Original Research Article

Determination of the Influence of Cassava Mill Effluents on Soil Microbial Community

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 $2.0x10^3$ CFU/ml to $2.3x10^4$ CFU/ml, $1.9x10^2$ CFU/ml to $1.5x10^4$ CFU/ml and $1.4x10^2$ CFU/ml to $2.2x10^2$ CFU/ml respectively. These values were lower compared to the values ($3.4x10^5$ CFU/g to $3.6x10^6$ CFU/g, $2.1x10^6$ CFU/g to $3.3x10^6$ CFU/g and $2.1x10^4$ CFU/g to $3.0x10^4$ 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.

Key words: Cassava mill effluents, total heterotrophic bacteria, total coliform, total fungi, impacted and unimpacted soil.

INTRODUCTION

Cassava is a single species crop (*Manihot esculenta*) though with several varieties. It is a dicotyledonous plant belonging to the botanical family. *Euphorbiacea* [15]. 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 [5]. 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 [12].

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 (Alves, 2010). Basically, cassava tuber contains about 70% water (Knipscheer *et al*., 2007). During cassava processing into garri, several by-products are derived including cassava peelings, cassava mill effluents (CME), sieves, air emission, high quality cassava flour (Ohimain, 2014).

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 (FAO, 2004).

According to Akani *et al.* (2006), 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*. (2014), 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. 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 (Akani *et al.,* 2006; Ezeigbo *et al*., 2014). Furthermore, CME contaminates agricultural farmland, surface water (creek, river, stream, pond etc) and percolates into sub-soil and groundwater resource (Ezeigbo *et al*., 2014). The discharge of effluents, sludge, and biosolid from food processing such as cassava on the land has been an age long practice (Oviasogie and Ndikwere, 2008). Sackey and Bani (2007), 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.

MATERIALS AND METHODS

Collection of samples

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

Microbiological Analysis

Enumeration of Total Heterotrophic Bacterial Count

Total heterotrophic bacterial count of the sample was determined using the pour plate method as described by Cheesbrough (2002). 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 37^0C for 24 hours. Thereafter, plates with colony growth were counted and recorded.

Determination of total fungi count

The spread plate method was used for the determination of total fungi count following standard procedures. One-millimeter (Iml) 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.

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. (2002). Biochemical test conducted includes Gram staining, triple sugar iron, indole, methyl red, Simon citrate utilization, catalase, oxidase Voges proskauer, coagulase, and sugar fermentation.

Identification of fungal isolates

The colonial morphologies of the fungal isolates on Saboraud Dextrose Agar were observed for colour and type of growth. Microscopic identification as described by Murray *et al*. (2003). 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 fugal isolates were matched based on the mycological atlas for fungal identification.

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 ($3.4x10^5$ CFU/ml – $3.6x10^6$ CFU/ml) yielded higher bacterial count when compared to soil samples impacted with cassava mill effluent ($2.0x10^3$CFU/ml – $2.3x10^4$CFU/ml). This is shown in Table 1.

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	2.2×10^3	3.6×10^6
B	SA2	2.0×10^3	3.4×10^5
C	SA3	2.3×10^4	3.2×10^6
D	SA4	2.8×10^3	3.5×10^6

The total coliform counts of the samples showed that the soil samples impacted with cassava mill effluent yielded low coliform count ($2.0x10^2$ CFU/ml – $1.5 x10^4$ CFU/ml) when compared to the control, soil without cassava mill effluent ($2.1x10^6$ CFU/ml – $3.3x10^6$ CFU/ml) as displayed on Table 2.

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)	TC count (CFU/g)

		(with effluent)	(without effluent)
A	SA1	1.9×10^2	2.4×10^6
B	SA2	2.6×10^2	3.3×10^6
C	SA3	2.0×10^2	2.5×10^6
D	SA4	1.5×10^4	2.1×10^6

The result of the fugal screening revealed that the cassava mill effluent had a negative impact on fugal 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.

Table 3. Total Fungal (TF) Count of Samples with cassava mill effluent and samples without cassava mill effluent (Control)

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

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 4: Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates

SN	Isolates Code	Colour/ Appearance	Gram Stain RXN	Shape	Arrangement	Catalase	Oxidase	Coagulase	Methyl red	Indole	Voges prokauer	Citrate	Glucose	Sucrose	Lactose	Slat	But	H_2S	Suspected Organisms
1.	$SA2_{III}$	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	-	+	+	A	A	-	*Bacillus spp*
2.	$SA1_{II}$	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	*Staphylococcus spp*
3.	$SA1_{III}$	Irregular Raised milky	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	+	A	A	+	*Micrococcus spp*
4.	$SA1_{IV}$	Irregular milky flat	-	Rod	Pairs	+	+	-	+	-	-	-	+	+	+	A	A	-	*Escherichia coli*
5.	$SA1_{V}$	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	-	+	+	A	A	-	*Bacillus spp*
6.	$SA2_{I}$	Irregular milky flat	+	Rod	Pairs	+	+	-	+	-	-	-	+	+	+	A	A	-	*Escherichia coli*
7.	$SA2_{II}$	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	*Staphylococcus spp*
8.	$SA2_{III}$	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	-	+	+	A	A	-	*Bacillus spp*
9.	$SA2_{IV}$	Irregular Milky Raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	+	A	A	+	*Micrococcus spp*
10.	$SA3_{I}$	Round Red Raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	+	A/G	A/G	-	*Klebsiella spp*
11.	$SA3_{II}$	Circular white convex	+	Cocci	Cluster	+	-	-	-	-	-	-	+	+	-	A	A	-	*Lactobacillus spp*
12.	$SA3_{III}$	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	-	+	+	A	A	-	*Bacillus spp*
13.	$SA3_{IV}$	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	-	+	+	A	A	-	*Bacillus spp*
14.	$SA3_{V}$	Round red raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	+	A/G	A/G	-	*Klebsiella spp*
15.	$SA4_{III}$	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	+	A	A	+	*Micrococcus spp*

16.	$SA2_{III}$	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	-	+	+	A	A	-	*Bacillus spp*
17.	$SA4_{I}$	Milky irregular edge	+	Rod	Singles	+	-	-	-	-	-	+	+	+	+	A	A	-	*Bacillus spp*
18.	$SA4_{II}$	Pink round irregular edges	-	Rod	Clusters	+	-	-	+	-	+	+	+	+	-	A	A	+	*Salmonella spp*
19.	$SA4_{III}$	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	+	A	A	+	*Micrococcus spp*
20.	$SA4_{IV}$	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	*Staphylococcus spp*
21.	$SA4_{V}$	Circular yellowish convex	+	cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	*Staphylococcus spp*
22.	$SA1_{II}$	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	*Staphylococcus spp*
23.	$SA3_{I}$	Round Red Raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	+	A/G	A/G	-	*Klebsiella spp*
24.	$SA4_{III}$	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	+	A	A	+	*Micrococcus spp*
25.	$SA4_{III}$	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	+	A	A	+	*Micrococcus spp*
26.	$SA3_{I}$	Round Red Raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	+	A/G	A/G	-	*Klebsiella spp*
27.	$SA2_{I}$	Irregular milky flat	+	Rod	Pairs	+	+	-	+	-	-	-	+	+	+	A	A	-	*Escherichia coli*
28.	$SA2_{I}$	Irregular milky flat	+	Rod	Pairs	+	+	-	+	-	-	-	+	+	+	A	A	-	*Escherichia coli*
29.	$SA4_{IV}$	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	*Staphylococcus spp*
30.	$SA4_{V}$	Circular yellowish convex	+	cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	*Staphylococcus 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.

Table 5: Fungal identification

Samples	Macroscopic features	Microscopic features	Suspected organisms
$SA1_I$	White – budding cells	Non septate	*Saccharomyces spp*
SA2	White with yellow to green surface	Septate	*Aspergillus spp*
SA3	White to dark colour	Non-septate	*Mucor spp*
$SA1_{II}$	Gray – coloured colonies	Septate	*Fusarium spp*
SA4	White with yellow submerged pigment	Septate	*Aspergillus spp*
SA1	Gray – coloured colonies	Septate	*Fusarium spp*
SA3	White with yellow to green surface	Septate	*Aspergillus spp*
SA4	White to dark colour	Non-septate	*Mucor spp*

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 biota. This corroborates the report of Adamu *et al.* (2020), which stated that the decrease in microorganisms 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 (2015), 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. 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*., (2001).

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.* (2020), who opined that the microorganisms isolated from casava mill effluent impacted site are *Staphylococcus aureus, Lactobacillus planetarium, Bacillus sustillis, Fusarium solani, Aspergillus nigger, Saccharomyces cerivasae, Klebsiella aergenes and Campylobacter pylori.* 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) (Linhares *et al.,* 2010). *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, (2008). 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*. (2002). 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 (Osakwe, 2012). 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.

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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