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The Impact of Cassava Effluent on the Microbial and Physicochemical Characteristics on Soil Dynamics and Structure

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Abstract

The effects of cassava effluent on soil microbial and physicochemical properties were studied using culture-dependent and standard analytical methods. Soil samples were collected from sites polluted with cassava effluent and from adjacent sites that were not impacted with the effluent pollution. The isolation and enumeration of microbial population was carried out using standard culture-based methods. Standard analytical methods were used to assay for physicochemical properties. The highest bacterial count of $3.61\times10^8 \pm 0.12$ CFU/g was recorded for polluted soil sampled from Ehor, while the lowest count of $1.3\times10^8 \pm 0.03$ CFU/g was recorded in Isihor. Isihor had the highest fungal count of $2.2 \times10^8 \pm 0.01$ CFU/g from soil contaminated with cassava effluent. The fungal counts of the polluted soil were significantly lower than the bacterial counts generally ($p < 0.05$). The heavy metal contents of the contaminated soils were relatively higher than the uncontaminated soil (control). Unlike in the control soils, pH of the polluted soils ranged from 4.0 - 4.78. The bacteria isolated were *Bacillus subtilis, Bacillus macerans, Pseudomonas aeruginosa, Klebsiella aoxytoca* and *Escherichia coli.* Eleven species of fungi belonging to the genera *Aspergillus*, *Penicillium a*nd *Rhizopus* were also isolated. The present study shows that the cassava effluent can have an increasing or limiting effect on the microbial diversity of the polluted soil which could also be attributed to the simultaneous impact on the physicochemical parameters of the soil.

Keyword: Biodiversity; Microbial density; Heavy metal; Pollution; Toxicants.

1. Introduction

The risk to human lives and aquatic organisms constituted by industrial and gaseous effluents cannot be over-stressed (Okafor, 2011). Most industries are responsible of releasing contaminants into the environment. Soil and water bodies are particularly polluted with toxicants from food processing and allied industries and inhabitants of the affected areas are exposed to health related risks as a result of this uncontrollable industrial discharge (Salami and Egwin, 2007). Soil is the uppermost layer of the earth's crust formed as a result of the microbial transformation of weathered rocks (Kolwan *et al.,* 2006). Soil is stratified into several layers and the topsoil is the most prolific. The topsoil consists of soil microorganisms which are involved in the degradation of organic matter and nutrient cycling. This has an effect on global geochemical nutrient (Bunning and Jimenez, 2003). The topsoil gets the ultimate effect from environmental pollutants. Such pollutants include hydrocarbon pollutants, palm oil mill effluent, human and animal wastes, wood waste, waste water from agro-allied industries and refineries, mining effluent as well as cassava mill effluent from cassava processing activities (Wade *et al.,* 2002; Walsh *et al.,* 2002; Ojumu, 2004; Arimoro and Osakwe, 2006).

Cassava (*Manihot esculenta*) belongs to the family Euporbaceae (Nwaugo *et al.,* 2008). It is one of the largest sources of energy-giving foods in the tropics (Fauquet *et al.,* 1990). Cassava is an essential food in Nigeria and other developing countries. Nigeria is the largest producer of cassava while the greatest exporter of this crop is Thailand (FAO, 2004). There has been great upsurge in the production and utilization of cassava in the past few years. This has led to the establishment of cassava milling engines in most environments with the consequence of an extensive ecological pollution associated with the effluent discharge. The unpleasant smell coming from the fermenting effluent calls for the establishment of laws to guide the discharge of cassava waste generated (Nwaugo *et al.,* 2008). In Niger Delta region of Nigeria, cassava tubers are processed for eating either as starch, garri, fufu, dried or wet cassava flour. Garri is widespread among all processed cassava products in Nigeria. Garri production is accompanied with the release of water, hydrocyanic acid, organic matter and sieves from the pulp. Hence, the remnants of the processing of cassava consist of solid and liquid wastes.

Reports have shown that the cassava effluent contains harmful cyanides, copper, mercury and nickel which have

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the capacity to affect native micro-biota (Aiyegoro *et al.,* 2007). Pollution from such effluent could result to a serious imbalance in the living and non-living entities of the ecosystem (Lemke *et al.,* 1997). This could lead to a reduction in the soil fertility. Unlike toxigenic organic matters that are susceptible to degradation, the metals that are discharge into the soil have the tendency to persist indeterminately where they accumulate in living organisms through food chain (Cossic *et al.,* 2002).

Cassava mills, which are mainly on small scale platform, are processed and managed by persons who lack knowledge of environmental safety. While on a small scale platform, there are many of them, when combined together they produce great negative effects on the ecosystem (Nwaugo *et al.,* 2008). Thus, it is essential to estimate the extent of such effects on both biotic and abiotic components of the ecosystem. Although Okafor and Uzuegbu (1987) studied the biochemical transformation associated with the fermentation of cassava effluent there are few reports on the ecological impacts of this effluent in some parts of Edo State, Nigeria. We evaluated the impact of cassava processing mill activities on culturable bacterial and the fungal diversity and the physicochemical status on the soil microbiota to ascertain the degree of pollution on the environment.

2. Materials and Methods

2.1. Samples Collection

Using disinfected soil auger, soil samples were collected in clean sterile bottles from a depth of 0-20 cm from soil polluted with cassava effluent in Oluku, Isihor and Ehor in Edo South region of Edo State, Nigeria. Soil samples, free of cassava effluent, were also collected from control sites outside the processing plant. Samples were collected during the rainy season between August and October, 2014.

2.2. Sample Preparation

One gram (1 g) of the soil samples was measured into a sterile test tube and 9 mL of sterile distilled water was added to make a stock solution; dilution was made from the stock solution. The 10^{-1} suspension was subsequently serially diluted to 10^{-10} dilution. Diluted samples were used for microbial analysis. On the other hand, soil samples were subjected to air-drying for seven days in the laboratory, ground and sieved through a 2-mm stainless-steel sieve and kept in a sealed polyethylene bag at ambient temperature (28±2^{o}C) for 24 h prior to physicochemical analysis.

2.3. Microbial Analysis

The aerobic heterotrophic bacterial and fungal populations were ascertained by a standard pour plate method. Heterotrophic bacteria were isolated using nutrient agar amended with 0.015 % (w/v) nystatin to inhibit fungal growth. The nutrient agar plates were incubated at 28 ± 2^{o}C for 24-48 h. Potato dextrose agar containing 0.05 % (w/v) chloramphenicol was used to isolate fungi upon incubation at 28 ± 2 ^{o}C for 72 h. Purification of representative bacterial and fungal colonies was done by sub-culturing and identifications were made as reported by Staley *et al.* (1989) and Talbot (1978), respectively.

2.4. Physicochemical Properties Analysis

Physicochemical parameters of effluent contaminated and control soil samples were examined. Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer AA Analyst 800 series Graphite Furnace AA) was used to analyze the digested samples for metals contaminant. Soil temperature and pH were determined using a mercury thermometer (Digital Thermometer (Model 6300), Spectrum Technologies, Inc. and pH meter (FieldScout pH 110 Meter), respectively. The Total Organic Carbon (TOC) was ascertained as reported by Nelson and Sommers (1982). Cation Exchange Capacity (CEC) was determined according to the method described by Chapman (1965). Total nitrogen using Kjedahl digestion and steam distribution method and percentage organic matter were investigated as reported by previous studies (Bremner, 1965). The electrical conductivity was determined by the method of Chopra and Kanzar (1988). P, NH^{4+}, NO^{3+} and NO^{3-} were estimated with the use of AAS (Perkin Elmer AA Analyst 800 series Graphite Furnace AA). The particle size analysis of the soil was achieved by hydrometer method as described by Bouyoucos (1962).

2.5. Statistical Analysis

Statistical analyses of data were achieved using Minitab16 software and Microsoft excel. Data were subjected to Anderson-Darling normality test. Comparisons of means were assessed statistically by subjecting data to one-way analysis of variance (ANOVA). Regression analysis was also done. A probability values (*p*-values) of less than 0.05 was considered as significant.

3. Results

The bacteria isolated from the studied areas were *Bacillus subtilis, Bacillus macerans, Pseudomonas aeruginosa, Klebsiella oxytoca* and *Escherichia coli*. On the other hand, eleven (11) species of fungi belonging to the genera *Aspergillus, Penicillium* and *Rhizopus* were isolated. Table 1 represents the occurrence and the distribution pattern of bacterial species or diversity in the studied areas; while Table 2 shows the occurrence and the distribution pattern of fungal diversity in the studied areas. The total heterotrophic bacterial and fungal population ranged from 1.3×10^8 - 3.61×10^8 CFU/g and 1.84×10^4 - 2.2×10^8 CFU/g, respectively. Table 5 represents the mean values and the standard error of physicochemical parameters of soil samples from cassava effluent polluted and control soil in Oluku, Isihor and Ehor during the period of study.

Table 1. The occurrence and distribution pattern of bacterial population in the in studied areas

Sampling sites	Month	August					September					October				
	Species	B_1	B_2	B_3	B_4	B_5	B_1	B_2	B_3	B_4	B_5	B_1	B_2	B_3	B_4	B_5
O		+	+	-	-	-	+	-	-	-	+	+	+	+	+	+
I		+	+	+	-	+	-	+	+	+	+	+	+	+	-	-
E		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C_O		-	-	-	+	+	-	-	-	+	+	-	-	-	-	-
C_I		+	-	-	-	-	-	+	+	-	-	+	+	+	-	-
C_E		+	+	+	+	+	-	-	-	-	+	-	-	-	+	+

Legend: O- Oluku; I- Isihor; E- Ehor; C_O- Control site at Oluku; C_I- Control site at Isihor; C_E- Control site at Ehor; + -present; - -absent; B_1- *Escherichia coli*; B_2- *Klebsiella oxytoca*; B_3- *Bacillus subtilis*; B_4- *Bacillus macerans*; B_5- *Pseudomonas aeruginosa*

Table 2. The occurrence and distribution pattern of fungal population in the studied areas

Sampling sites	Month	August			September			October		
	Species	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3
O		+	-	+	+	+	-	+	+	+
I		+	+	+	-	+	+	+	+	+
E		+	+	+	+	+	-	+	-	+
C_O		-	-	+	+	-	-	-	-	-
C_I		-	-	-	+	-	-	-	-	+
C_E		+	-	-	-	+	-	-	+	-

Legend: O- Oluku; I-Isihor; E-Ehor; C_O-Control site at Oluku; C_I-Control site at Isihor; C_E-Control site at Ehor; + -present; - -absent; F_1- *Rhizopus* spp; F_2-*Aspergillus* spp;F_3- *Penicillium* spp.

Table 3. Effect of cassava effluent on bacterial load of soil (CFU/g) during the period of study

S	August		September		October	
	Control Soil	Polluted soil	Control Soil	Polluted soil	Control Soil	Polluted soil
O	$1.2\times10^8 \pm 0.02$	$2.14\times10^8 \pm 0.04$	$3.0\times10^8 \pm 0.01$	$3.1\times 10^8 \pm 0.12$	$2.3\times10^8 \pm 0.02$	$3.2\times10^8 \pm 0.02$
I	$1.4\times10^8 \pm 0.04$	$1.72\times10^8 \pm 0.04$	$1.5\times10^8 \pm 0.03$	$1.6\times10^8 \pm 0.01$	$1.0\times10^8 \pm 0.05$	$1.3\times10^8 \pm 0.03$
E	$1.41\times10^8 \pm 0.04$	$3.61\times10^8 \pm 0.12$	$3.3\times10^8 \pm 0.15$	$2.16\times10^8 \pm 0.03$	$1.1\times10^8 \pm 0.01$	$2.0\times10^8 \pm 0.04$

Legend: S-sampling site; O- Oluku; I- Isihor; E- Ehor; Values are means of triplicates; ± - standard deviation

Table 4. Effect of cassava effluent on fungal load of soil (CFU/g) in during the period of study

S	August		September		October	
	Control Soil	Polluted soil	Control Soil	Polluted soil	Control Soil	Polluted soil
O	$1.2\times10^5 \pm 0.01$	$1.84\times10^4 \pm 0.08$	$2.0\times10^6 \pm 0.02$	$2.1\times 10^5 \pm 0.12$	$1.3\times10^6 \pm 0.01$	$2.2\times10^8 \pm 0.01$
I	$1.3\times10^6 \pm 0.03$	$2.45\times10^4 \pm 0.31$	$1.0\times10^4 \pm 0.03$	$0.6\times10^8 \pm 0.01$	$2.0\times10^2 \pm 0.05$	$1.4\times10^7 \pm 0.03$
E	$1.1\times10^4 \pm 0.04$	$8.4\times10^4 \pm 0.49$	$1.3\times10^6 \pm 0.05$	$1.16\times10^7 \pm 0.03$	$2.3\times10^4 \pm 0.01$	$2.0\times10^7 \pm 0.03$

Legend: S-sampling site; O- Oluku; I- Isihor; E- Ehor; Values are means of triplicates; ± - standard deviation

Table 5. Physicochemical parameters of soil samples from cassava effluent polluted and control soil in Oluku, Isihor and Ehor during the period of study

Parameters	Oluku		Isihor		Ehor	
	Polluted soil	Control soil	Polluted soil	Control soil	Polluted soil	Control soil
Temp (°C)	25.00±0.1	25±0.30	20.65±0.1	27.52±0.12	26.52±0.6	25±0.66
pH	4.00±0.2	5.6±0.01	4.78±0.26	5.58±0.44	4.78±0.3	6.6±0.55
EC (µs/Cm)	182±0.17	175±0.14	192±0.00	187±0.00	160±0.00	150±0.00
Cl^- (mg/kg)	47.17±1.0	43±1.00	59.33±0.0	40.67±0.02	50±0.08	49±0.88
SO_4^{2+} (mg/kg)	7.9±0.7	7.4±0.1	9.67±0.36	6.0±0.72	4.00±0.1	3.0±0.99
NO_3^- (mg/kg)	4.0±0.4	3.0±0.02	4.00±0.79	0.67±0.00	7±0.03	8±0.00
PO_4^{3-} (mg/kg)	5.24±0.4	5.24±0.03	6.43±0.34	6.88±0.61	7.22±0.1	5.22±0.01
Fe (mg/kg)	12.0±0.1	12.6±0.1	4.58±0.01	4.38±1.00	5.88±1.1	4.33±0.11
Zn^{2+} (mg/kg	1.87±0.0	1.78±0.02	1.89±0.01	1.44±0.01	1.43±0.1	1.33±0.01
Mn^{2+} (mg/kg)	0.69±0.0	0.61±0.00	0.05±0.00	0.01±0.05	0.06±0.0	0.01±0.01
Cu^{2+} (mg/kg)	2.17±0.0	2.12±0.00	1.69±0.07	1.09±0.06	2.9±0.07	1.9±0.00
O.C (%)	1.21±0.40	0.21±0.20	2.15±0.06	1.26±0.07	2.36±0.5	136±0.03
T.N (%)	0.31±0.0	0.11±0.002	0.37±0.02	0.130±0.01	0.44±0.4	0.33±0.03
Moisture (%)	13±0.1	12±0.01	12±0.02	11±0.02	13±0.05	13±0.03
Sand (%)	89±0.68	87±0.034	86±1.22	87±0.97	89±1.23	76±0.03
Silt (%)	4±0.8	3±0.9	4±0.02	4±0.06	4±0.07	3±0.02
Clay (%)	7±0.51	6±0.50	7±0.32	6±0.38	8±0.38	5±0.01

Legend: EC-electrical conductivity; O.C - organic carbon; T.N - total nitrogen Values are means of triplicates; ± - standard deviation

4. Discussion

The results reveal the following bacterial isolates, which are common micro-biota of the soil: *Bacillus subtilis, Bacillus macerans, Pseudomonas aeruginosa, Klebsiella oxytoca* and *Escherichia coli*; while eleven (11) species of fungi belonging to the genera *Aspergillus, Penicillium,* and *Rhizopus* were isolated. These bacteria and fungi species were isolated by previous authors (Knowles, 1988; Ehiagbonare *et al.,* 2009). These isolates occurred more in cassava effluent polluted soil than in the control soils in all the studied areas (Tables 1 and 2). Cassava effluent polluted soil from Ehor had the highest bacterial count of 3.61×10^8 CFU/g in August while the lowest bacterial count of 1.3×10^8 CFU/g was recorded at Isihor in October. These values are similar to those reported by Okoh *et al.* (1999) and Aiyegoro *et al.* (2007).

The fungal counts for the polluted and control soil ranged from 1.84×10^4-2.2×10^8 CFU/g and 2.0×10^2-2.0×10^8 CFU/g, respectively. This suggests that the cassava effluent has effects on the fungal diversity of the polluted soil. The fungal counts were significantly lower than the bacterial counts ($p < 0.05$); and this is in agreement with the report from Aiyegoro *et al.* (2007). The relative increase in the fungal growth in the polluted soil experiments may be due to the acidic pH of the soil which ranged from 4.0-4.78 (Table 5). On the contrary, the relative low fungal diversity, observed in the control soil, could be attributed to the near neutral pH of 5.58-6.6 (Table 5). However, the differences in the soil pH values of the different months for the polluted soil were not observed to be statistically significant ($p < 0.05$). These bacteria may have acquired the genetic traits that enabled them to survive in such an acidic environment. The low pH of the soil could explain the presence of cyanogenic glycosides in the cassava effluent contaminated soil. Factors like low pH, high negative soil charges, and low clay content were reported as soil conditions that increase cyanide mobility (Fuller, 2004). In addition, the high organic carbon contents of the cassava effluent may have contributed to the proliferation of these aerobic microorganisms as reported by (Okwute and Isu, 2007; Nwaugo *et al.,* 2008). Top soil was indicated to harbor the richest microbial diversity because it contains a higher amount of organic matter and oxygen which decreases with depth (Maloney *et al.,* 1997). Higher bacterial counts of 3.3×10^8 CFU/g were observed in the control soil samples than those in the polluted soil in September from Ehor (Table 3). A similar trend was also observed in control soil samples from Oluku and Isihor in August (Table 4). In the present investigation, there was a drastic increase in the amount of rainfall in August and September, upon which there was a decline; this trend did not correlate generally with the total microbial counts. The soil was made up of sandy, silt and clay particles. The mean percentage of sandy particles of the polluted soil ranged from 86-89 % while that of the control ranged from 76-87 %. Similar results were observed by Uzoije *et al.* (2011). The percentage silt and clay contents were relatively low (Table 5). The organic carbon content ranged from 1.21-2.36 % for the polluted soil. The organic carbon content was higher in all the polluted soil experiments than in the control experiments. This increase could be a result of the high organic matter and organic carbon content of the cassava effluent. There were no significant differences in the amount of organic carbon among the various months ($p > 0.5$). This suggests that

the cassava effluents are perhaps of similar nutrient quality. However, the total organic carbon contents of the polluted soil were significantly higher than those from the control sites in all the studied areas ($p < 0.05$). There was a significant positive relationship between organic carbon and temperature ($p < 0.05$). The total nitrogen ranged from 0.31-0.44%. The total nitrogen recorded was probably due to nitrogen mineralization as a result of the degradation of organic matter. Electrical conductivity is used as a means of appraising soil salinity. The values recorded in the soil samples may be due to the increase in the concentration of soluble salts. With the exception of iron, the heavy metal levels for all sites were significantly higher than the levels observed in the control sites ($p > 0.05$). This implies that the soils receiving the effluent have some levels of heavy metal enrichments. The high concentration of heavy metals like zinc, copper, and manganese in the effluent contaminated soils could also be attributed to the wearing off or abrasion of the cassava milling machine parts and emission of the metals through the exhaust of the machine (Osakwe, 2010).

Intensive research is necessary to examine the impact of cassava effluents on soil microbiomes on non-cultural microorganisms using metagenomics approach to examine the bacterial and fungal strain diversity, the relationships between the microorganism and the ecosystem, phylogenetic relationships among the microorganism.

5. Conclusion

The results of the present study reveal that the cassava effluent has impacts on the microbial diversity of the receiving soil. This is indicated by the significant increase observed in the microbial density of the polluted soil and the simultaneous impact on the physicochemical parameters of the soil.

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