



# The Influence of Cassava Mill Effluents on Soil Microbial Community in Akpabuyo, Cross River State, Nigeria

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## Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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## ABSTRACT

The influence of cassava mill effluent on the microbial community of the soil was determined using viable counts method. Soil samples were collected from different sites (Locations A, B, C and D) impacted with cassava mill effluent, and also from sites unimpacted with cassava mill effluent, which served as control. The result of analysis of the impacted soil showed that the counts of Total Heterotrophic Bacteria, Total Coliforms and Total Fungi ranged from  $2.0 \times 10^3$  CFU/ml to  $2.3 \times 10^4$  CFU/ml,  $1.9 \times 10^2$  CFU/ml to  $1.5 \times 10^4$  CFU/ml and  $1.4 \times 10^2$  CFU/ml to  $2.2 \times 10^2$  CFU/ml respectively. These values were lower compared to the values ( $3.4 \times 10^5$  CFU/g to  $3.6 \times 10^6$  CFU/g,  $2.1 \times 10^6$  CFU/g to  $3.3 \times 10^6$  CFU/g and  $2.1 \times 10^4$  CFU/g to  $3.0 \times 10^4$  CFU/g respectively) obtained from the unimpacted soils (control). The identification process of the isolates revealed the presence of the following bacteria; *Bacillus* spp., *Micrococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Salmonella* spp. The fungal isolates from the effluents were identified to be *Aspergillus* spp., *Rhizopus* spp. and *Mucor* spp. The results obtained indicates that effluents from cassava mill effluent have a great impact on the microbial population of the soil. Since its application causes change that affects the microbial population and diversity, its continuous disposal could lead to decreased soil fertility and consequent low crop yield. Therefore, cassava mill effluents should be subjected to treatments that reduces their harmful substance content before disposal.

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## 1. INTRODUCTION

“Cassava is a single species crop (*Manihot esculenta*) though with several varieties. It is a dicotyledonous plant belonging to the botanical family. *Euphorbiaceae*” [1]. “Cassava is a weedy perennial and branched shrub that can grow up to 5 meters in heights. It has large, spirally arranged, lobed leaves of very variable forms. During growth, the shrubs produce several tuberous roots as reserves made of up to 35% starch which may reach up to 1m in length and together may weigh up to 40kg. Cassava produces small regular female and male flowers in small clusters. The shrub produces a form of non-fleshy fruit capsule” [2]. “Cassava thrives in tropical and subtropical regions of the world as it requires warm temperatures for optimal growth. The plants require at least 8 months of warm weather, thriving in regions with warm, moist climates with regular rainfall. Cassava can be grown in many types of soil, producing even in poor soil but will be optimally productive in well-draining, sandy, clay loamy with a pH between 5:6 and 6:5” [3].

“Although cassava is a perennial crop, the storage roots can be harvested from 6 to 24 months after planting (MAP) on cultivar and the growing conditions. In the humid low land and tropics, the root can be harvested after 6-7 months. In regions with prolonged periods of drought or cold, the farmers usually harvest after 18-24 months. Moreover, the roots can be left in the ground without harvesting for a long period of time, making it a very useful crop as a security against famine. Basically, cassava tuber contains about 70% water” [4]. “During cassava processing into garri, several by-products are derived including cassava peelings, cassava mill effluents (CME), sieves, air emission, high quality cassava flour” [5]. “Traditionally, garri production is associated with the discharge of large amounts of water, hydrocyanic acid and organic matter in the form of peels and sieves from the pulp as waste products. When these waste products are improperly disposed, they are left in mounds which generate offensive odours and unsightly scenarios” [6].

According to Akani et al. [2], “the deleterious effects of cassava mill effluent on soil can be traced to the high levels of cyanogenic glucosides, biochemical oxygen demand and soluble carbohydrates and proteins in the

effluent. Cyanide released from the cassava effluents are highly lethal, it is fairly mobile in the soil and destroy microbes”. In Nigeria, these byproducts (mainly solid and liquid wastes) are discharged into the ecosystem without treatment. Elijah et al. [7] opined that “wastewater of cassava processing units could pose more intense problem in near future probably due to lack of effluent treatment facility, as effort of Nigerian Government is ongoing to boost cassava-based products”. “These wastes stream could lead to environmental impacts especially on soil fertility, water and air quality” [8,9]. Ezeogo et al. [10] reported that “cassava mill effluent has noticeable effect on the physicochemical and microbiological properties of soil”.

“The solid wastes are consumed by domestic animals such as goat in some part of Nigeria. The liquid wastes are also consumed by domestic animals such as goat, but instances of toxicity leading to death of flora and fauna have been reported in literatures” [2,11]. “Furthermore, CME contaminates agricultural farmland, surface water (creek, river, stream, pond etc) and percolates into sub-soil and groundwater resource” [11]. “The discharge of effluents, sludge, and biosolid from food processing such as cassava on the land has been an age long practice” [12]. Sackey and Bani [13], have reported instances of CME flowing into vegetation, abandoned into living communities. This work therefore was aimed at assessing the impact of cassava mill effluents on soil bacteriological indices of soil quality. It will ascertain the impact of the cassava mill effluents on the microbial community of soils around it.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Samples

The cassava mill effluent samples were collected aseptically from Akpabuyo, Cross River State, Nigeria, in a sterile container and were transported to the Microbiology laboratory, Cross River University of Technology, Calabar, for further analysis.

### 2.2 Microbiological Analysis

#### 2.2.1 Enumeration of total heterotrophic bacterial count

Total heterotrophic bacterial count of the sample was determined using the pour plate method as

described by Cheesbrough [14]. Serial dilution was prepared from the liquid sample. Exactly one millimeter (1ml) was taken from each selected dilution ( $10^4$ ,  $10^5$ ,  $10^6$ ) into sterile petri dishes. The molten sterilized Nutrient and MacConkey agar were poured into the plates, swirled to spread the inoculum evenly within the agar medium and allowed to solidify then incubated at  $37^{\circ}\text{C}$  for 24 hours. Thereafter, plates with colony growth were counted and recorded.

## 2.2.2 Determination of total fungi count

The spread plate method was used for the determination of total fungi count following standard procedures. One-millimeter (1ml) of diluents from the samples were aseptically transferred into already sterilized solidified Sabouraud Dextrose Agar (SDA) plates, swirled and then incubated at ambient temperature for 3-4 days. Colony growth was counted and expressed as colony forming unit per millimeter.

## 2.2.3 Biochemical characterization and identification of bacterial isolates

All bacterial isolates were characterized and identified based on their cultural, morphological, microscopic and biochemical features following the methods described by Cheesbrough [14]. Biochemical test conducted includes Gram staining, triple sugar iron, indole, methyl red, Simon citrate utilization, catalase, oxidase Voges proskauer, coagulase, and sugar fermentation.

## 2.4 Identification of Fungal Isolates

The colonial morphologies of the fungal isolates on Sabouraud Dextrose Agar were observed for colour and type of growth. Microscopic identification as described by Murray et al. [15]. This was done by preparing wet mount using lactophenol cotton blue to observe the microscopic characteristics of the fungi such as type of hyphae (whether septate or non-septate). A drop of Lactophenol blue was placed on a clean microscope slide, with the aid of an inoculating needle, a small portion of growth

midway between the colony center and edge was gently removed and placed in the dropped Lactophenol blue on the slide. With two sterile dissecting needles, the fungus was gently teased apart so that it is thinly spread out in the Lactophenol. After which, a coverslip was placed on the edge of the Lactophenol and slowly lowered. Then the slide was placed under the microscope for examination. Both microscopic and macroscopic features of the fungal isolates were matched based on the mycological atlas for fungal identification.

## 3. RESULTS

The enumeration of the total heterotrophic bacteria of the samples from different locations revealed that the cassava mill effluent has a negative effect on the soil microbiota, as the soil samples without cassava mill effluent ( $3.4 \times 10^5$  CFU/ml –  $3.6 \times 10^6$  CFU/ml) yielded higher bacterial count when compared to soil samples impacted with cassava mill effluent ( $2.0 \times 10^3$  CFU/ml –  $2.3 \times 10^4$  CFU/ml). This is shown in Table 1.

The total coliform counts of the samples showed that the soil samples impacted with cassava mill effluent yielded low coliform count ( $2.0 \times 10^2$  CFU/ml –  $1.5 \times 10^4$  CFU/ml) when compared to the control, soil without cassava mill effluent ( $2.1 \times 10^6$  CFU/ml –  $3.3 \times 10^6$  CFU/ml) as displayed on Table 2.

The result of the fungal screening revealed that the cassava mill effluent had a negative impact on fungal loads as the soil without the effluent had higher fungal load compared to the soil samples impacted with the effluent, as showed in Table 3.

Biochemical characterization and identification processes revealed that *Salmonella* species, *Micrococcus* species, *Bacillus* species, *Staphylococcus* species, *Escherichia coli*, *Klebsiella* species etc (Table 4). Were the suspected bacterial isolates observed in the samples.

**Table 1. Total heterotrophic bacterial (THB) count of samples with cassava mills effluent and samples without effluent (control)**

Location	Samples	THB count (CFU/g) (with effluent)	THB count (CFU/g) (without effluent)
A	SA1	$2.2 \times 10^3$	$3.6 \times 10^6$
B	SA2	$2.0 \times 10^3$	$3.4 \times 10^5$
C	SA3	$2.3 \times 10^4$	$3.2 \times 10^6$
D	SA4	$2.8 \times 10^3$	$3.5 \times 10^6$

**Table 2. Total coliform (TC) count of samples with cassava mill effluent and samples without cassava mill effluent (Control)**

Location	Samples	TC count (CFU/g) (with effluent)	TC count (CFU/g) (without effluent)
A	SA1	$1.9 \times 10^2$	$2.4 \times 10^6$
B	SA2	$2.6 \times 10^2$	$3.3 \times 10^6$
C	SA3	$2.0 \times 10^2$	$2.5 \times 10^6$
D	SA4	$1.5 \times 10^4$	$2.1 \times 10^6$

**Table 3. Total fungal (TF) count of samples with cassava mills effluent and samples without cassava mill effluent (Control)**

Location	Samples	TF count (CFU/g) (with effluent)	TF count (CFU/g) (without effluent)
A	SA1	$1.6 \times 10^2$	$2.4 \times 10^4$
B	SA2	$2.0 \times 10^2$	$3.0 \times 10^4$
C	SA3	$1.4 \times 10^2$	$2.5 \times 10^4$
D	SA4	$2.2 \times 10^2$	$2.1 \times 10^4$

**Table 4. Cultural, morphological and biochemical characteristics of bacterial Isolates**

SN	Isolates Code	Colour/ Appearance	Gram RXN	Stain		Catalase	Oxidase	Coagulase	Methyl red	Indole	oges prokauer	Citrate	Glucose	Sucrose	Lactose	T	S	I	Suspected organisms
				Shape	Arrangement											Slat	Butt	H <sub>2</sub> S	
1.	SA2 <sub>III</sub>	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	+	+	A	A	-	<i>Bacillus</i> spp.	
2.	SA1 <sub>II</sub>	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus</i> spp.	
3.	SA1 <sub>III</sub>	Irregular Raised milky	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus</i> spp.	
4.	SA1 <sub>IV</sub>	Irregular milky flat	-	Rod	Pairs	+	+	-	+	-	-	-	+	+	A	A	-	<i>Escherichia coli</i>	
5.	SA1 <sub>V</sub>	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	+	+	A	A	-	<i>Bacillus</i> spp.	
6.	SA2 <sub>I</sub>	Irregular milky flat	+	Rod	Pairs	+	+	-	+	-	-	-	+	+	A	A	-	<i>Escherichia coli</i>	
7.	SA2 <sub>II</sub>	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus</i> spp.	
8.	SA2 <sub>III</sub>	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	+	+	A	A	-	<i>Bacillus</i> spp.	
9.	SA2 <sub>IV</sub>	Irregular Raised Milky	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus</i> spp.	
10.	SA3 <sub>I</sub>	Round red raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	A/G	A/G	-	<i>Klebsiella</i> spp.	
11.	SA3 <sub>II</sub>	Circular white convex	+	Cocci	Cluster	+	-	-	-	-	-	-	+	+	A	A	-	<i>Lactobacillus</i> spp.	

SN	Isolates Code	Colour/ Appearance	Gram RXN	Stain		Catalase	Oxidase	Coagulase	Methyl red	Indole	oges prokauer	Citrate	Glucose	Sucrose	Lactose	T	S	I	Suspected organisms
				Shape	Arrangement											Slat	Butt	H <sub>2</sub> S	
12.	SA3 <sub>III</sub>	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	+	+	A	A	-	<i>Bacillus</i> spp.	
13.	SA3 <sub>IV</sub>	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	+	+	A	A	-	<i>Bacillus</i> spp.	
14.	SA3 <sub>V</sub>	Round red raised	-	Rod	Cluster	+	+	+	+	-	-	+	+	+	A/G	A/G	-	<i>Klebsiella</i> spp.	
15.	SA4 <sub>III</sub>	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus</i> spp.	
16.	SA2 <sub>III</sub>	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	+	+	A	A	-	<i>Bacillus</i> spp.	
17.	SA4 <sub>I</sub>	Milky irregular edge	+	Rod	Singles	+	-	-	-	-	-	+	+	+	A	A	-	<i>Bacillus</i> spp.	
18.	SA4 <sub>II</sub>	Pink round irregular edges	-	Rod	Clusters	+	-	-	+	-	+	+	+	-	A	A	+	<i>Salmonella</i> spp.	
19.	SA4 <sub>III</sub>	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus</i> spp.	
20.	SA4 <sub>IV</sub>	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus</i> spp.	
21.	SA4 <sub>V</sub>	Circular yellowish convex	+	cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus</i> spp.	
22.	SA1 <sub>II</sub>	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus</i> spp.	
23.	SA3 <sub>I</sub>	Round red raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	A/G	A/G	-	<i>Klebsiella</i> spp.	
24.	SA4 <sub>III</sub>	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus</i> spp.	
25.	SA4 <sub>III</sub>	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus</i> spp.	
26.	SA3 <sub>I</sub>	Round red raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	A/G	A/G	-	<i>Klebsiella</i> spp.	
27.	SA2 <sub>I</sub>	Irregular milky flat	+	Rod	Pairs	+	+	-	+	-	-	-	+	+	A	A	-	<i>Escherichia coli</i>	
28.	SA2 <sub>I</sub>	Irregular milky flat	+	Rod	Pairs	+	+	-	+	-	-	-	+	+	A	A	-	<i>Escherichia coli</i>	
29.	SA4 <sub>IV</sub>	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus</i> spp.	
30.	SA4 <sub>V</sub>	Circular yellowish convex	+	cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus</i> spp.	

**Table 5. Fungal identification**

Samples	Macroscopic features	Microscopic features	Suspected organisms
SA1 <sub>i</sub>	White – budding cells	Non septate	<i>Saccharomyces</i> spp.
SA2	White with yellow to green surface	Septate	<i>Aspergillus</i> spp.
SA3	White to dark colour	Non-septate	<i>Mucor</i> spp.
SA1 <sub>ii</sub>	Gray – coloured colonies	Septate	<i>Fusarium</i> spp.
SA4	White with yellow submerged pigment	Septate	<i>Aspergillus</i> spp.
SA1	Gray – coloured colonies	Septate	<i>Fusarium</i> spp.
SA3	White with yellow to green surface	Septate	<i>Aspergillus</i> spp.
SA4	White to dark colour	Non-septate	<i>Mucor</i> spp.

The result of the total fungal screening showed that *Aspergillus* species, *Saccharomyces* species, *Mucor* species, *Rhizopus* species and *Fusarium* species (Table 5). Were the fungal isolates obtained.

#### 4. DISCUSSION

The results obtained showed that the microbial population of the soil samples that were not impacted with cassava mill effluent had higher microbial load when compared to the soil samples impacted with cassava mill effluents. This could be attributed to the harmful effect of cyanide acid content of cassava mill effluent on the soil microbiota. This corroborates the report of Adamu et al. [16], which stated that the decrease in microbial load is as a result of effluent disposal at the milling sites, which resulted in death of many microorganisms due to high cyanide concentration. In a related study, Clems [17], reported that the presence of cyanide acid in the soil and fermented cassava could lead to the inhibition of microbial growth as it increases the soil pH which makes the soil usually very acidic. This is in agreement with the findings of Adoni et al. [18], who further reported the deleterious effect of CME on soil structure, soil microorganisms and soil characteristics compared to control soil samples. The lower level of microbial load observed from the soil with cassava effluents could also be traced to the fact that constant introduction of cassava mill effluents into soil could lead to high level of heavy metals with iron having the highest concentration. This agrees with the findings of Uzochukwu et al. [19]. It also corroborates the findings of Agbo et al. [20], who reported that increase in heavy metals could be traced to the use of wastewater for irrigation and probably due to extensive use of organic fertilizer and/or solid manure. The study further revealed that CME effect on the physicochemical determinants (pH, Ca, Mg, K) and heavy metals determinants (Fe, Zn, Co, Ni, Pb and Mn) was concentration determinant.

The organisms isolated from this research work includes *Klebsiella* species, *Escherichia coli*, *Bacillus* species, *Lactobacillus* species, *Micrococcus* species, *Staphylococcus* species, *Aspergillus* species, *Fusarium* species, *Mucor* species and *Saccharomyces* species. This observation is in agreement with the reports of Adamu et al. [16], who opined that the microorganisms isolated from cassava mill effluent impacted site are *Staphylococcus aureus*, *Lactobacillus planetarium*, *Bacillus sustillis*, *Fusarium solani*, *Aspergillus nigger*, *Saccharomyces cerivasa*, *Klebsiella aergenes* and *Campylobacter pylori*. Ojorbor et al. [21], Adoni [18] and Ezeogo et al. [10] in separate research studies, identified similar microorganisms from cassava mill effluent receiving soil. The presence of *Lactobacillus* species in this study could be attributed to the fact that it has the ability to thrive between the pH gradient of inside cell and outside cell in the presence of large amount of acetate and lactate. Also, they are known to thrive in acidic environment (< 4.5). *Staphylococcus* isolation could be traced to the fact that *Staphylococcus* species are normal flora of the skin, nose and could find its way during processing procedure of the cassava and sample collection. This agrees with the work of Boucher and Corey [22]. The presence of *Micrococcus* spp. in this study could be because they are normal flora of the skin and also present in places like soil, water, dust and even animal skin. This is in tandem with the works of Gang et al. [23]. The presence of *E. coli* and *Klebsiella* species observed could be as a result of poor handling of cassava during preparations and *E. coli* do not need much nutritional requirement to survive in non-aquatic environment. The presence of fungi like *Saccharomyces* species in this work indicates that it partakes in cassava fermentation processes. *Fusarium* species are also present because they are soil borne organism.

The organisms found on the soil with cassava effluent were able to survive because they could

withstand high acidic conditions, thus the lower population of fungal species. The pH level of the soil determined the availability of nutrients and therefore affects the potency and physical property of the soil [24,25]. From this study, the results have clearly proven that cassava effluent has a great impact on the microbial population of the soil, hence can be detrimental to crop yield. Therefore, cassava effluent should not be disposed in farm lands, but instead disposal sites should be mapped out, the effluents treated before disposal. This result implies that cassava mill effluents have inhibitory properties as it poses great threat to soil microorganisms which in turns affect soil fertility and crop yields.

## 5. CONCLUSION

This study revealed that effluents from cassava mills have a negative impact on the microbial community of the soil. Its application causes change that affects the microbial population and diversity. The continuous disposal of these effluents could lead to decreased soil fertility and consequent low crop yield. Therefore, there is need for cassava mill effluents to be subjected to treatments that reduces its harmful substances before their disposal.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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