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Assessment of Microbial Quality of Cassava Mill Effluents Contaminated Soil in a Rural Community in the Niger Delta, Nigeria

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Abstract

This study investigated the effect of cassava mill effluents on receiving soil microbial quality from smallholder cassava processors in the Niger Delta region of Nigeria. Triplicate soil samples were collected at 0 - 20 cm depth using soil auger. The samples were analyzed following standard microbiological protocol. Results of the microbial density in the cassava mill effluents contaminated soil ranged from 0.060 - 1.16 x 10^6 cfu/g (total heterotrophic bacteria), 0.35 - 6.60 x 10^3 cfu/g (coliform counts) and 0.64 - 5.96 x 10^3 cfu/g (total fungi). Analysis of variance revealed that there was significant variation ($P < 0.05$) among the various cassava mill effluents contaminated soil locations apart from total fungi. Furthermore, the control sample depicted significant higher density compared to the contaminated locations. The microbial diversity include *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Bacillus, Micrococcus, Proteus, Enterobacter, Aspergillus, Penicillium, Mucor, Rhizopus* species and *Saccharomyces cerevisiae.* The similarity interaction between locations with regard to the microbial diversity ranged from 50.00 - 92.30%. The similarity index is above critical level of significance ≥ 50% for similarity. This inferred that microorganisms found in the various locations of study are significantly similar. Hence, there is the need to manage cassava mill effluents in a sustainable manner so as to avoid its impact on the soil microflora.

Keywords: *Cassava Processing; Effluents; Environmental Impacts; Soil Microflora*

Introduction

Cassava is one of the major staple food in Nigeria. Cassava is propagated by stem. Cassava matures between 6 - 12 months of planting depending on the variety. But in some communities that cassava cultivation is a major source of livelihood they can still be left in the plantation for 1 - 2 years after planting. These usually take place where the farmers have additional source of livelihood.

During cassava processing into products such as gari, three major wastes streams are generated including liquid (cassava mill effluents), solid (cassava peels and seivates) and gaseous emissions (associated pollutant gases generated during frying and other emission from processing environment) [1].

Quality of cassava mill effluents is assessed based on their physical, chemical and biological constituents (including microbiology). Several studies have been carried on with regard to physical and chemical composition of cassava mill effluents as wells as its contaminated soil (point of discharge) in different processing facilities predominantly among smallholders [2-4]. Small scale cassava processors dominated the enterprise especially in local area, where they are used to produce gari, lafun, etc.

Cassava mill effluents are known to be toxic to the environment and its associated biota [5]. For instance, cassava mill effluent without additives such as palm oil are known to induce toxicity of domestic animals such as goat, sheep [6], decrease plant productivity [7,8] and even prevent it from germination/growing [6]. In addition, instances, of cassava mill effluents altering the physical, chemical and microbial characteristics of the receiving environment have been widely reported by authors [2,6,7,9-17].

Environmental risks associated with cassava mill effluents on the receiving soil have been documented. For instance, Izah., et al. [4] used nine pollution indices to show the effect of cassava mill effluents in the environment. Izah., *et al*. [3] have also assessed geoaccumulation factor, enrichment factor and quantification of contamination in soil receiving cassava mill effluent from a rural community in the Niger Delta region of Nigeria.

Studies on possible treatment of cassava mill effluents and possible utilization for animal feed have been carried out. For instance, *Saccharomyces cerevisiae* can be used for the improvement of some physicochemical [18], cyanide content [19] and heavy metals characteristics of cassava mill effluents [20]. Comparison on the heavy metals and cation composition of *S. cerevisiae* biomass cultured in cassava mill effluents for animal feed utilization have been reported by Izah., *et al* [19,20].

Cassava mill effluents often lead to strongly objectionable odor in ambient air. Odor from the cassava processing environment typically emanates from the decomposition/degradation of effluents through the mineralization of its nutrient constituents. Instances of odor resulting from cassava mill effluents being perceived from several meters away from the point of discharge have been reported by authors [6,21].

Microorganisms have been widely described as ubiquitous organisms. This could be due to their ability to survive in different environment depending on the species, physiological and adaption mechanisms it possesses. Studies have been widely carried out with regard to microbiological studies of cassava mill effluents contaminated soil. Some of this studies were carried out in Amanuke, Awka-North Local Government Area of Anambra State [10], Akaeze, in Ebonyi State [22], Oluku, Isihor and Ehor in Edo South region of Edo State [15], Abagboro, Osun state [23], Aba, Abia state [13], Elele, Rivers state [24], Mmahu in Ohaji/Egbema Local Government Area in Imo State [9], Ovia North East Local Government Area of Edo State [6]. But information about the effect on Ndemili, a rural community in Ndokwa West local government area of Delta state is deficient in literature, hence, the need for this study.

Materials and Methods

Study Area

Ndemili is a community in Ndokwa west local government area of Delta state. Ndemili is located in latitude N06º01' and longitude E006º17'. Farming is a major source of livelihood to resident of the area [2-5,18-20]. Cassava, yam, maize, oil palm etc are some of the notable farm produce cultivated in the area [2-5,18-20]. Cassava processing stages for gari production involves manual peeling of the tuber and washing, motorized grating using liter power engine, hydraulic/thread-like dewatering process and manual frying using fuel wood. The cassava mill effluents generated are discharged into the environment without treatment (Figure 1). Like other Niger Delta region, Ndemili is characterized by two distinct seasons namely; wet and dry seasons. The relative humidity and temperature of the area is 50 - 95% and 28 ± 8ºC respectively all year round [2-5,18-20].

Figure 1: *Cassava mill effluents contaminated soil from smallholder cassava processors in a rural community in the Niger Delta region of Nigeria.*

Field Sampling

Soil samples were collected in triplicate across six disturbed and one undisturbed soil locations. The samples were collected in the dry season month of March 2017 using soil auger. The soil samples were wrapped in sterile aluminum foil packs and labeled appropriately before being transported to the laboratory.

Microbiological Analysis

Enumeration of bacterial and fungal counts

Two bacteria groups were enumerated using two different media viz: nutrient agar and MacConkey Agar. Nutrient agar was used to enumerate obligate and facultative bacteria, while MacConkey Agar was used to enumerate coliforms. Furthermore, fungi (mould and yeast) growth was enumerated using potatoes dextrose agar.

Pour plate method by Benson [25] and Pepper and Gerba [26] were used for the microbial enumeration. An aliquot of serially diluted soil samples were pipetted into sterile petri dishes (appropriately labeled) and prepared media was poured and stirred to both clockwise and anti-clock wise directions to achieve homogenous distribution. The plates were allowed to solidify plated in the various media. The agar plates containing the Nutrient Agar were incubated on inverted at 37°C for 24 - 48 hours, while the agar plate meant for fungal were incubated on inversion at 30°C for 3 - 5 days. At the end of the incubation period, growth on the plates were counted and expressed as colony forming unit per gram (cfu/g) of the soil sample.

Identification of total viable bacteria and fungi count

Serially diluted samples were streaked in specialized media. For instance, in Mannitol salt agar, after 24 hours of aerobic incubation at 37°C, growth with yellow pigment is an indication of *Staphylococcus aureus*. In Blood agar, growth with swarming characteristics after incubation suggested the presence of *Proteus* species. Growth on MacConkey agar was streaked in fresh nutrient agar. From there it was further streaked in Levine's Eosin Methylene Blue (EMB) Agar and incubated at 37ºC for 24 hours. The presence of small and nucleated colonies with greenish metallic sheen indicates *E. coli* and *Enterobacter* sp respectively [25]. The total heterotrophic bacteria isolates

were identified based on biochemical test. Growth from blood agar and mannitol salt agar and MacConkey agar was streaked in nutrient agar and incubated on inverted at 37°C for 24 hours from where biochemical test viz: coagulase, oxidase, urease, gram reaction, motility, catalase, citrate and indole test were carried out following the procedure previously described by Cheesbrough [27]. Then after, the resultant characteristics was compared with those of know taxa following the guide provided by Holt., et al. [28] (Bergey's Manual of Determinative Bacteriology) and Cheesbrough [27].

The samples were also streaked in potatoes dextrose agar supplemented with chloramphenicol and incubated for 3 - 5 days at 30°C. The fungi isolates were identified based on the macroscopic/colonial and microscopic characteristics. The colonial/ macroscopic characteristics were compared with the guide provided by Ellis., *et al*. [29] and Benson [25]. The microscopic morphology was determined using the scheme of Pepper and Gerba [26], Benson [25]. Wet mount preparation of the isolate was made on a clean grease-free glass slide using Lactophenol cotton blue stain. Sterile wire loop was used to collect a loop of the isolate and placed in lactophenol cotton blue stain and it was well mixed. The isolate was viewed under the light microscope.

The yeast isolates were identified using conventional microbiological techniques based on their cultural, morphological, and physiological/biochemical characteristics (viz: using carbon fermentation and assimilation techniques, glucose-peptone-yeast extract broth, lacto-phenol cotton blue stain and growth based on temperature) as described by Kurtzman and Fell [30], APHA [31], Benson [25] and have been applied by Iwuagwu and Ugwuanyi [32], Abioye., *et al.* [33], Okoduwa., *et al.* [34], Izah., *et al.* [5, 18 - 20]. The resultant morphology of the fungi (mould and yeast) identification was compared with the scheme provided by Ellis., *et al.* [29] and Benson [25].

Statistical Analysis

Statistical Package for Social Sciences was used to carry out the statistical analysis. The data was expressed as mean ± standard error. One way analysis of variance was used to show significant variation at P = 0.05 among the various locations. Where significant difference exist, Duncan multiple range test statistics was used to show the source of variation. Sorenson qualitative index by Ogbeibu [36] was used to determine the microbial diversity similarity between the various locations. Critical level of significance = 50% for similarity.

Results and Discussion

The microbial density of soil contaminated with cassava mill effluent from smallholder cassava processors in a rural community in the Niger Delta region of Nigeria is presented in table 1. The total heterotrophic bacteria count ranged from 0.060 - 1.16 x 10^6 cfu/g in the cassava mill effluents contaminated soil and 62.83 x 10^6 cfu/g in the control. Typically, there was significant variation ($P < 0.05$) among the various locations. The control result was significantly higher compared to the contaminated locations.

Locations	Total Heterotrophic Bacteria x 10^5 cfu/g	Coliform count x 10^3 cfu/g	Total Fungi x 10^3 cfu/g
LA	3.56 ± 0.72ab	1.25 ± 0.08b	5.96 ± 4.62a
LB	8.57 ± 0.61bc	3.83 ± 0.18c	4.57 ± 0.61a
LC	0.60 ± 0.06a	0.35 ± 0.04a	0.64 ± 0.11a
LD	7.00 ± 1.22abc	2.01 ± 0.13b	4.40 ± 0.55a
LE	11.63 ± 0.24c	6.60 ± 0.44d	1.33 ± 0.05a
LF	62.83 ± 4.94d	10.73 ± 0.38e	39.47 ± 2.82b

Table 1: *Microbial density of soil contaminated with cassava mill effluent from smallholder cassava processors in a rural community in the Niger Delta.*

Each data is expressed as mean ± standard error (n = 3); Different letters along the column indicate significant variation (P < 0.05) according to Duncan statistics

The coliform counts ranged from 0.35 - 6.60 x 10^3 cfu/g (in the contaminated location) and 10.73 x 103 cfu/g in the control. Significant variation ($P < 0.05$) exist among the various locations. Statistically, the contaminated locations had lower coliform counts compared to the control. The fungal counts ranged from 0.64 - 5.96 x 103 cfu/g (in the contaminated location) and 39.47 x 10^3 cfu/g in the control. Apart from the control, there was no significant difference ($P > 0.05$) in the microbial density of fungi in the cassava mill contaminated locations.

The lower microbial density in the contaminated sites could be due to the characteristics of the effluents. The acidic pH of the discharged effluent as previously reported [18,37] could have caused acidification [38], thereby affecting the indigenous microflora. Furthermore, Akani [39], Okechi., *et al*. [10] had earlier reported cyanide which is highly lethal and fairly mobile in soil to be destructive to microorganisms. The findings of this study has similar trend with the work of Nwaugo., *et al*. [22] and Omomowo., *et al*. [40], Eze and Onylide [24], Ezeigbo., *et al*. [13] but contrary to the work of Ehiagbonare., *et al*. [6] and Okechi., *et al*. [10] which had reported a higher microbial count in the contaminated soil compared to control. Furthermore, the trend in the findings of this study has also been observed in the population of lipolytic, cellulolytic, phosphate solubilizing and nitrifying bacteria as reported by Nwaugo., *et al*. [22], Ibe., *et al* [9].

Table 2 presents the microbial diversity of soil contaminated with cassava mill effluent from smallholder cassava processors in a rural community in the Niger Delta region of Nigeria. The microbial isolates found include *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus, Bacillus, Enterobacter* and *Micrococcus* species (bacteria), and *Saccharomyces cerevisiae* (yeast), *Penicillin, Aspergillus, Rhizopus* and *Mucor* species (mould). The similarity of the microbial isolates between the various locations based on Sorenson qualitative index is presented in figure 2. The similarity interaction between locations with regard to the microbial diversity ranged from 50.00 - 92.30%. The similarity index is above critical level of significance ≥ 50% for similarity. This inferred that microorganisms found in the various locations of study are significantly similar.

Microorganisms	LA	LB	LC	LD	LE	LF
E. coli	+	+	+	+	+	+
Proteus sp	-	-	-	-	+	-
Staphylococcus aureus	+	+	+	+	+	+
Bacillus sp		+	+	+	+	+
Enterobacter sp	+			+	+	
Pseudomonas aeruginosa	-	+	+	-	+	+
Micrococcus	-	-	+	+	-	-
Penicillin sp	+	+	-	-	-	-
Aspergillus sp	+	+	+	+	+	+
Rhizopus sp	+	-	+	+	+	-
Mucor sp	-	+		+	+	+
Saccharomyces cerevisiae	-	-	+	+	-	-

Table 2: *Microbial diversity of soil contaminated with cassava mill effluent from smallholder cassava processors in a rural community in the Niger Delta.*

+ = present; - = absent

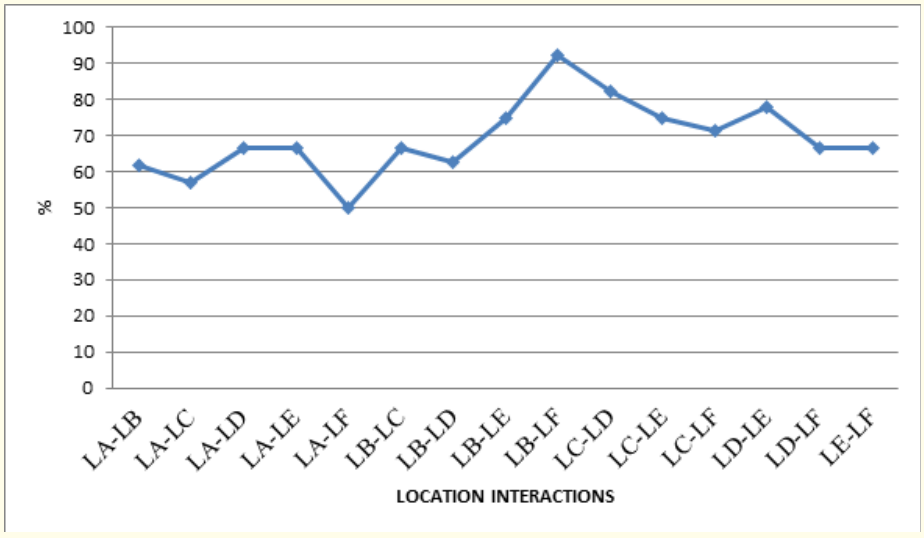

Figure 2: *Similarity index of microbial diversity found in the fish soil samples between each of the markets.*

The occurrence of these microbes in the cassava mill effluents contaminated soil could be due to human effects. For instance, *E. coli* and *Enterobacter* species is an indication of fecal coliform. Fecal materials may have entered the soil through human activities and/or domestic animals such as goat or fowl that roam about the vicinity in their quest for food. *Staphylococcus aureus* are known to be normal flora to humans. Therefore, their presence in the soil may be connected to human activities in the processing sites. Other microbes such as *Bacillus, Micrococcus* species, *Pseudomonas aeruginosa, Penicillin, Aspergillus, Rhizopus* and *Mucor* species have been widely reported in environmental samples and food. The microbes identified in this study are comparable to the findings of Nwaugo., *et al*. [22] and Omomowo., *et al*. [40], Eze and Onylide [24], Ezeigbo., *et al*. [13], Okechi., *et al*. [10], Ehiagbonare., *et al*. [6], Omotioma., *et al*. [41] on cassava mill effluents, its contaminated soil and surface water.

Conclusion

This study investigated the effect of cassava mill effluents on the microbial quality of contaminated soil. The study found that cassava mill effluents significantly reduce the microbial density while it does not significantly affect the diversity. Therefore, sustainable management practices should be adopted through biotechnological advances to minimize the impacts of cassava mill effluents on the soil and its microflora composition.

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