

Effects of cassava mill effluent (CME) on bacteria diversity of soil and aquatic environments in South-South Nigeria

Abstract

The effects of cassava effluent on the bacteria diversity of Nkissa River in South-south area of Nigeria and the adjoining soil were investigated. Results obtained in soil analysis showed changes in temperature (28.6-32.6 °C), pH (7.2-10.3) and toxicity of cassava (TOC) (24.2-41.3 mg/g). Highest values were obtained near the waste pit while control soil had the least values. Cyanogenic potential was highest near the pit. Total heterotrophic bacteria count ranged from 3.7×10^4 - 6.6×10^6 CFU/g. Phosphate solubilizing bacteria count ranged from 2.2×10^2 - 2.9×10^3 CFU/g. In all cases, highest values were obtained 100 m from the waste pit, followed by the control while the least was in the pit edge. The water analysis showed that dissolved oxygen (DO), biochemical oxygen demand (BOD), total suspended solids (TSS) and total dissolved solids (TDS) were adversely affected by the cassava effluent as values from the upstream were significantly ($p < 0.05$) lower than those from the discharge point (DP) to the downstream areas (DS I and DS II). The metallic ions were not significantly affected. The cyanogenic potentials of the water samples were quite low (1.03-0.42 mg/l). *Klebsiella Corynebacterium*, *Acinetobacter*, and *Morexalla* species which were absent from Upstream (US), were found from the Discharge/Fallout Point (DFP) to the Downstream (DS) samples. *Escherichia*, *Lactobacillus*, *Bacillus* and *Micrococcus* species were found in all the water samples analyzed. The cassava effluent utilization test showed that *Alcaligenes*, *Xanthomonas*, *Lactobacillus*, *Corynebacterium* and *Micrococcus* species are good metabolizers of the effluents indicated. However, *Escherichia* and *Enterobacter* species did not utilize the effluent at all. Results indicated adverse effects of the cassava mill effluent (CME) on soil parameters and water qualities which call for regulations on the disposal of CME to avoid environmental degradation.

Keywords: bacteria diversity, cassava effluent, environmental degradation, soil, water, mill effluent, sheep, cyanogenic glucoside, environmental conditions,

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Introduction

Cassava is a single species crop, (*Manihot esculenta*) though with several varieties. It is a dicotyledonous plant belonging to the botanical family *Euphorbiaceae*. It contains laticifers and produces latex.¹ The cassava plant is said to originate in Northeast Brazil with an additional Centre of origin in Central America, from these Centers, the crop spread to several parts of the world including Africa, Asia and America especially in the tropical zones.¹ There are several varieties and cultivars but generally cassava is said to be of two main varieties based on the characteristics and contents of Cyanogenic glycoside of its root/tubers. These are bitter and sweet; the bitter variety has its Cyanogenic glucoside distributed throughout the tuber and in high concentration while the sweet variety has low Cyanogenic glucoside, mainly in the peel of the tuber. The fresh/pulp of the sweet variety therefore has low Cyanogenic glucoside. However, the growing environmental conditions could influence the Cyanogenic glucoside concentrations of each variety.²

Cassava is grown in tropical lowland under warm, moist climate with a temperature range of 25-30 °C and rainfall of 100-150cm per year well distributed. It can however, grow under lower rainfall levels too but not doing very well. The best soil type for it is a light sandy loam and well- drained soil of medium fertility as high fertility

encourages more vegetative growth than tube formation. Cassava is a perennial plant, usually harvested within 12-18months naturally. Generally, cassava crop is cultivated for its tubers/roots, but cases of other parts of the plant being put into effective uses abound especially the leaves. Cassava leaves has been used as vegetable for human consumption in a few East African countries like Tanzania, Angola, and Malawi. The root tubers produced over 20-30% of the total harvest has been used in animal feed supplements. In animal feed, the leaves serve as forage material.¹ The leaves are harvested and feed to domestic animals like goats, sheep and cattle while the cassava tubers peels are served to pigs as feed supplements. Before utilization of the tuber it is inevitably peeled; about 0.5-2.0% of the tuber is the peel while the edible part is 80-90%. This edible fleshy tuber is composed of 60-70% water, 30-35% carbohydrate, 1-2% protein while fiber, fat and mineral matter make up the remaining. Two important factors that influence the use of cassava tuber are the high content of Cyanogenic glucosides and lack of good storage or keeping quality of the fresh tuber. This implies that the tubers will only be consumed after elaborate processing to reduce the Cyanogenic glucoside content and improve the keeping quality.⁴ Processing of cassava tubers for human consumption gives four types of products; these include the meal, flour, chips and starch production.^{5,6} The processing of these cassava tubers result in generation of several types of waste which include

not only the peel but the effluent. The effluent include the milky colloid pressed out of the fresh tuber paste, the latex, the wash water, etc. These wastes are automatically discharged into the surrounding environment causing pollution. The above mentioned pollution effects from cassava wastes becomes more pronounced as specific mills are now established to process cassava tuber to obtain garri and starch, the two forms in which the tubers are mainly consumed. In most cassava tuber preparations, the processing mills are established near water bodies or in free land spaces. The discharge of the cassava mill waste results in offensive odour emanating from the biodegraded products of the waste.^{7,8} In this case, the cassava mill effluent (CME) wastes affect the soil or water microbiological properties which in turn affect the general productivity of the particular ecosystem.⁹ Since cassava contains some minerals that affect the soil or water quality, assessment of the impacted habitat therefore involves the factors to obtain an appropriate view of the situation. Having established that human activities for economic, food or industrial objectives impact on the environment; the impacted environment should be assessed in order to determine the premeditative approach for a safer, greener and healthier habitat. Therefore, this study evaluates the effect of cassava mill effluent (CME) on bacteria diversity of soil and aquatic environments in South-South Nigeria.

Materials and methods

Study Area

The study area was Okwuzi, community of Egbema, River State Nigeria. It is a rainforest area with tropical climate. Egbema has vegetation characterized by tall trees and green grasses. The people are mainly fishermen and farmers with cassava and yam being the main crop grown along with palm trees and vegetables. Nkissa River empties into the Orashi River in Egbema; the river is an all season one. Two large cassava-processing plants were established in 2001 and 2002 on either sides of the river and processes between one and two (1-2) tones of cassava tubers daily.

Collection of samples

Soil samples were collected using shiprek soil auger disinfected with cotton wool soaked in 70% ethanol at 0-15cm depth. Four sampling areas of the mill were chosen, the areas sampled were the pit edge, 5m, 10m and 100m away while the sample from 250 m away served as the control. Sterile universal bottles were used to collect the soil samples for microbiological analysis. The biological indices-bacterial diversity was analyzed within 2-4hours of collection as described by Nwaugo et al.,¹⁰ Sampling of the Nkissa River was done at four different points, one was Upstream(US) which served as control, about 120 m before the Discharge/fallout point (DFP), and then two samples Downstream (DSI and DSII) Downstream I was 120m from the DFP, while the second Downstream II was 120m from DS I. At each sampling point, only the water column which was already disturbed due to the flow of the water was collected. Three water samples were collected at each sampling point and pooled together to form the sample for that particular point. Sampling was done ten (10) times at two weeks intervals for microbiological analysis. Nutrient agar, Mineral agar and MacConkey agar was used.

The physicochemical analysis

The temperature was determined by the use of mercury in bulb thermometer as described by Chessbrough.¹¹ The pH was determined

using Jenway Hanna 1910 multipurpose tester. The dissolved oxygen (DO) was measured with the (DO₂ meter Jenway, model 9071, USA). The biological oxygen demand (BOD) was determined using titrimetric method as described.¹² The total dissolved solids (TDS) and total suspended solids (TSS) were determined by the method of APHA.¹³ Total carbon content of the samples was determined according to the of Walky and Black¹⁴ while total nitrogen contents were determined by Kjeldahl method of Theroux and Eldrige.¹⁵ The cyanogenic potentials (Hydrogen Cyanide contents) of the samples were determined using redox titration (Iodimetry) as described by Olayiwola and Oladipo.¹⁶

Microbial analysis

Determination of bacteria load

Each of the soil samples was serially diluted on a ten-fold dilution. Exactly 0.1 ml from 10⁻⁶ dilution factor was transferred to various media, triptone soy agar, MacConkey agar, Tribututyryn agar, mineral salt agar and phosphate solubilizing medium for determination of total heterotrophic count, total coliform count, nitrifying bacteria and phosphate solubilizing bacteria respectively, using spread plate technique as described by Agwaranze et al. (2017).¹⁷ The plates were incubated at 37 °C for 28 hours. Discrete colonies were counted and determined as cfu/g. The same procedure was adopted for the water samples and reported as cfu/ml.

Identification of Isolates

The discrete colonies were sub-cultured on fresh nutrient agar medium and incubated at 37 °C for 24 hours to obtain pure cultures. The pure cultures obtained were characterized using microscopy, biochemical and sugar fermentation tests as described by Collins and Lyne and Cowan and Steel.^{18,19}

Statistical Analysis

All the analyses were conducted in triplicate and the mean data±SD (standard deviation) were reported.

Results

The result of the physicochemical analysis of the soil samples shows that temperature ranged from 28.6-32.6 °C. The pH values were within the basic range (7.2-10.3). Toxicity of Cassava was highest at the edge (41.3 mg/g) and lowest at the control (24.2 mg/g) while the cyanogenic potential ranged from 0.62 to 5.21 (Table 1). Table 2 presents the physicochemical parameters of the water samples. The result revealed that pH was highest at DSI (7.8) and lowest at DSII (6.7). Temperature ranged from 28.2 °C to 29.1°C while TDS and BOD ranged from 540 mg/L to 175 mg/l and 20 mg/L to 60 mg/L respectively. In the analysis of bacterial diversity, results obtained indicated that total heterotrophic bacteria (THB) have the highest counts in all the soil samples analyzed (Table 3). This group (THB), like all other bacterial groups, increased from 3.7×10⁴ cfu/g (pit edge soil) to 7.4×10⁶ cfu/g in the 100 m away soil. The 100 m away soil value of total heterotrophic bacteria was more than the control in soil. The lipolytic bacteria (LB) count ranged from 0.9×10¹ cfu/g to 2.4×10³ cfu/g, nitrifying bacteria (NB) from 0.4×10¹ cfu/g to 2.9×10³ cfu/g while phosphate solubilizing bacteria (PSB) ranged from 2.2×10² cfu/g to 2.4×10⁴ cfu/g. The bacteria load of the water samples show that the highest counts for THBC and CEUBC (6.7×10⁴ and 4.2×10⁴ respectively) were observed in DSI while the

highest count for CBC (2.4×10^2) was observed in DSII (Table 4). The least bacteria count was obtained in US for CBC (1.5×10^2), THBC (4.5×10^4) and CEUBC (2.1×10^2). The highest occurring grow was the THB, while the least was the coliform. The cassava effluent utilizing bacterial (CEUB) were more than the coliform bacteria (CB). Table 5 presents the bacteria diversity of soil contaminated with the cassava effluent and their prevalence on the various sampling points. The bacteria genera were *Escherichia*, *Enterobacter*, *Staphylococcus*, *Lactobacillus*, *Micrococcus* and *Bacillus*, *Alcaligenes*, *Klebsiella*, *Corynebacterium* and *Acinetobacter*. *Bacillus* species was 100%

in 5 m away and control, 80% and 70% in pit edge and 10 m away respectively. Also, *Corynebacterium*, *Morexella* and *Acinetobacter* species were not observed in both control and 10 m away while *Klebsiella* species was isolated in all samples except the control. The bacteria diversity of the water samples contaminated with the effluent shows that *Bacillus* species was 100% in DSI, 80%, 70% and 60% in DFP, DSII and US respectively (Table 6). The least isolated bacteria were the *Alcaligenes* in all the sampling points. *Corynebacterium*, *Morexella*, *Acinetobacter* and *Klebsiella* species were not isolated from the upstream (US).

Table 1 Physico-chemical characteristics of the soil contaminated with cassava effluent

Parameters	Sampling points				
	EDGE	5 M	10 M	100 M	CONTROL
pH	10.3 ± 0.10	10.1 ± 0.01	8.4 ± 0.20	7.6 ± 0.20	7.2 ± 0.02
TEMPERATURE	32.6 ± 0.02	30.1 ± 0.02	29.2 ± 0.02	28.7 ± 0.02	28.6 ± 0.02
TOC (mg/L)	41.3 ± 0.02	35.0 ± 0.00	30.8 ± 0.01	24.4 ± 0.01	24.2 ± 0.00
C/N Ratio	19.48 ± 0.12	15.30 ± 0.10	13.12 ± 0.21	8.01 ± 0.08	7.87 ± 0.06
CYNOGENIC POTENTIAL (mg/L)	5.21 ± 0.09	4.66 ± 0.14	3.22 ± 0.12	0.92 ± 0.06	0.62 ± 0.04

Values are mean of three independent experiments

Abbreviations: TOC, toxicity of cassava; C/N Ratio, carbon and nitrogen ratio

Table 2 Physico-chemical characteristics of the water contaminated with cassava effluent

Parameters	Sampling points			
	US	DFP	DSI	DSII
pH	6.8 ± 0.02	7.1 ± 0.01	7.8 ± 0.02	6.7 ± 0.02
TEMPERATURE	28.2 ± 0.20	28.9 ± 0.21	29.1 ± 0.09	28.8 ± 0.20
TDS (mg/L)	540 ± 0.00	1750 ± 0.02	1320 ± 0.06	920 ± 0.04
TSS (mg/L)	20 ± 0.00	60 ± 0.00	40 ± 0.00	31 ± 0.00
DO (mg/L)	3.0 ± 0.00	4.0 ± 0.00	25 ± 0.00	30 ± 0.01
BOD (mg/L)	20 ± 0.00	60 ± 0.00	50 ± 0.00	30 ± 0.00
CYNOGENIC POTENTIAL (mg/L)	ND	1.03 ± 0.04	0.42 ± 0.01	Traces

Values are mean of three independent experiments

Abbreviations: BOD, biological oxygen demand; DO, dissolved oxygen; TDS, total dissolved solids; TSS, total suspended solids; ND, not detected, US, upstream, DFP, discharge/fallout point; DS I, downstream one, DS II, downstream two.

Table 3 Bacterial load of soil Samples Contaminated with effluent

Group	Sampling points				
	Edge	5m	10m	100m	Control
THB	3.7×10^4	3.9×10^4	3.9×10^5	3.4×10^6	6.6×10^6
LB	0.9×10^1	0.9×10^1	1.7×10^2	2.4×10^3	2.2×10^3
NB	0.4×10^1	0.4×10^1	0.4×10^2	2.7×10^3	2.9×10^3
PSB	2.2×10^2	2.4×10^2	2.4×10^3	2.7×10^3	2.9×10^3

Abbreviations: THB, total heterotrophic bacteria; LB, lipolytic bacteria; NB, nitrifying bacteria, PSB, phosphate solubilizing bacteria

Table 4 Bacterial load of water Samples Contaminated with Effluent

Group	Sampling points			
	US	DFP	DS I	DS II
CBC	1.5×10^2	1.7×10^2	1.9×10^2	2.4×10^2
THBC	4.5×10^4	4.6×10^4	6.7×10^4	5.1×10^4
CEUBC	2.1×10^2	3.7×10^3	4.2×10^4	3.9×10^4

Abbreviations: BC, coliform bacterial count; THBC, total heterotrophic bacteria count; CEUBC, cassava effluent utilizing bacteria count; US, upstream, DFP, discharge/fallout point; DS I, downstream one, DS II, downstream two

Table 5 Relevance of Organisms Observed in Various Soil Samples Analyzed

Organisms	Sampling sites			
	Control	Pit edge	5m away	10m away
<i>Enterobacter</i> sp	4(40%)	2(20%)	4(40%)	2(20%)
<i>Escherichia</i> sp	2 (20%)	2(20%)	3(30%)	2(20%)
<i>Staphylococcus</i> sp	6(60%)	7(70%)	5(50%)	5(50%)
<i>Lactobacillus</i> sp	2(20%)	8(80%)	6(60%)	5(50%)
<i>Micrococcus</i> sp	2(20%)	5(50%)	7(70%)	7(70%)
<i>Bacillus</i> sp	10(100%)	6(60%)	10(100%)	5(50%)
<i>Alcaligenes</i> sp	1(10%)	8(80%)	5(50%)	4(40%)
<i>Klebsiclla</i> sp	-	4(40%)	4(40%)	5(50%)
<i>Corynebacterium</i> sp	-	5(50%)	6(60%)	-
<i>Morexella</i> sp	-	5(50%)	2(20%)	-
<i>Aemietobacter</i> sp	-	5(50%)	4(40%)	-

Table 6 Diversity of Bacteria from Effluent Contaminated Water

Organisms	Sampling sites			
	US	DFP	DS I	DS II
<i>Enterobacter</i> sp	5(50%)	2(20%)	3(30%)	2(20%)
<i>Escherichia</i> sp	4(40%)	2(20%)	4(40%)	2(20%)
<i>Staphylococcus</i> sp	4(40%)	6(60%)	7(70%)	5(50%)
<i>Lactobacillus</i> sp	2(20%)	5(50%)	7(70%)	5(50%)
<i>Micrococcus</i> sp	2(20%)	5(50%)	7(70%)	5(50%)
<i>Bacillus</i> sp	6(60%)	8(80%)	10(100%)	7(70%)
<i>Alcaligenes</i> sp	1(10%)	3(30%)	5(50%)	5(50%)
<i>Klebsiclla</i> sp	-	2(20%)	4(40%)	4(40%)
<i>Corynebacterium</i> sp	-	3(30%)	5(50%)	5(50%)
<i>Morexella</i> sp	-	2(20%)	2(20%)	2(20%)
<i>Aemietobacter</i> sp	-	2(20%)	4(40%)	2(20%)

Abbreviations: US, upstream; DFP, discharge/fallout point; DS I, downstream one; DS II, downstream two

Discussion

Results obtained in this study suggest adverse effects of the impact of cassava mill effluent on soil physicochemical and biological parameters. Analysis of these results showed that values obtained in pH and temperature was highest at the point nearest to the waste pits (edge of the waste pit). The same pit edge soil had highest organic carbon and cyanogenic potential. The pH of the control soil in the present study was 7.2 which are good for the growth of plants as nutrients are at optimum availability between pH of 6 and 7 in the soil. Akani et al.^{9,20,21} have reported similar situations in their studies. The TOC and low C/N ratio observed with increasing effects of the cassava mill effluent (CME) could be attributed to the contents of the effluent. CME is known to be high in organic carbon, which could then reduce the C/N ratio of impacted soil but increased the TOC. Shanhinroksar et al.^{22,23} agreed that soil impaction with organic matter results in decreased C/N ratio especially if the impacting material has low Nitrogen content. This agrees well with this study, CME has very low protein but high carbohydrate contents hence the observed low C/N

ratio. The high cyanogenic potentials observed near the waste pit agreed with the studies of Nwabueze and Odunsi.^{8,24} These researchers had reported values of 2.91-4.11 and 2.17-5.0 mg HCN kg from cassava peels and tuber pastes respectively. The high cynogenic potential had been attributed to the high cynogenic glucosides contained in cassava. The values obtained by Nwabueze and Odunsi.^{8,24} do not differ much from the results obtained in this study, especially as it was cassava effluent that was studied too. The high change in pH and temperature very close to the waste pit could be attributed to the high oxidative and reductive biochemical transformations taking place there. The breakdown of organic matter in the effluent was exothermic, which caused the increase in temperature while the metabolism of the little protein content released ammonia (NH₃). The ammonia dissolved in the available moisture to cause the reported increase in pH value. A similar observation had been reported by Chinyere²⁵ in similar studies on cassava effluent.

In biological indices analyzed, results showed that the bio-loads of all the bacterial groups increased with distance away from the waste pit suggesting adverse growth conditions towards the pit, this had been by Aderiye and Laleye^{6,7} Values obtained at 100m away were not statistically higher than those from control. This observation suggests that the high content of cassava mill effluent suppressed bacterial growth but at very low concentrations, this suppressed bacterial growth could be due to cumulative effects of some components of the cassava wastes which inhibited the bacterial growth.¹⁵ Karin²⁶ reported that organic matter when added to the soil in small concentrations encouraged bacterial growth. This observation agreed well with this study. Conditions and nutrients at 100m sampling point could have been optimum for bacterial growth, hence the results obtained. Several authors Nwaugo et al.,^{10,26-28} have stated that total heterotrophic bacterial counts are in all cases higher than the other specific bacterial groups in the soil. Similarly; Nwaugo et al.²¹ and Prescott et al.²⁹ reported that very slight change in environmental factors affect nitrifying bacteria adversely. THB were the most prevalent in all the soil samples analyzed, NB were the least, NB were the most adversely affected bacterial group in the study. Some bacterial species in the specialized group (LB, NB and PSB) may equally be found in the THB, making the THB more abundant than any other group.

In the water analysis of this work, twelve microbial species were isolated. Most of them had been reported earlier in similar work. Desse and Taye^{5,9} have observed some of them in fermenting tuber and vegetable. While some are natural saprophytes like *Corynebacterium*, *Bacillus*, *Micrococcus*, *Alcaligenes*, *Acinetobacterium* species, others like *Staphylococcus*, *Escherichia* and *Enterobacter* species could occur due to human activities. Some of the organisms in the water body could have come from the processing plants or effluent as some of them which were not in the US, were found in the DFP and DS samples. Some of the other organisms increased in prevalence in the presence of the cassava effluent indicating that the effluent was utilized as nutrient. This was the case of *Alcaligenes*, *Lactobacillus*, *Micrococcus* and *Bacillus* species. These organisms were those, which could utilize the effluent. Abiona et al.,^{3,4} stated that effluent from cassava was nutritive enough to support microbial growth, which agree with this work. Cassava effluent supplied both nutrients and organisms to the Nkissa River from the DFP, but was too harsh or in a state not very appropriate for immediate microbial utilization. A similar situation had been reported by.³⁰⁻³¹ Highest bio-load occurred in the DSI but decreased in DSII and in DFP was higher than US values. The THB was the highest, followed by CEUB before

CB, which was very low. This means that survival of the CB in the downstream portion of the river was due to the metabolism of the intermediates of the effluent. From the results, CB was not utilized of the cassava effluent. CEUB occurred in the US indicating that the cassava effluent degraders could be found anywhere without the presence of the effluent. However, the presence of the effluent ensured better adaptation to its metabolism hence the increase in number and prevalence.

Conclusion

This study therefore revealed adverse environmental effects of cassava mill effluent on soil biological parameters. Again, it also calls for serious rehabilitation, if the soil will be used for agricultural and other purposes as the factors important in soil health are negatively affected. The results obtained indicated that similar organisms were observed in each environment. Further observations in the study suggest the need for proper legislation against indiscriminate disposal of industrial wastes into our environment whether organic or inorganic, biodegradable or not.

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Conflict of interest

The author declares there is no conflict of interest.

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