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Long-Term Impact of Cassava Mill Effluent on Some Chemical and Biological Properties of Soils

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ABSTRACT

Effluent generated from cassava processing, when discharged to the soil, alters the nature of soil properties. Hence this study was carried out to evaluate the impact of long-term discharge of cassava mill effluent on soil chemical and biological properties. Using a target sampling technique, four micro pits were dug, two at the polluted site and two 50 m away from the polluted site which served as the control. Soil samples were collected from the pits at varying depths of 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm and 20-50 cm. Soil samples collected were analysed for chemical and biological properties and data generated were subjected to t-test analysis to assess the impact of cassava effluent on some selected soil chemical properties and biological properties. The results of the chemical properties indicated that the polluted site had higher organic matter (mean=25.97 g kg^{-1}) relative to the control site (mean= 15.42 g kg^{-1}). Total nitrogen was higher in the polluted site (mean = 1.29 g kg^{-1}) relative to the control site (mean = 0.74 g kg^{-1}). Available phosphorus was higher in the polluted site (mean = 13.5 mg kg^{-1}) relative to the control site (mean = 8.67 mg kg^{-1}). Total exchangeable bases (TEB) was higher in the polluted site (mean= 6.7 $cmol_c kg^{-1}$) relative to the control site (mean=4.05 cmolc kg^{-1}). Effective cation exchange capacity (ECEC) was higher in the polluted site (mean = 8.25 cmolckg-1) relative to the control site (mean=4.78 $cmol_c kg^{-1}$) whereas the pH was lower in the polluted site (mean=5.71) relative to the control site (mean=6.8). The results of the biological properties showed that the Total Fungal Count was higher in the polluted site (mean=1.12 x 105 CFU g^{-1}) relative to the control (mean=0.33 x 105 CFUg^{-1}) whereas Total Heterotrophic Bacterial Count was lower in the polluted site (mean=2.29 x105 CFUg^{-1}) relative to the control (mean=5.72 x 105 CFUg^{-1}). The t-test analysis result revealed that cassava effluent had a significant positive impact on organic matter, total nitrogen, available phosphorus, ECEC and total fungal population whereas it had a significant negative impact on soil pH and total bacterial population.

Keywords: Cassava mill effluent, soil chemical properties, soil biological properties, soil pollution.

INTRODUCTION

Soil and water bodies are particularly polluted with toxicants from food processing and allied industries (Salami and Egwin 2007). Major pollutants from food processing include hydrocarbons, palm oil mill effluent, human and animal

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wastes, wood waste, waste water from agro-allied industries as well as cassava mill effluent from cassava processing activities (Wade *et al*. 2002; Arimoro and Osakwe 2006).

Cassava (*Manihotes culentus* Crantz) which belongs to the family *Euphorbiaceae* (Nwaugo *et al.* 2008) is widely cultivated in the tropical and subtropical regions of the world for its starchy tuberous roots with more than 200 calories/day of food value (FAO 2004). It is a staple food of nearly one billion people in Africa, South America, Asia and Pacific (ANU 2007) and in Nigeria, the estimated cassava production is approximately over 34 million metric tons (FAO 2004).

The highly perishable nature of harvested cassava roots and the presence of cyanogenic glucosides in bitter cultivars call for immediate processing of the storage roots into more stable and safer products. Cassava can be processed by either washing, exposure to air, heating or pressing. Consequently, a lot of processing equipment and technology has been developed by various governmental and private organisations in Nigeria to facilitate the processing of cassava roots to reduce losses (IITA 2005).

In Nigeria, cassava is processed into traditional delicacies which include *garri, fufu, lafun flour* etc, some of which are fermented products (Oti 2002). Among all the products obtained from cassava, *garri* is the most common in Nigeria and its production is done in varying scales, small, medium and large (Uzoije *et al.* 2011).

During cassava processing, much effluent and solid wastes are generated and released into the environment. One of the major recipients of this effluent is the soil (Orhue *et al.* 2014). On the average, 2.62 m^3 ton^{-1} of solid residue and 3.68 m^3 $ton^{-1}$of water residues are generated via cassava processing in Nigeria. (Horsfall *et al*. 2006; Isabirye *et al*. 2007).

Cassava effluents when discharged into the soil result in changes in soil properties (Nwakaudu *et al*. 2012). For intance, Eghoaye and Dada (2004) investigated cassava effluent polluted soils and observed an increase in soil acidity, potassium, sodium, phosphorous, and organic carbon and a decrease in calcium nitrogen and magnesium. Akpan *et al*. (2011) reported increased pH, N, organic carbon, exchangeable acidity and decreased Mg, K, P in soil treated with cassava mill effluent. In another study (Ogboghodo *et al*. 2001), cassava effluent was found to increase the number of organisms in the soil ecosystem which may be associated with an increase in soil pH, organic carbon and total nitrogen.

The Nigeria government is working towards increased cassava cultivation with a harvest target of 150 million tons annually (IITA 2011). Consequently, in the past few years, there has been a great upsurge in the production of cassava and establishment of more cassava processing mills in the southern parts of the country with the consequence of an extensive ecological pollution associated with the effluent discharge into the soil (Igbinosa and Igiehon 2015).

Previous studies conducted in Nigeria to evaluate the impact of cassava mill effluent on soil properties indicated conflicting findings and were primarily based

on short-term impact, thus necessitating a more intensive and accurate study. Therefore the major objective of this study was to evaluate the long-term impact of cassava mill effluent on soil chemical and biological properties.

MATERIALS AND METHODS

Study Area

The study was conducted at an active 11-year-old cassava mill located in Iho-Dimeze, Ikeduru in Imo State Southeastern Nigeria which lies between latitude 4^{o} 45^{1} N to 7^{o} 15^{1} N and longitude 6° 50^{1} E to 7^{o} 25^{1} E. Soils of the study area are derived from coastal plain sands (Onweremadu *et al.* 2007). The study area falls within the humid tropical climate with a mean annual rainfall, higher than 2500 mm, and temperature ranging from 26 °C to 30 °C while relative humidity is about 70%. The rainfall pattern is bimodal with peaks in the month of July and September (Ihem *et al.* 2014). Secondary vegetation dominates the area and farming is a major socio-economic activity in the area with the main crops cultivated being maize (*Zea mays*), oil palm (*Elaeis guineensis*), cassava (*Manihot esculenta*), plantain (*Musa sapientum*) etc.

Characterisation of Cassava Mill Effluent

Cassava mill effluent (CME) is a colloidal of fine particles of cassava starch in water (Sackey and Bani 2007) with total suspended and dissolved solids of about 789 and 799 mgL^{-1}, respectively (Orhue *et al.*, 2014). It is high in organic matter and highly acidic in nature (Sackey and Bani, 2007) with a pH of 4.6 and a range of 2.5-4.20 reported by Adejumo and Ola (2011) and Rim-Rukeh (2012), respectively. An investigation into the elemental content of CME revealed 0.19, 0.18, 1.48, 0.58 and 0.82 mg L^{-1} nitrogen, phosphorus, calcium, potassium and magnesium, respectively (Orhue *et al.* 2014). Further studies on the chemical properties of the effluent showed biological oxygen demand (BOD) in the range of 13.0-73.0 mg L^{-1}, chemical oxygen demand (COD) ranging from 320 to 365 mgL-1, dissolved oxygen ranging from 1.10 to 2.60 mgL^{-1} and hydrocyanic acid ranging from 54.10 to 63.20 (Rim-Rukeh 2012). Studies on the biological properties of CME indicated presence of *Streptococcus* spp, *Bacillus* spp, *Staphylo coccus aureus, Lactobacillus* spp, *Micrococcus* spp and *Pseudomonas* spp bacterial species (Ehiagbonare *et al.* 2009; Rim-Rukeh 2012) while *Mucor* spp, *Aspergillus* spp, *Penicillium* spp and *Saccharomyces cerevisae* fungal species have been identified (Ehiagbonare *et al*. 2009).

Field Studies and Sample Collection

Guided by the target sampling technique, two micro profile pits were dug in the polluted site and another two pits 50 m away from the polluted site which served as the control. Composite soil samples were collected from the profile pits dug at five varying depths of 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm and 20 cm-50 cm, summing to a total of 20 samples used for the study. The soil samples collected were air-dried, sieved using a 2-mm sieve and subjected to laboratory analyses.

Laboratory Analyses

Chemical Properties

Soil pH was determined in 1:2.5 solute/suspension ratio using glass electrode of a pH meter (Thomas 1996). Exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) were extracted with NH_4OAc buffered at pH 7.0 (Thomas 1982). Exchangeable K^{+} and Na^{+} content of extracts were read on flame photometer while exchangeable Ca^{2+} and Mg^{2+} were determined using atomic absorption spectrophotometer. Exchangeable acidity (Al^{3+} and H^{+}) was extracted with 1 N KCl (Thomas 1982) and determined by titrating with 0.5 N NaOH using phenolphthalein as an indicator. Effective cation exchange capacity (ECEC) was obtained by summation of basic and acidic cations; organic matter (OM) was determined by wet oxidation method (Nelson and Sommers 1982) and total nitrogen by micro Kjeldahl apparatus method (Bremner and Mulvaney 1982) while available phosphorus was determined using Bray II solution (Olson and Sommers 1982).

Biological properties

The total aerobic heterotrophic bacterial (THC) and total fungal count (TFC) expressed in colony forming unit per gram soil ($CFUg^{-1}$) were ascertained by a standard pour plate method as described by Igbinosa and Igiehon (2015). Using this method, 1 g of the soil samples was measured into a sterile test tube and 9 mL of sterile distilled water was added to make a stock solution. The 10^{-1} suspension was subsequently serially diluted to 10^{-10} dilution and the diluted samples were used for microbial analysis. Heterotrophic bacteria were isolated using nutrient agar amended with 0.015 % (w/v) nystatin to inhibit fungal growth. The nutrient agar plates were incubated at 28 ± 2 ^{0}C for 24 - 48 h. Potato dextrose agar containing 0.05 % (w/v) Chloramphenicol was used to isolate fungi upon incubation at 28 ± 2 ^{0}C for 72 h. After incubation, total counts of fungi and heterotrophic bacteria were determined using the colony counter. The identification of bacteria species was based on their morphological characteristics and biochemical tests carried out on the isolates using gram staining technique of Fawole and Oso (2004) while the fungal isolates were further characterised based on their morphological and microscopic features using lacto phenol cotton blue staining techniques (Hunter and Bamett 2000).

Statistical Analysis

Data obtained from laboratory analyses were subjected to *t*-test analysis at 5% level of probability to assess the impact of cassava mill effluent on selected soil chemical and biological (bacteria and fungi) properties.

RESULTS AND DISCUSSION

Impact of Cassava Mill Effluent on Some Chemical Properties of the Soils

Figures 1-6 show the results of chemical properties of the soils. The results indicated higher soil pH in the control site relative to the polluted site, with the

former and latter having mean values of 6.60 and 5.71, respectively (Figure 1). Orhue *et al.* (2014) investigated the properties of cassava effluent and noted a low pH value of 5.07. Hence lower soil pH values recorded in the polluted site could be due to the acidic nature of the cassava mill effluent occasioned by the presence of hydrogen cyanide, resulting in decreased soil pH (Ogboghodó *et al*. 2001). Soils of the polluted site had more organic matter (OM) content than that of the control site with values from 12.37-35.07 g kg^{-1} in polluted site and 11.51-20.27 g kg^{-1} in the control site (Figure 2) and these values decreased with soil depth. Higher organic matter values observed in soils of the polluted site are chiefly due to the organic nature of cassava effluent (Aquino *et al*. 2015). These findings are in agreement with the report of Akpan *et al*. (2011) who observed a higher organic matter level in cassava effluent polluted soils relative to the control site soils. Cassava mill effluent contributed positively to total nitrogen content of the soils as the values were higher in polluted sites (0.61 g kg^{-1}- 1.76 g kg^{-1}) than in the control sites (0.55 g kg^{-1}- 0.99 g kg^{-1}) with values decreasing with depth (Figure 3). Available P followed a similar distribution trend with total nitrogen in the soils as it was more in the polluted sites (17.32 mg kg^{-1}) than in the control sites (7.39 mg kg^{-1}) and decreased with depth (Figure 4). The highest value (32.58 mg kg^{-1}) of available P observed in the surface soil (0-5cm) of the polluted site could be due to a higher level of organic matter in the surface soil as increasing organic matter increases P availability (Brady and Weil 2010). Soils of the polluted site had more ECEC than soils of the control site, with values ranging from 5.69-12.67 cmolc kg^{-1} and 4.28-5.58 $cmol_c$ kg^{-1} in the former and the latter, respectively. The higher ECEC observed in the polluted site could be due to its higher organic matter content (Figure 5). It has been reported that organic matter contributes to ECEC of soils (Havlin *et al.* 2012). It was also observed that ECEC decreased with depth in the two sites. TEB of the polluted and control sites soils differed with the polluted soils having higher values relative to the control site soils. the values varied from 5.07 $cmol_c$ kg^{-1} -11.89 $cmol_c$ kg^{-1} and 3.28 $cmol_c$ kg^{-1} - 5.16 $cmol_c$ kg^{-1} in the polluted and control site soils, respectively. These findings suggest that cassava mill effluent has positive effects on TEB of soils (Figure 6) which supports the report of Isitekhale and Adamu (2016) who observed higher exchangeable bases in cassava mill effluent treated soils.

Impact of Cassava Mill Effluent on Total Heterotrophic Bacterial Count (THC) of the Soils

Figure 7 shows the impact of cassava mill effluent on the total heterotrophic bacterial count in the soils. Cassava mill effluent negatively affected total heterotrophic bacterial count as a lower count was observed in the polluted soils compared to the control soils. Mean values of 5.72× 105 $CFUg^{-1}$ and 2.29×105 $CFUg^{-1}$ were recorded in the control and polluted sites, respectively. The ratio of bacterial count of the control soils to the polluted soils was high at lower depths and varied from 1.42- 33.07, which reveals the high impact of cassava effluent on

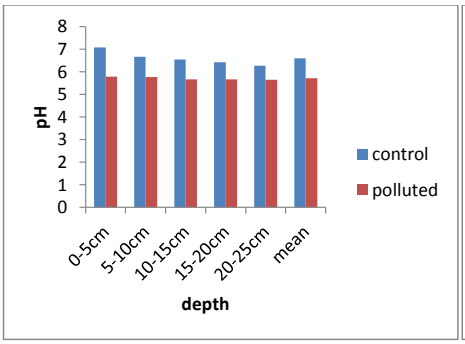

Figure 1: Impact of cassava effluent on soil pH

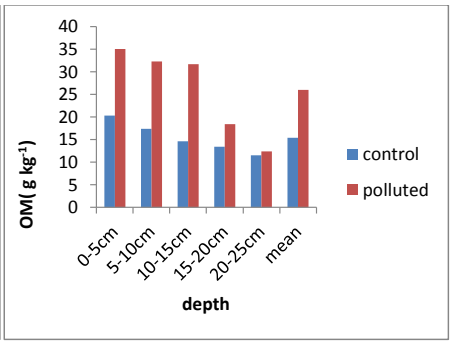

Figure 2: Impact of cassava effluent on soil organic matter (OM)

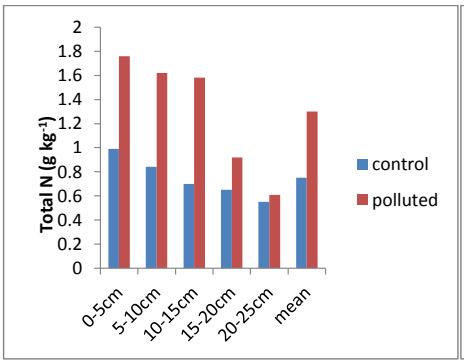

Figure 3: Impact of cassava effluent on soil total nitrogen

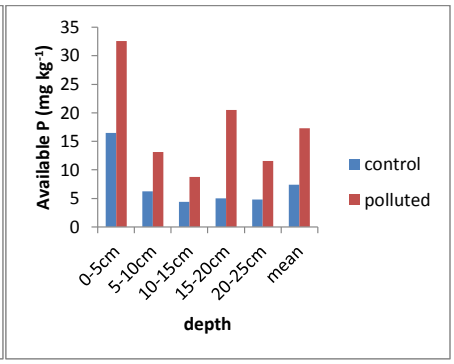

Figure 4: Impact of cassava effluent on soil available phosphorus

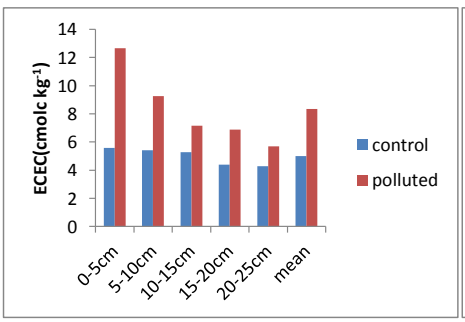

Figure 5: Impact of cassava effluent on soil ECEC

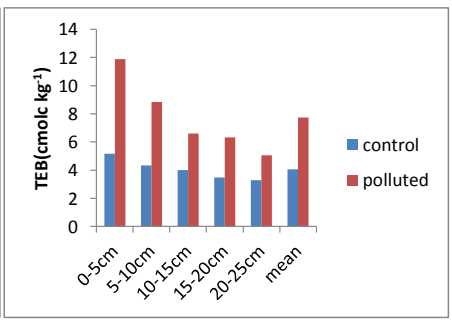

Figure 6: Impact of cassava effluent on soil TEB

total heterotrophic bacterial population in soils and could be due to lower pH of the polluted soils occasioned by the presence of cassava mill effluent. In a related study, Ibe *et al*. (2014) as well as Obueh and Odesiri-Eruteyan (2016) made a similar observation and attributed their findings to the acidic nature of the effluent due to the presence of cynogenic glucocide in cassava mill effluent. The results further revealed a decreasing total heterotrophic bacteria count with depth in both

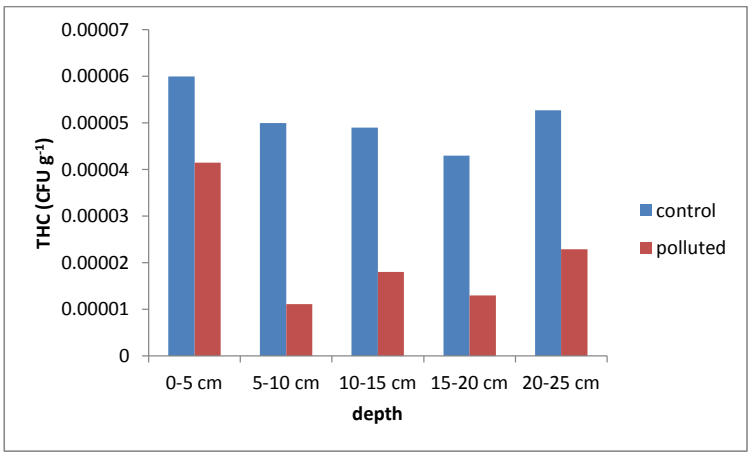

Figure 7: Impact of cassava mill effluent on total heterotrophic bacterial count

sites which could be due to a reduced organic matter level since organic matter is known to contribute to soil biomass (Maloney *et al.* 1997).

Impact of Cassava Mill Effluent on Total Fungal Count (TFC) of the Soils

The results of the impact of cassava mill effluent on fugal population of the soils are presented in Figure 8. Total fungal count was better in the polluted site (0.15×10^5 - 1.25×10^5 $CFUg^{-1}$) relative to the control site (0.05×10^5 - 0.85×10^5 $CFUg^{-1}$) which suggests that cassava mill effluent favours fungi diversity and could be due to the starchy nature of the cassava effluent which serves as source of energy to the fungi as well as higher nitrogen content of the polluted site which favours microbial diversity. It could also be attributed to lower pH of the polluted site. Das (2011) noted that yeast grows readily at low pH. These findings are in agreement with the report of Igbinosa and Igiehon (2015). The varying ratio (0.25- 0.68) of total fungal count of the control site to the polluted site (C:P) site indicates that cassava mill effluent impacted on total fungal count of soils. The decreasing total fungal count with depth observed in the two sites could be due to decreasing organic matter content of the soils with depth. It has been reported that increasing organic matter content of soils, enhances microbial growth (Brady and Weil 2010). The decrease with depth could be also due to a decrease in soil aeration with depth since fungi are aerobes (Das 2011).

Identification of Bacterial Isolates

The results of identification of bacteria isolated are presented in Table 1. *Staphylococcus aureus, Escherichia coli, Baccilus* spp, *Pseudomonas* spp, *Streptococcus* spp and *Conyne bacterium* spp. bacteria species were identified in the soil samples investigated, similar to the assertions of Igbinosa and Igiehon (2015). The results revealed that *Escherichia coli* was observed in the polluted site only, an indication that cassava mill effluent enhances the proliferation of

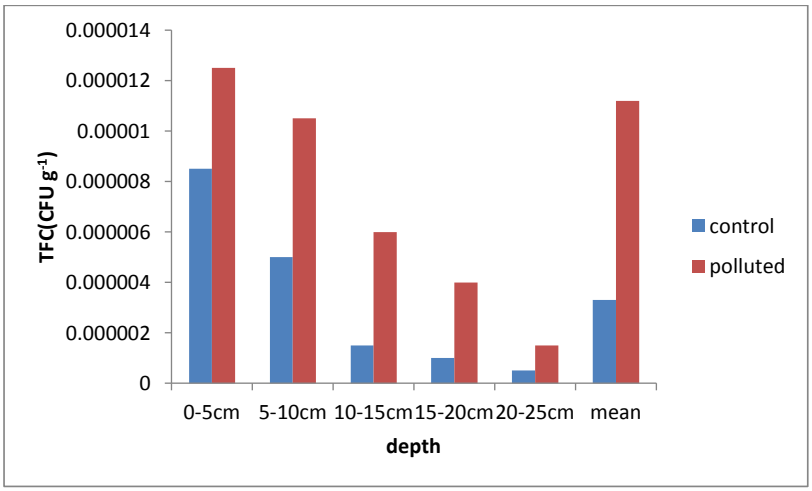

Figure 8: Impact of the cassava mill effluent on total fungal count

the bacterium specie which could be due to significant glucose level in cassava effluent (Uzochukwu *et al*. 2001) and the higher pH of the polluted site (Table 1) which enhances faster growth of the bacterium (Don 2008). *Streptococcus* spp was more prominent in the control site whereas *Pseudomonas* spp was more prominent in the polluted site. These findings suggest that cassava mill effluent promotes the proliferation of Pseudomonas spp but inhibits the growth of *Streptococcus* spp. However, cassava effluent did not influence the growth of *Baccilus* spp, *Staphylococcus aureus* and *Conynebacterium* spp (Table 1) as the aforementioned bacteria species were identified in the both sites. The findings further revealed that most of the bacteria species identified were observed at the upper depths which could be due to the high organic matter of the depths. It has been reported that organic matter is particularly important as the prime habitat for immense numbers and variety of soil fauna and microflora (Bullock 2005).

Identification of Fungal Isolates

Table 2 shows the various fungi species identified in the soil samples studied. A total of six fungi species were identified namely, *Candida* spp, *Penicillum* spp, *Rhizopus* spp, *Aspergillus niger*, *Rhodonturula* spp and *Fusarium* spp. Findings suggest that cassava mill effluent promoted the proliferation of *Candida* spp and *Fusarium* spp but inhibited the proliferation of *Rhodonturula* spp as the former were identified in the polluted site only whereas the latter was identified in the control site only. Penicillum spp was more prominent in the polluted site relative to the control site, an indication that the cassava effluent favours the growth of the fungus which could be due to a higher quantity of decaying organic matter in the polluted site occasioned by the deposition of cassava effluent into the soil. Bullerman (2003) reported that the presence of decaying organic materials in an

TABLE 1
Identification of the bacterial isolates

Samples	Media	morphological characteristics	gram reaction	OX Test	MOT Test	IND Test	SPORE Staining	CAT Test	CIT Test	COA test	Sugar ferm test S H_2S	B	G	Possible bacteria
A1,A2,A3,B1,B2,B3	N.A	Milkish raised non mucoid colonies	Gram positive cocci	-	-	-	-	+	-	-	R -	Y	-	*Staphylococcus aureus*
B1,B2,B3,B4	N.A	Milkish flat mucoid colonies	Gram negative rod	-	-	+	-	-	-	-	Y -	Y	+	*Escherichia coli*
A1,A2,B1,B2,B3	N.A	Milkish raised non mucoid colonies with zone of clearance	Gram positive cocci in chains	-	-	-	-	-	-	-	R	R	- -	*Streptococcus* spp
A1,A2,A3, A4,B1,B2,B3,B4	N.A	Milkish flat non mucoid colonies with rough edges	Gram positive rod	-	-	-	+	+	-	-	R -	R	-	*Bacillus* spp
A1, A2,A3, B1, B2,	N.A	Bluish green raised pigmented colonies	Gram negative rod	+	+	-	-	+	-	-	R -	R	-	*Pseudomonas* spp
A1,A2,A3,A4,B1,B2, B3,B4	N.A	Milkish raised needle pointed colonies	Gram positive rod	-	-	-	-	+	-	-	R -	R	-	*Conynebacterium* spp

Notes: N.A=nutrient agar, S=slope colouration, B=butt colouration, G=gas production, H2S= hydrogen sulphide production, Y=yellowish colouration (acidic), R=reddish colouration (alkaline), mot= motility, Ind= Indole, Cat= catalase, Cit= citrate, Coa=coagulase, A1- 0-5 cm Polluted, A2- 5-10 cm polluted, A3-10-20 cm polluted, A4- 20-50 cm polluted, B1-0-5 cm control, B2-5-10 cm control, B3- 10-20 control, B4-20-50 cm control

TABLE 2
Identification of fungal Isolates

Sample(s)	Morphological characteristics	Microscopic characteristics	Possible fungi
A1, A2,A3,A4	Creamy raised non mucoid colonies	Budded yeast cells	*Candida* spp
A1,A2A3,A4,B 1,B2	Whitish broom-like cottony colonies with greenish centre	Septate hyphae with Candida borne on Sterigmata	*Penicillium* spp
A2, B2,	Whitish broom-like cottony colonies	Unbranched hyphae with terminal spores	*Rhizopus* spp
A1,B1	Whitish broom-like cottony colonies with yellowish green centre	Septate hyphae with candida borne on sterigmata	*Aspergillus niger*
A1	Whitish broom-like cottony colonies turning purple	Septate hyphae with spores	*Fusarium* spp
B2	Orange broom-like cottony colony	Non Septate hyphae with spores	*Rhodonturula* spp

Notes: A1= 0-5 cm polluted; A2= 5-10 cm polluted; A3=10-20 cm polluted; A4-=20-50 cm polluted; B1=0-5 cm control; B2=5-10 cm control; B3= 10-20 control; B4=20-50 cm control

TABLE 3
Comparison of the chemical and biological properties of soils of the polluted and control sites using *t*-Test

Soil property	Polluted	Control	T-test value (p<0.05)	Remark
Soil pH (H_2O)	5.716	6.601	0.002	Significant(-)
Soil organic matter	15.059	8.951	0.030	Significant(+)
Total nitrogen	1.29	0.728	0.028	Significant(+)
Available phosphorus	17.32	7.392	0.012	Significant(+)
Total exchangeable bases	7.651	4.059	0.014	Significant (+)
ECEC	8.159	5.091	0.036	Significant(+)
THC	229200	535100	0.0043	Significant(-)
TFC	146700	33000	0.0977	Significant(+)

Notes: ECEC=Effective cation exchange capacity; THC=Total heterotrophic bacterial count; TFC= Total fungal count

area promotes the proliferation of *Penicillum* spp. A comparison of the presence of *Rhizopus* spp and *Fusarium* spp in the polluted and control sites suggests that cassava mill effluent does not influence the proliferation of the two fungi species. Irrespective of the sites, most of the fungi species were identified in the upper depths with higher organic matter and soil aeration, revealing the positive contribution of organic matter and soil aeration to fungi proliferation (Zhang *et al.* 2013; Das 2011).

Comparison of the Chemical and Biological Properties of Soils of the Polluted and Control Sites Using t-test Analysis.

The results of the *t*-test statistical analysis conducted to compare some chemical (pH, organic matter, total nitrogen, available phosphorus, total exchangeable bases and ECEC) and biological properties (total heterotrophic bacterial count and total fungal count)of the cassava mill effluent in polluted and control sites are presented in Table 3. For chemical properties, the analysis indicated significant ($p< 0.05$) and positive impact of cassava effluent on organic matter, total nitrogen, available phosphorus and ECEC. The implication of these findings is that increasing the concentration of cassava effluent in the soils will significantly result in an increase in the values of the aforementioned chemical properties. However, cassava effluent significantly and negatively impacted on the soil pH as there was a negative significant ($p< 0.05$) difference between the polluted and control sites which indicates that increasing the concentration of cassava effluent in the soils will significantly decrease the pH of the soils. For biological properties, whereas significant negative ($p<0.05$) difference was observed between the total fungal count of the polluted and control sites, there was significant positive ($p< 0.05$) difference between the total heterotrophic bacterial count of the polluted and control sites. From the findings, it can be inferred that long-term discharge of cassava into the soil will result in a significant decrease in bacterial population of the soils but will result in an increase in the fungi population of the soils.

CONCLUSIONS

The results of this study revealed that long-term discharge of cassava mill effluent into the soil significantly increased soil organic matter, total nitrogen, available phosphorus, total exchangeable bases and effective cation exchange capacity but significantly decreased soil pH. Generally, long-term discharge of cassava effluent into the soil increased total fungal count but decreased total heterotrophic bacterial count. Specifically, the long-term discharge of the effluent promoted the proliferation of *Escherichia coli* and *Pseudomonas* spp bacteria species as well as *Candida* spp, *Fusarium* spp and *Penicillium* spp fungi species but inhibited the growth of *Streptococcus* spp bacterium specie and *Rhodonturula* spp fungus specie.

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