp*. were only isolated in non-polluted and polluted soils of Odukpani. The cassava effluent increased pH, N, organic carbon, exchangeable acidity, microorganisms and decreased CEC, available P, Mg and K.

KEY WORDS: Cassava Mill Effluent, Soil Pollution, Soil Properties, Bacteria, Fungi.

INTRODUCTION

Cassava is regarded as a typical subsistence crop in the tropics. recent studies have shown that over 50 percent of the harvested crop is sold to get income by the farmers. Cassava production in Cross River State has witnessed an increasing trend over the past twenty or more years. In Obubra and Odukpani areas the total production from 1998 to 2009 was about one million metric tones (Oko, *et al*., 2004). It was also observed by Ogbohodo, *et al*. (2006) that bacteria and fungi population increased with time as the soil was polluted with cassava mill effluent and it was also observed that some bacteria present in the soil at the beginning of the experiment and up to the tenth week of yg pollution with effluent became extinct at the end of the experiment.

FAO (2008) found that the total bacteria (*Lactobacillus planetarium, Pseudomonas aeruginosa, Bacillus spp* and *Viltro* spp.) obtained from the contaminated soil with cassava waste water was more than that in the soil without contaminant. According to Nwankwo *et al*. (2005), organisms isolated during the fermentation of cassava tubers, as practised for "fufu" production included *Bacillus subtilus, Pseudomonas alealigenes, Lactobacillus planetarium, Leuconostic mensenteriodes* and *Pseudomonas aeruginosa*. The effluent from cassava greatly affected the activities of the microorganisms in the polluted soil and the soil became more acidic in nature (Ngaba and Lee, 1979).

The cassava effluent has great influence on the chemical properties of the soil. Studies by Ebhoaye and Dada (2004) using fresh cassava processing effluent and aged effluent obtained from a cassava processing mill in Ekpoma, Edo State showed increase in the level of pH, organic carbon, phosphorus, sodium, potassium and decrease in calcium magnesium and nitrogen in the soil after treatment with the effluent. The increase in pH and sodium may be due to the higher calcium and magnesium components of the effluent used (Asadu, *et al*., 2007). There was no marked difference on the level of exchangeable acidity and particle sizes.

The objective of this investigation, therefore was to analyse both the polluted and non-polluted soils for their physical, chemical and microbiological properties over five years pollution with the effluent.

The study is necessary in these areas because cassava production and processing are on the increase and could cause soil and water pollution in the area. According to Oko *et al.* (2004), the majority of people living in Odukpani Local Government Area use the liquid from cassava for starch making and the effluents are drained into the soil. In Obubra Local Government Area, the liquid is drained into pits and eventually covered. In order to avert hazardous effects of effluent in the soil, it is necessary to find out the effects of emptying cassava mill effluent into agricultural and waste land.

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MATERIALS AND METHODS

The investigation was carried out in three (3) cassava production villages each in Obubra and Odukpani Local Government Area of Cross River State. These areas have been receiving effluent for a minimum period of five years.

Polluted and non-polluted soils were sampled using an auger at soil depths of 0-15cm and 15-30cm and analyzed for physicochemical and microbial properties. The samples for microbial analysis were put in labelled polythene bags and placed in a cooler with ice block (Jensen, 1962). The samples for the physico-chemical properties were air-dried, crushed and made to pass through a 2 mm sieve and stored in labelled polythene bags for the analysis. Samples for microbial properties were analyzed for bacterial and fungal population count by the methods of Zuberer, 1994 and Alef, 1995. Biochemical analysis for bacteria were determined by methods of Collins and Lyne (1979) and Cowan (1985).

Particle size distribution was determined by hydrometer method (IITA,1979). Soil pH was measured in a water suspension ratio of 1:2.5 using a glass electrode pH meter (IITA, 1979). Percentage organic carbon was determined by the wet oxidation method described by Nelson and Sommers (1982). Total nitrogen was determined by macro Kjeldahl digestion method (Jackson, 1969). The available P was determined using Bray P1 as described by IITA, (1979). Exchangeable cations (Ca, Mg, K and Na) were leached from the soil sample using 1N NH_4OAc buffered at pH 7.0 (Chapman, 1965). Exchangeable acidity was determined by leaching the soil with 1N KCl solution and the extract titrated with standard NaOH solution (IITA; 1979). Cation Exchange Capacity (CEC) was determined by 1N neutral NH_4OAc at pH, Chapman (1965).The relationship between the population of bacteria isolate and fungal isolate in both polluted and non-polluted soil was determined using correlation analysis and tested at 5% probability level according to the procedure outlined by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The particle size distribution and chemical properties for polluted and non-polluted soils of the study areas are in Tables 1 and 2.

PARTICLE SIZE DISTRIBUTION AND TEXTURAL CLASS

The texture of the soils ranged from sandy to loamy sand in the polluted soils. There was no marked difference in the particles size distribution of the soils of the study area.

SOIL pH

The pH ranged from 5.1 to 6.6 in polluted soils and 4.3 to 5.4 in non-polluted soils. The pH was generally higher in polluted soils than non-polluted soils (Tables 1 and 2). These results agree with the findings of Ebhoaye and Dada (2004), who found an increase in soil pH after treatment with the cassava effluent. The critical level for good plant growth in this area. The pH values of polluted soils within this range and even above in some areas in indicating nutrient availability and micro floral activities to support plant growth.

CATION EXCHANGE CAPACITY (CEC) ($cmol^{+}kg^{-1}$)

The CEC increased with increase in the soil pH indicating high level of nutrient availability. Increase in CEC provides a medium for adsorption of plant nutrients and improved conditions for micro-organisms (Sohi, *et al*., 2009).

ORGANIC CARBON (%)

The organic carbon accumulation was higher at the top soil than the sub-soil in all the locations studied, and particularly more on polluted soils than on non-polluted soils.

AVAILABLE P ($mg\ kg^{-1}$)

Available P was higher in polluted soils than non-polluted soils. This was probably due to the cyanogenic compounds from the cassava effluent which led to high organic carbon in the polluted soils. Total content was higher in polluted soils than non-polluted ranging nitrogen from 0.08% to 0.53% (Ebhoaye and Dada,2004).

EXCHANGEABLE BASE ($cmol\ ^{+}kg^{-1}$)

The exchangeable cations were generally low in polluted soils, soils than non-polluted soils, probably due to low Ca, Mg, K and Na contents of the effluent (Orhue, *et al*. 2005 and Buol, *et al*. 1973). Base saturation was high in polluted soils ranging between 48.81-63.51%.

EXCHANGEABLE ACIDITY($cmol\ ^{+}kg^{-1}$)

There was difference in exchangeable acidity in polluted and non-polluted soils. The exchangeable acidity (EA) ranged from 1.70 to 3.16 cmol kg^{-1} in polluted soils and 1.93 to 3.68 cmol kg^{-1} in non polluted soil. The EA was higher in non polluted soils than polluted soils.

MICROBIAL ISOLATES

Microbial isolates from polluted and non-polluted soils of Obubra are shown in Tables 3 and 4. The bacteria isolated from polluted and non-polluted soils of Obubra were *Lactococcus lactis*, *Bacillus subtilis, Lactobacillus lactis* and the fungal isolates identified were *Aspergillus niger, Fusarium* spp. and *Rhizopus* spp.

The microbial isolates from polluted and non-polluted soils in Odukpani, are shown in Tables 5 and 6. The bacterial isolates from polluted and non-polluted soils of Odukpani were *Lactobacillus lactis, Bacillus subtilis*, *Lactococcus lactis* and *Pseudomonas aeruginosa*. The fungal isolates identified from polluted and non-polluted soils of Odukpani were *Penicillium* spp., *Rhizopus* spp. and *Aspergillus niger*.

The polluted soils in Obubra had higher population of bacteria than non-polluted soils with mean colony count of 86×10^5 and 121×10 cfu/g for non-

higher in polluted soils than non-polluted soils in Obubra. This increase in bacteria and fungi in the polluted soils help in the rapid decomposition of organic matter and also help in releasing of essential nutrients from the soils for plants growth. The hydrocarbon utilizing bacteria (HUB) were *Bacillus substilis* and catalase positive organisms. They made use of the pollutants to form the substrate and can catabolize glucose and sugar to pyruvate and decarboxylate the pyruvate in the effluent to acetaldehyde which later reduce to ethanol by alcohol dehydrogenase with Nicotinamide adenine dinucleotide phosphate (NADP). The reaction affects crop production because during this process energy and Co_2 are liberated for effective respiration by the plants. The hydrocarbon utilizing fungi (HUF) were *Aspergillus niger* and *Fusarium* spp. They were slow decomposers and secondary consumers which lived on already prepared substrate.

In polluted soils of Odukpani, the bacteria with the higher population was *Pseudomonas aeruginosa* with 78 x 10^5 cfu/g but they served as the hydrocarbon-utilizing bacteria and also they have a functional tricarboxylic acid cycle which oxidize pyruvate completely to CO_2 for photosynthesis (Willey et al., 2008). Carbon dioxide have positive physiological effects by increasing the rate of photosynthesis in which some plants and bacteria use the energy from sunlight to produce sugar for effective metabolism. The appearance of *Penicillium* spp. may be due to the favourable condition and available substrate in Odukpani area. The mean colony count of bacteria in non-polluted soils of Odukpani area were 125 x 10^5cfu/g and polluted soils were 180 x 10^5cfu/g. Thus the result of Odukpani area also showed that the population of microorganisms were higher in polluted than non-polluted soils.

The correlation coefficient (r) and coefficient of determination (r^2) of correlation analysis for bacterial isolates and fungal isolates in both sludge-polluted and non-polluted soils of Odukpani are presented in Table 7 with the exception of *Pseudomonas aeruginosa* versus *Rhizopus spp*. which had a significant ($P < 0.05$) r-value of -0.9058, all other correlation of bacteria and fungi were not significantly ($p<0.05$) different. The relationship between bacteria isolates and fungal isolates was not statistically significant ($P < 0.05$). The bacterial-fungal relationship in non polluted soil of Obubra had negative values of the correlation coefficient r with the exceptions of *Lactococcus lactis* vs. *Fusarium spp.*, and *Lactobacillus lactis* vs. *Rhizopus* in Table 8. The correlation was not significant ($P > 0.05$) with the exception of *Lactobacillus lactis* vs. *Rhizophus spp*. with a positive correlation coefficient of 0.8799. In the soil polluted with sludge, the bacterial-fungal isolate relationship was positive with exception of *Lactobacillus lactis* vs. *Rhizophus spp., Lactococcus lactis* vs. *Aspergillus spp*. and *Bacillus subtilis* vs. *Aspergillus* spp. However, the correlation coefficient (r) was not statistically significant ($P < 0.05$).

Generally, the coefficient of determination (r^2) was higher in polluted soils than non-polluted soils.

CONCLUSION AND RECOMMENDATION

The effects of cassava mill effluent on some physical, chemical and microbiological properties showed that there were no effects on soil physical properties. The pH in the polluted soils increased and thus reduced the level of CEC. Total nitrogen, organic carbon and exchangeable acidity were also increased in polluted soils. Magnesium, Available P and K were low in polluted soils than non-polluted soils ranging from 0.53-2.27,3.12-5.82,0.10-0.11 $cmol^+kg^{-1}$ and 0.07 - 3.00,2.74-5.86, 0.06-0.43 $cmol^+kg^{-1}$ respectively. Calcium and exchangeable acidity were moderate ranging from 1.80-3.00 and 1.70 – 3.16 $cmol^+kg^{-1}$ Total mean count of bacteria and fungi were higher in polluted soils than non-polluted soils in both Odukpani and Obubra Local Government Areas.

Also in both areas, *Bacillus substilis* was a constant occurring hydrocarbon-utilizing bacteria found in both non-polluted and polluted soils. *Aspergillus niger* was also found to be the hydrocarbon utilizing fungus. It was persistent and prevalent in both polluted ad non-polluted soils.

The effluent had effects on the following physical, chemical and microbial properties of the soil .There was no marked difference in the soil texture between polluted and non-polluted soils. The pH ranged from 4.5 to 6.6 and pH of 5.5 to 6.5 is the average optima range for plant growth and high nutrient availability in this area (Nigam, 2000 and Agbede, 2009). Also the beneficial microorganisms are available at this point for the decomposition of organic matter releasing essential nutrients to the soil. The cyanogenic compounds in the effluent increased the total nitrogen content, organic carbon, exchangeable acidity, microorganisms and reduced CEC, available phosphorus, magnesium, potassium and calcium. In effect government should create awareness to farmers on the need for proper waste management of cassava effluent by providing reservoirs for storing the effluent. This will minimize environmental pollution. Scientists should be provided with research tools to determining the value of effluent through chemical analysis and evaluation on how it could serve as a raw material for oil and gas production and starch.

The result obtained generally showed non-significant relationship between bacteria and fungi.

Table 1: Physicochemical Properties of polluted and non-Polluted Soils of Obubra

Locations	Depth (cm)	Silt (%)	Clay (%)	Sand (%)	pH	Org.C (%)	TN (%)	Avail.P ($mgkg^{-1}$)	Exch Bases Ca ($cmol^{+}kg^{-1}$)	Mg ($cmol^{+}kg^{-1}$)	K ($cmol^{+}kg^{-1}$)	Na ($cmol^{+}kg^{-1}$)	EA ($cmol^{+}kg^{-1}$)	ECEC $cmol^{+}kg^{-1}$)	BS (%)	Textural class
Obubra	0-15	15.47	9.13	75.40	5.4	1.25	0.50	2.94	2.27	3.00	0.43	0.07	3.68	9.45	61.00	Sandy loam
Non-polluted	15-30	17.27	15.39	67.34	5.1	0.60	0 50	2.74	3.50	1.10	0.10	0.07	2.39	7.15	66.71	Sandy loam
							0.50									
Mean		16.37	12.26	71.37	5.3	0.93		2.84	2.89	2.05	0.27	0.07	3.04	8.30	63.86	
							0.00									
SD		1.27	4.43	5.70	0.2	0.46		0.14	0.87	1.34	0.23	0.00	0.91	1.63	4.04	
							0.00									
CV(%)		7.78	36.13	7.99	3.8	49.40		4.98	30.10	65.37	86.46	0.00	30.01	19.59	6.32	
							0.53									
Obubra	0-15	15.47	5.13	79.40	5.3	1.69		4.10	2.27	0.87	0.10	0.08	2.59	6.34	59.14	Loamy sand
							0.50									
Polluted	15-30	9.73	6.97	83.30	5.1	1.00		3.12	3.00	2.27	0.11	0.12	3.16	8.66	63.51	Loamy sand
							0.52									
Mean		12.6	6.05	81.35	5.2	1.35	0.02	3.61	2.64	1.57	0.11	0.10	2.88	7.50	61.33	
SD		4.06	1.30	2.75	0.1	0.49	3.85	0.69	0.52	0.09	0.01	0.03	0.40	1.64	3.09	
CV(%)		32.22	21.49	3.39	1.9	36.30		19.11	19.70	63.06	0.09	30.00	13.89	21.87	5.04	

Table 2: Physicochemical Properties of polluted and non-Polluted Soils of Odukpani

Locations	Depth cm	Silt (%)	Clay (%)	Sand (%)	pH	Org.C (%)	TN (%)	Avail.P $mgkg^{-1}$	Exch Bases Ca	Mg	K	Na ($cmol^{+}kg^{-1}$)	EA ($cmol^{+}kg^{-1}$)	ECEC ($cmol^{+}kg^{-1}$)	BS (%)	Textural class
Odukpani	0-15	3.70	7.70	88.60	4.5	0.91	0.08	4.04	2.20	0.67	0.06	0.06	2.25	7.24	41.30	Sandy loam
Non-polluted	15-30	2.20	11.90	85.90	4.3	0.80	0.03	5.86	1.20	0.07	0.06	0.06	1.93	3.32	41.87	Sandy loam
Mean		2.95	9.80	87.25	4.4	0.86	0.06	4.95	1.70	0.37	0.06	0.06	2.09	5.28	41.59	
SD		1.06	2.97	1.90	0.1	0.08	0.04	1.29	0.71	0.42	0.00	0.00	0.23	2.77	0.40	
CV(%)		35.93	30.31	2.19	2.3	9.30	66.67	22.60	41.76	114.67	0.00	0.00	10.83	52.50	0.97	
Odukpani	0-15	4.37	7.70	87.93	6.6	1.30	0.10	5.82	1.80	1.10	0.10	0.05	1.91	4.96	61.49	Sand
Polluted	15-30	4.03	13.03	82.93	6.4	0.86	0.08	5.30	1.80	0.53	0.10	0.07	1.70	4.20	48.81	Loamy sand
Mean		4.20	10.37	85.43	6.5	1.08	0.09	5.56	1.80	0.82	0.10	0.06	1.81	4.58	55.15	
SD		0.24	3.77	3.54	0.1	0.31	0.01	0.37	0.00	0.40	0.00	0.01	0.15	0.54	8.97	
CV(%)		5.72	36.34	4.14	2.2	28.81	11.1	6.61	0.00	49.15	0.00	23.57	8.29	11.73	16.26	

Table 3: Bacterial Isolates from Polluted and Non-Polluted Soils in Obubra

Sampling Locations	Bacterial Isolates	Count (cfu/g)
Obubra (Non-polluted soils)	*Lactococcus lactis*	48.0×10^5
	Bacillus subtilis	22.0×10^5
	Lactobacillus lactis	16.0×10^5
	Mean colony count	86.0×10^5
Obubra (Polluted soils)	*Lactococcus lactis*	52.0×10^5
	Lactobacillus lactis	34.0×10^5
	Bacillus subtilis	35.0×10^5
	Mean colony count	121.0×10^5

Table 4: Fungal Isolates from Polluted and Non-Polluted Soils in Obubra

Sampling Locations	Fungal Isolates	Count (cfu/g)
Obubra (Non-polluted soils)	*Fusarium* spp.	4×10^3
	Rhizopus spp.	9×10^3
	Aspergillus niger	8×10^3
	Mean colony count	21×10^3
Obubra (Polluted soils)	*Aspergillus niger*	4×10^3
	Fusarium spp.	9×10^3
	Rhizopus spp.	8×10^3
	Mean colony count	22×10^3

Table 5: Bacterial Isolates from Polluted and Non-Polluted Soils in Odukpani

Sampling Locations	Bacterial Isolates	Count (cfu/g)
Odukpani (Non-polluted soils)	*Lactococcus lactis*	25×10^5
	Bacillus subtilis	28×10^5
	Lactobacillus lactis	55×10^5
	Pseudomonas aeruginosa	17×10^5
	Mean colony count	125×10^5
Odukpani (Polluted soils)	*Lactococcus lactis*	17×10^5
	Lactobacillus lactis	47×10^5
	Bacillus subtilis	38×10^5
	Pseudomonas aeruginosa	78×10^5
	Mean colony count	180×10^5

Table 6: Fungal Isolates from Polluted and Non-Polluted Soils in Odukpani

Sampling Locations	Fungal Isolates	Count (cfu/g)
Odukpani (Non-polluted soils)	*Penicillium* spp.	4×10^3
	Rhizopus spp.	8×10^3
	Aspergillus niger	18×10^3
	Mean colony count	30×10^3
Odukpani (Polluted soils)	*Aspergillus niger*	10×10^3
	Penicillium spp.	10×10^3
	Rhizopus spp.	14×10^3
	Mean colony count	34×10^3

Table 7: Correlation coefficient (r) and coefficient of determination r^2 (%) of correlation between bacterial isolates population and fungal isolates population in polluted and non- polluted soils of Odukpani Local Government Area.

Bacterial isolate vs	Fungal isolate	Non-polluted soil (r)	Non-polluted soil (r^2)	Polluted soil (r)	Polluted soil (r^2)
Lactococcus lactis	*Aspergillus niger*	0.0381^{ns}	0.15	-0.4385^{ns}	19.23
P. aeruginosa	*Aspergillus niger*	0.4140^{ns}	17.14	-0.0620^{ns}	0.38
Lactobacillus lactis	*Aspergillus niger*	0.3620^{ns}	13.10	0.5650^{ns}	31.92
Bacillus subtilis	*Aspergillus niger*	-0.2068^{ns}	4.28	0.7123^{ns}	50.73
Lactobacillus lactis	*Rhizopus spp.*	-0.0834^{ns}	0.70	0.2697^{ns}	7.27
P. aeruginosa	*Rhizopus spp.*	-0.3024^{ns}	9.14	-0.9058^{*}	82.05
Lactobacillus lactis	*Rhizopus spp.*	0.296^{ns}	8.76	-0.5762^{ns}	33.20
Bacillus subtilis	*Rhizopus spp.*	-0.0676^{ns}	0.46	0.5140^{ns}	26.42
Lactobacillus lactis	*Pencillium spp.*	-0.1472^{ns}	2.17	0.3508^{ns}	12.31
P. aeruginosa	*Pencillium spp.*	-0.3115^{ns}	9.70	-0.0930^{ns}	0.86
Lactobacillus lactis	*Pencillium spp.*	-0.3419^{ns}	11.69	-0.6285^{ns}	39.50
Bacillus subtilis	*Pencillium spp.*	0.3042^{ns}	9.25	-0.5960^{ns}	35.52

Table 8: Correlation coefficient (r) and coefficient of determination r^2 (%) of correlation between bacterial isolates population and fungal isolates population in polluted and non- polluted soils of Obubra Local Government Area.

Bacterial isolate vs	Fungal isolate	Non-polluted soil (r)	Non-polluted soil (r^2)	Polluted soil (r)	Polluted soil (r^2)
Lactococcus lactis	*Fusarium spp.*	0.2333^{ns}	5.44	0.373^{ns}	13.91
Bacillus subtilis	*Fusarium spp.*	-0.0431^{ns}	0.19	0.6431^{ns}	41.36
Lactobacillus lactis	*Fusarium spp.*	-0.2500^{ns}	6.25	0.0601^{ns}	0.36
Lactobacillus lactis	*Rhizopus spp.*	-0.8322^{ns}	70.26	0.1712^{ns}	2.93
Bacillus subtilis	*Rhizopus spp.*	-0.6259^{ns}	39.18	0.2208^{ns}	4.88
Lactobacillus lactis	*Rhizopus spp.*	0.8799^{*}	77.42	-0.4369^{ns}	19.09
Lactobacillus lactis	*Aspergillus niger*	-0.0501^{ns}	0.25	-0.7554^{ns}	57.06
Bacillus substilis	*Aspergillus niger*	-0.1183^{ns}	1.40	-0.7815^{ns}	61.07
Lactobacillus lactis	*Aspergillus niger*	-0.4740^{ns}	22.47	0.5357^{ns}	28.69

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