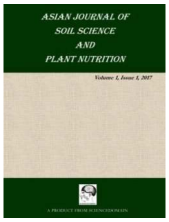

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The Impact of Long-term Cassava Mill Effluent Discharge on Soil pH and Microbial Characteristics in Cross River State

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Authors' contributions

This work was carried out in collaboration between all authors. Author JFA designed the study, managed the literature searches, draft the article and critically revise the article. Author MOE supervised field survey, involved in data collection, critical revision of the article. Author IAI involved in data collection, performed the statistical data analysis and interpretation and also managed the literature searches. All authors read and approved the final manuscript to be published.

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ABSTRACT

Increasing level of cassava effluent discharge on agricultural soils has become a source of concern for environmentalists including soil scientists. Therefore, this study assessed the impact of such activities on soil pH and microbial characteristics. The result obtained showed that the soil samples collected after 25 m away from the point of effluent discharge were slightly acid to slightly alkaline in pH, while those at the point of effluent discharge up to 10 to 25 m away from the point of effluent discharge were acidic. This showed that cassava mill effluent discharge increased soil acidity which could suppress bacterial growth in the soil within the point of discharge. The control soil had the highest microbial count than the impacted soils. The microbial species isolated included bacteria such as *Lactococcus lactis, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus variance and Staphylococcus aureus* while the fungal isolates include *Aspergillus niger, Fusarium spp, Penicillium spp, Mucor spp and Rhizopus spp.* The result further showed that in impacted soil *Bacillus subtilis* (27.78%), *and Rhizopus spp* (28%) had the highest frequency of occurrence for bacteria and fungi respectively while in the control soils *Lactococcus lactis* (26.67%), and *Rhizopus*

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spp (27.27%) and *Penicillium spp* (27.27%) were the most dominant bacteria and fungus isolated across the different sampling distances. Hence, there should be an increasing awareness of the emergency created by cassava processing mills and management practices should be adopted to remediate the impacted soil.

Keywords: Bacteria; cassava effluent; fungi; soil pH.

1. INTRODUCTION

Cassava is an important food security crop in Nigeria. It produces the feed that feeds the populace and also provides the primary source of livelihood for the rural poor. It is seen to have a high poverty-reduction potential for Nigeria due to its low production cost [1] and [2]. Currently, Nigeria is the World largest producer of cassava accounting for 19 per cent of total world production and about one-third of the total national output of cassava comes from the Niger Delta region where many livelihoods depend on cassava as a main source of food and income [3]. This upsurge in the production and utilization of cassava in recent years has led to the establishment of cassava mills in many production areas. The cassava production system in Cross River State and elsewhere in Nigeria is characterized by small-scale farmers that cultivate less than 2 hectares of land and is subsistent in nature, primarily cultivated for the traditional food market. However, any surplus cassava harvest in this production system is sold to local processors.

Raw cassava produce can be processed into diverse traditional delicacies and industrial product which include; garri, fufu, starch, lafun, chips, pellets, cake, flour etc, some of which are fermented products [4] and [5]. However, processing of cassava into other products, for example garri involves several unit operations vis-a-vis, peeling, washing, grating, pressing and fermenting, sieving, roasting and drying [6] in [7]. The processing of cassava has increased extensively in recent times and this processing has equally increased the environmental pollution associated with the disposal of the effluents. This is so because cassava processing traditionally generate a lot of waste (e.g water, hydrocyanic acid, peels and sieves from the pulp) from cassava mills which are usually discharged on land indiscriminately and this in turn affects soil and groundwater including biota (plant and micro-organism) [8] and [9] within the confinement where such activities are carried out.

Garri, starch and /or fufu production makes use of large volume of water which results in the discharge of large amount of waste water. The wastewater is discharged indiscriminately by most processor and allowed to accumulate on the soil, even near residential homes, producing offensive odours and unsightly scenarios [8] and [6]. This wastewater from the processing factory contains cellulose, carbohydrate, nitrogenous compounds, varying concentration of heavy metals and cyanoglycosides. Cyanogens and glycosides are easily hydrolyzed into hydrogen cyanide which is toxic to soil, biota and aquatic animals, thus posing serious threat to humans and environment [10,11] and [12].

Soil gets the ultimate impacts of the toxicants from cassava processing and other industrial effluents and this is detrimental to agricultural activities in the area where such activities are carried out. Top soil (0-20 cm) consists of large proportion of microorganisms which are involved in the degradation of organic matter and nutrient cycling. Long-term discharge of this effluent into the soil could result in a serious imbalance in the microbial population, which in turn could result in alteration of soil fertility toward a negative direction. Studies that have been carried out on the effect of cassava effluent on soils properties have consistently showed that there are always some microbial changes in the soil properties when the effluent are discharged on it [13] and [14]. This however, shows that cassava mill effluent discharge has the tendencies to alter soil properties causing changes in soil pH and microbial loads [15,16] and [7].

Interestingly, Nigeria government is currently working towards increased cassava cultivation with harvest target of 150 million tons annually and the establishment of hundreds of cassava processing centers [17]. This means that there will be great increase in the effluent water generated. According to Okunade and Adekulu [18], “there is neither a specific method of disposal or of treatment of the cyanide-laden wastewater resulting from cassava processing in Nigeria nor any government policy guidelines”. Therefore, there is a need to assess the impact of cassava mill effluent on soil pH and microbial characteristics which are the key indicators of productive soils and thus calls for regulation in the discharge of the waste generated. The aim of this research was to assess the long-term impact of cassava mill effluent discharge on soil pH and microbial load in the surrounding soils.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was conducted in two agricultural zones of Cross River State, viz; Central and Southern Agricultural Zone. Obubra and Odukpani were chosen to represent Central and Southern Agricultural Zone respectively, due to predominant of cassava milling plants. In each of the selected local government areas, investigation was done in mills that have received effluent for a minimum of ten years. In Odukpani local government area, Usung Odot, Ndon Nyam and Ikot Nyong were the three sampling areas whereas in Obubra local government area Wankade II, Ochon Town and Apiapom were the sampling areas. Obubra (longitude 6° 5' and 8° 20' E and latitude 5° 4 [1] and 6° 101N) and Odukpani (latitude 5°25'N and longitude 8°0'E) falls within the rainforest zone of Cross River State where the rainy season lasts for about 7-8 months with the dry months having less than 60 mm of rainfall. It has temperature with average daily maximum of 35°C. The rainy season has a short dry period called "August Break" or short dry season, which last between 2 to 4 weeks. The underlying geological material of Odukpani consists of coastal plain sand, made up of unconsolidated sandy sediments underlain by massive deposit of limestone. The geological formation of Obubra area consists predominantly of basement complex formation of sedimentary rocks, sandstone, shale, basalt, siltstone and quartz, mica and schist. The Vegetation of the area is humid tropical rainforest.The area is used predominantly for horticultural and arable crop cultivation where crops like maize, watermelon, fluted pumpkin, cassava and yam are grown.

2.2 Samples Collection

Soil auger was used to collect soil samples from a depth of 0-20 cm from soil impacted with cassava effluent discharge in Wankade II, OchonTown and Apiapom in Obubra LGA and Usang Odot, Ndon Nyam and Ikot Nyong in Odukpani LGA. The soil samples from the cassava processing plant were collected at different distances: from the point of discharge, 10 m, 25 m, 50 m and 100 m away from the site using sterile plastic bags. The bulk soil samples from the 100 m distance served as control. At each sampling distance, three samples were collected and pooled to give a composite sample for that particular sampling distance, and 5 point (point of discharge, 10 m, 25 m, 50 m and 100 m) were considered in all. All the samples were transported to the Laboratory for analysis. At each sampling distance, three samples were collected and pooled to give a composite sample for that particular sampling distance.

2.3 Sample Preparations

The samples were air-dried, gently crushed to pass through a 2-mm sieve and then stored in fresh clean polyethylene bags in the refrigerator at 2°C between 7-14 days to maintain the stability of the samples without significant alteration in their biological properties.

2.4 Determination of Soil pH

Soil pH was determined using a 1:2.5 (w/v) soil-water ratio by pH-meter with a glass electrode pH meter [19].

2.5 Microbial Analysis

2.5.1 Enumeration of total heterotrophic bacteria and fungi

The total aerobic bacterial and fungal counts were carried out using pour plate technique as described by Fawole and Oso [20], after ten-fold serial dilution. The media of choice were Oxoid Nutrient agar, Agar-Agar extract and Saboroud Dextrose agar, prepared according to the manufacturer's instruction.

2.5.2 Characterization and identification of microbial isolates

The bacteria isolates were identified by biochemical test (gram reaction, motility, indole, catalase, coagulase, oxidase, urease and citrate). The resultant characteristics were compared with those of known taxa using the keys provided in the Bergey's Manual of Determinative Bacteriology by Holt et al. [21] and the scheme of Cheesbrough [22]. The cultural characteristics of the bacteria isolated were compared with the culture characteristics presented by Dubey and Maheshwari [23]. Fungal isolates were examined macroscopically and microscopically using the needle mounts technique. Their identification was performed according to the scheme of Barnett and Hunter [24] and Larone [25].

2.6 Statistical Analysis

Data collected were analyzed using descriptive and inferential statistics; mean, percentages, frequency distributions and coefficient of variation (CV) were used for descriptive statistic. The data collected were also analyzed using one way analysis of variance (ANOVA) and the significant means separated by Duncan's Multiple Range tests.

3. RESULTS

3.1 Lateral Distribution of Soil pH

The lateral distribution of soil pH in the studied soil is presented in Table 1. In Obubra local government area, the lowest soil pH value of 4.4 was observed at the point of discharge, whereas the highest soil pH value of 5.7 was observed at 50 and 100 m away from the point of effluent discharge. Similarly, in Odukpani local government area, the lowest soil pH value of 4.3 was observed at the point of discharge, whereas the highest soil pH value of 7.4 was observed at 25 m away from the point of effluent discharge. The soils at the point of discharge consistently had lower value of soil pH and tend to increase as distance increases. This means that the further the sampling point away from the cassava effluent discharge points, the higher the value of soil pH.

3.2 Lateral Distribution of Microbial Load

In Table 2 is presented the microbial population at various distances from the mill. The result showed that sample collected at the point of effluent discharge and 10 m away from the point of effluent discharge recorded the least average bacterial count value of 8.0 x10^5 cfu/g (Wankade soil) and 8.0 x10^5 cfu/g cfu/g (Ochon Town), and the highest average fungal count value of 5.0 x10^3 cfu/g (Ndon Nyam and Ikot Nyong soil) and 5.0 x10^3 cfu/g (Usang Odot) compared to samples collected at other distances away from the point of effluent discharge.

Table 1. Effect of cassava effluent on soil pH along a gradient from the point of discharge

Discharge distance	Obubra local government area			Odukpani local government area		
	Wankade II	Ochon Town	Apiapom	Usang Odot	Ndon Nyam	Ikot Nyong
Point of discharge	4.7	4.9	4.4	4.3	4.6	4.5
10 meters away	5.0	4.9	4.8	4.9	4.8	4.7
25 meters away	5.3	5.1	5.4	7.4	6.4	6.1
50 meters away	5.6	5.7	5.6	6.7	5.6	5.8
100 meters away	5.7	5.0	5.7	5.4	5.6	5.6
Mean	5.26	5.12	5.18	5.74	5.40	5.34
SD	0.42	0.34	0.56	1.28	0.72	0.70
CV (%)	7.9	6.5	10.8	22.3	13.4	13.1

Table 2. Effect of cassava effluent on bacterial load of soil (CFU/g) x10^5 along a gradient from the point of discharge

Discharge distance	Obubra local government area			Odukpani local government area		
	Wankade II	Ochon Town	Apiapom	Usang Odot	Ndon Nyam	Ikot Nyong
Point of discharge	8.0	8.0	11.5	10.0	9.0	10.0
10 meters away	8.5	8.0	14.0	10.0	11.0	11.0
25 meters away	8.6	12.0	16.0	14.0	12.0	14.0
50 meters away	15.0	10.5	15.0	14.0	16.0	17.0
100 meters away	17.0	15.0	17.0	11.0	14.0	16.0
Mean	11.42	10.70	14.70	11.8	12.4	13.6
SD	4.25	2.95	2.11	2.05	2.70	3.05
CV (%)	37.2	27.6	14.4	17.4	21.8	22.4

Table 3. Effect of cassava effluent on fungal load of soil (CFU/g) x10^3 along a gradient from the point of discharge

Discharge distance	Obubra local government area			Odukpani local government area		
	Wankade II	Ochon Town	Apiapom	Usang Odot	Ndon Nyam	Ikot Nyong
Point of discharge	4.0	3.0	2.0	3.0	5.0	5.0
10 meters away	4.0	3.0	3.0	5.0	5.0	3.0
25 meters away	3.0	2.0	1.0	5.0	4.0	3.0
50 meters away	2.0	2.0	2.0	4.0	3.0	2.0
100 meters away	2.0	2.0	1.0	3.0	3.0	4.0
Mean	3.0	2.4	1.8	4.0	4.0	3.4
SD	1.0	0.55	0.84	1.0	1.0	1.14
CV (%)	33.3	22.8	46.5	25.0	25.0	33.5

The populations of the different bacterial groups were lower in the impacted soil than the control. Bacterial counts were generally higher than fungal count. In general, the microbial load of the impacted soil was lower than the control sample. The relatively low count of impacted soil could be attributed to the effluent making the soil acidic due to the presence of cyanide.

The analysis of variance (ANOVA) results presented in Table 4 showed that pH at the point of discharge was not significantly different from that of 10 meter away from point of discharge but was significantly different ($p = 0.01$) from those of 25, 50 and 100 meters away in soils under Obubra local government area. Similarly, in soils under Odukpani local government area pH at the point of discharge was not also significantly different from that of 10 meters away but was significantly different ($p = 0.01$) from those of 25, 50 and 100 meters away. The ANOVA results at $P \leq 0.05$ showed that there was a significant difference in total bacterial counts between soils at point of discharge and soils from 50 and 100 meters away from discharge point in Obubra. Although soils at 10 and 25 meters away were not significantly different from soil at point of discharge but were significantly different from that of 100 meters away. Similarly, in Odukpani total bacterial count at point of discharge and 10 meters away were significantly different from those of 25, 50 and 100 meters away. Fungal count in Obubra from the point of discharge and 10 meters away were significantly different $p<0.05$ from those of 25, 50 and 100 meters away. Conversely, fungal count in Odukpani from the point of discharge and other distance from the point of discharge were not significantly different $p>0.05$.

The occurrence of isolated microorganisms is presented in Table 5. The results revealed the isolation of such bacteria as *Lactococcus lactis, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus varians and staphylococcus aureus* while the fungal isolates include *Aspergillus niger, Fusarium spp, Penicillium spp, Mucor spp and Rhizopus spp.* Bacterial and fungal species were encountered in both impacted and control soils in course of this study, but their occurrences and levels of predominance were different. In impacted soil *Bacillus subtilis* (27.78%) *and Rhizopus spp* (28%) were the dominant bacteria and fungi isolated while in the control soils *Lactococcus lactis spp* (26.67%) and *Rhizopus spp* (27.27%) and *Penicillium spp* (27.27%) were the most dominant bacteria and fungus across the different sampling distances.

4. DISCUSSION OF RESULTS

The soil pH at the point of discharge and 10 m away from discharge point were highly acidic. This can be detrimental to agricultural activities in where such cassava mills are located, as soil pH determines the availability of nutrients and the potency of toxic substances as well as the physical properties of the soil [26]. This is consistent with some other reports [6], [16] and [27]. The soil pH values of the soils around the point of effluent discharge indicate a generally high tendency for high availability of the metals; hence, this increases the risk of heavy metals uptake by crops. It has also been reported by McBride [28] in Sullivan and Lewis [29] that soil acidity influences many chemical and biological characteristics of soil, including availability of nutrients and toxicity of metals which can also affect microbial communities in many ways. Acidic soils can significantly reduce nodule numbers, nodulation and nodule function, and N-fixing capabilities within the roots of legumes [30]. This may result in reduced plant vigor and productivity, as well as significant crop loss.

Table 4. ANOVA results of the cassava effluent discharge on soil pH and microbial properties of the studied soil

Discharge distance	pH		Bacterial count		Fungal Count	
	Obubra	Odukpani	Obubra	Odukpani	Obubra	Odukpani
Point of discharge	4.67^{c}	4.47^{d}	9.17^{c}	9.67^{b}	3.00^{a}	4.33
10 meters away	$4.9b^{c}$	4.80^{cd}	10.17^{bc}	10.67^{b}	3.33^{a}	4.33
25 meters away	5.27^{ab}	6.63^{a}	12.20^{bc}	13.33^{a}	2.00^{b}	4.00
50 meters away	5.63^{a}	6.03^{ab}	13.50^{ab}	15.67^{a}	2.00^{b}	3.00
100 meters away	5.47^{a}	5.53^{bc}	16.33^{a}	13.67^{a}	1.67^{b}	3.33
F-value	8.01***	15.12***	6.76**	10.87***	7.23***	1.0^{NS}

*** and *** denote significant level at 5 and 1% respectively; NS = not significant; means within a column not sharing a letter in common differ significantly (p≤0.05) from each other following Duncan multiple range test (DMRT)*

Table 5. The microorganisms isolated from the impacted and control soil samples and their percentage occurrence

Organisms isolated	Occurrence of isolates					Frequency of occurrence	
	Point of discharge	10	25	50	100	Impacted soils	Control soil
Bacterial							
Lactococcus lactis	-	+	+	+	+	4(22.22)	8(26.67)
Bacillus subtilis	-	+	+	+	+	5(27.78)	6(20)
Pseudomonas aeruginosa	-	-	+	+	+	3(16.67)	5(16.67)
Micrococcus varians	-	-	+	+	+	2(11.11)	4(13.33)
Staphylococcus aureus	-	+	+	+	+	4(22.22)	7(23.33)
Fungal							
Aspergillus niger	+	+	-	+	+	3(12)	1(9.09)
Penicillum spp	+	+	-	-	+	4(16)	3(27.27)
Fusarium spp	+	+	+	-	+	6(24)	2(18.18)
Mucor spp	+	+	-	-	+	5(20)	2(18.18)
Rhizopus spp	+	+	+	+	+	7(28)	3(27.27)

+ = microbes is present; - = microbes is absent; values in parenthesis are percent occurrence

Beneficial soil microbes and plants prefer a near-neutral pH range of 6 to 7, so increased soil acidity is often accompanied by shifts in the types of microbes in soils and their activities [29]. This means significant changes in the rate of decomposition which can lead to immobilization of basic nutrients and decreased nutrient availability to plant. The acidic nature of soils at point of effluent discharge up to 10 to 25 m away from the effluent discharge means reduced microbial function and this can adversely affect soil health and productivity. Soil fungi are favoured by a low soil pH, implying that at lower soil pH the soil is much more fungi-dominated [31]. This may allow the invasion of fungal root pathogens and also change the way organic residues are decomposed, and this may in turn immobilized soil carbon and plant nutrients slowing turnover and nutrient release [32]. Generally, the soil pH of the impacted soils were lower (i.e more acidic) than those of the unimpacted soil. This is no doubt due to the presence of hydrogen cyanide present in the effluent [14].

The result of microbial load analyzed showed that the bioloads of all the bacteria groups increased with distance away from the point of discharge (see Tables 2 and 3). This observation suggests an adverse growth conditions towards the discharge point. However, most values obtained at 50 m away from the discharge point were higher than those from control. Fungal counts were higher in impacted soils than control soils. The relative increase in the fungal growth in the impacted soil may be due to the acidic pH environ of the soil which in most cases were as low as 4.3 (Table 1) and more robust nature of fungi which enables them to withstand the more acidic environment of the soil than bacteria [33]. On the contrary, the relative low fungal diversity, observed in the control soil, could be attributed to the near neutral pH in most location. The low pH of the soil could explain the presence of cyanogenic glycosides in the cassava effluent impacted soil [34]. Microbes in soil are important to healthy soil processes and good soil quality. [32], aptly stated that many aspects of most nutrient cycles are controlled by soil microbes, in that without microbes, organic matter decomposition simply would not occur, legumes would not be able to fix nitrogen, and ammonia would not be converted to plant-available nitrate. The microorganisms isolated from the effluent impacted soil were in line with the previous works of Ezeigbo et al. [35] and Igbinosa and Igiehon [34] who isolated similar organisms.

5. CONCLUSION

The results obtained from this study have indicated adverse environmental effects of cassava mill effluents discharge on soil pH and microbial loads. Analysis of results showed that soil pH close to the point of effluent discharge was highly acidic and the microbial loads especially bacterial around these points were very low. The result further showed that soil pH-driven changes can have a major influence on microbial population. It is very clear from this investigation that any imbalance in the microbes in soils can lead to reduced soil microbial health which can impact on general crop productivity. Hence, understanding more precisely the response of beneficial soil microbial populations to acidification caused by cassava mill effluent discharge and its resultant effect on soil health will provide valuable insights to sustainable agriculture and healthy environmental management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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