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Research Article

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Effect of Cassava Processing Effluent on Microbial Diversity and Physicochemical Constituents of Soils

Obire Omokaro[1*] and Deeyah Faith Epsibari[2]

[1,2]*Department of Microbiology, Rivers State University, P.M.B 5080, Port Harcourt, Nigeria.*

[*]Corresponding author
Obire Omokaro, Department of Microbiology, Rivers State University, P.M.B 5080, Port Harcourt, Nigeria.

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Abstract

This paper examines the effect of cassava processing effluent on the microbiological and physicochemical constituents of soils at Luyor Gwara in Khana, River State, Nigeria. The parameters of concern were investigated using standard analytical techniques. Bacterial and fungal counts were reported as colony forming units (CFU) and spore forming units (SFU) respectively. Mean values of total heterotrophic bacterial and fungal count of the polluted soils ranged from 1.18 × 107CFU/g to 1.90 × 107CFU/g and from 1.4 × 106 SFU/g to 7.0 × 106 SFU/g respectively. While bacterial and fungal counts in the control soil ranged from 3.0 × 106CFU/g to 4.0 × 106CFU/g and 1.0 × 106 SFU/g to 3.0 × 106 SFU/g respectively. Except for station C that had lower fungal counts, the bacterial and fungal counts were higher in polluted soils than in control soils. There was statistical significant difference in the total heterotrophic bacterial and fungal count at p≤0.05. The bacteria frequencies were Staphylococcus aureus (20.58%), Bacillus spp (17.64%), Escherichia coli (14.7%), Corynebacterium spp (11.8%), Lactobacillus spp (8.82%), Pseudomonas spp (8.82%), Alcaligenes faecalis (8.82%), Klebsiella spp (5.88%), and Kurthia spp (2.94%). While fungi were Aspergillus niger (30%), Penicillium spp (20%), Microsporium canis (15%), Fusarium spp (10%), Mucor spp (10%), Saccharomyces cerevisiae (10%), and Epidermophyton floccosum (5%). The soil temperatures ranged from 27.8 to 25.4℃, pH from 6.99 to 7.84, Electrical conductivity from 172 to 60µs/cm, Sulphate ranged from 184 to 34.5mg/kg, Nitrate from 61.77 to 31.10mg/kg, Phosphate from 10.5 to 2.1mg/kg, and total Organic Carbon ranged from 0.51 to 0.045%. Generally, physicochemical constituents were higher in the cassava effluent polluted soils than in the control. The cassava effluent however impacted negatively on the soil microbial populations and diversity which will sure affect the soil ecology and fertility. The presence of potential pathogens poses serious health hazard by disease associated with these organisms.

Keywords: Soil, Cassava effluent, microbial diversity, physicochemical constituents.

Introduction

Cassava (Manihot esculenta Crantz) was brought to Africa in the 16th century by the Portuguese traders from Brazil around 1550 [1]. Cassava is a root tuber crop that is widely cultivated in the southern parts of Nigeria for its edible root tubers. It is mainly a food crop whose tubers are quite rich in carbohydrate (85.9%) with very small amount of protein (1.3%) in addition to cyanogenic glucosides [2].

From the nutritional and economic point of view, Cassava is the most important and major food item [3, 4]. Cassava root tubers and leaves are not consumed raw because they are highly toxic. Cassava is often classified as sweet or bitter according to the cyanide content of the root tubers. Tubers containing less than 50mg HCN/kg are classified as sweet or non toxic while tubers with greater amounts are classified as bitter or toxic [5, 6]. The cassava that is mostly grown in the Niger Delta region of Nigeria are the bitter and high cyanide toxic variety. Fermentation is therefore necessary in processing cassava tubers to reduce the cyanide levels and render it non-toxic before consumption [7].

In Nigeria and in other countries, Cassava can be processed into several traditional delicacies such as “Garri”, Fufu, Lafun, Tapioka, etc. [3, 4]. However, Garri is the most popular of all in the Niger Delta and it is a dry granular fermented product of cassava which can be consume directly or further processed into a dough known as “Eba” before consumption with soup. Garri production is done in household scale, co-operative scale and on industrial

scale i.e, small, medium and large scale). The household scale is the most popular in the Niger Delta. Garri processing from Cassava involves, peeling of the skin off the root tubers to expose the flesh which is washed with large volumes of water before grating the tubers into a pulp. The pulp is allowed to ferment for 24hours to 48 hours while being allowed to dewater by pressing. The dewatered pulp is sieved and dried by dry frying into dry granulated flour [8]. Large volume of effluent containing hydrocyanic acid and other toxic substances are generated during the washing, grating and pressing processes. Unfortunately, these effluents are discharged indiscriminately without any form of treatment onto surrounding soils [9].

Soil is composed of minerals, gases, liquid, organic matter, and both microorganisms and macro-organisms which together support life [10, 11]. Soil has a profile of many layers and the 0 - 15 cm being the layer of microbiological activity. Microorganisms in this topmost layer play key roles in the decomposition of organic matter and in the recycling of nutritional elements such as carbon, nitrogen, phosphorus, sulphur, etc [12]. The topsoil being the exposed portion of the earth crust, receives the greatest impact from pollutants that are discharged.

Luyor Gwara in Khana Local Government Area is an agrarian area in Rivers state, Nigeria. Cassava is one of the crops produced in large quantities, most of which are processed by every household to garri. Cassava mills produce large volumes of cassava processing effluent that are indiscriminately discharged onto the surrounding soil, where they accumulate and sink, thereby posing serious health and environmental hazard. The discharge of untreated cassava processing effluent containing hydrocyanic acid and other toxic substances onto soils would surely result in the ecological imbalance of the biological and chemical constituents of the soil. This in turn will lead to reduced soil fertility and productivity and food insecurity. There is therefore the need to investigate the soil microbial population and diversity and the physicochemical parameters of soil as to ascertain the impact of cassava processing effluent on soil microorganisms and on the physicochemical constituents of soil polluted with cassava effluent.

The aims and objectives of this study therefore were to cultivate, enumerate and characterize the total culturable aerobic heterotrophic bacteria and fungi in cassava effluent polluted soils and unpolluted soil (control) and to determine some of the physicochemical properties. Comparison of results of cassava effluent polluted soils and control will ascertain the effect of cassava effluent on the microbial populations and diversity and physicochemical constituents of the soils.

Materials and Methods

Cassava Effluent Polluted Soil Sample Collection

Soil samples were collected from five (5) locations in a cassava mill at Luyor Gwara in Khana Local Government Area of Rivers State with the aid of an ethanol disinfected auger borer. Cassava effluent polluted soil samples were collected from the point of discharge of cassava effluent and from three (3) other locations 15m away from each location after the point of discharge of cassava effluent. These locations were designated stations A (point of effluent discharge), B, C, and D (effluent receiving pit). Another soil sample not exposed to cassava effluent was collected outside the location of the cassava mill and this served as the control soil. Soil samples for the microbiological analysis were collected with the aid of a sterile augur borer from 0 – 15 cm top soil layer into sterile sample bottles labeled A, B, C, D, and Control respectively. Samples were placed in cool box containing ice packs to suppress metabolic activities of the soil microorganisms. While another batch of soil samples for the physicochemical analysis of soil constituents were collected from each location into separate sterile polythene bags and appropriately labeled. The samples were immediately transported to the laboratory for immediate processing and analysis.

Media Preparation

Three microbiological media were used for the primary isolation and culturing of the isolates. They include Nutrient agar, Sabouraud dextrose agar and MacConkey agar. These media were all prepared according to manufacturers' instruction before use [13]. The diluent used for serial dilution was normal saline because it helps to reactivate microorganisms. Serial dilution was made serially in one –tenth stepwise for each of the five soil samples up to 10-5 dilution [14].

Cultivation of Bacteria and Fungi

From the dilutions of 10-2 and 10-4 of each soil sample, 0.1ml (aliquot) was transferred asceptically onto fresh prepared Nutrient agar plates and SDA plates, using the spread plate method with the aid of a sterile glass bent rod [14, 15]. The inoculated plates were inverted and labeled accordingly and incubated at 370C for 24 hours for Nutrient agar plates and at 280C for 5- 7 days for SDA plates. The discrete colonies which developed were counted and the average counts for duplicate cultures were recorded as total viable aerobic heterotrophic bacteria and fungi in the sample with regards to size of colony, colour, and edge.

Isolation and Enumeration of Bacteria and Fungi and Preparation of Stock Culture

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types which appeared on the cultured plates into freshly prepared Nutrient agar plates which were incubated at 370C for 24 hours. Pure cultures of fungi were obtained by subculturing disctete colonies onto freshly prepared Sabouraud dextrose agar plates and incubated at 280C for 5-7 days. The following standard characterization tests were performed: Macroscopic examination of fungal growth was carried out by observing the colony morphology – colour (pigmentation), texture and surface appearance. Microscopic examination was done by using the wet mount technique and observing them under the microscope [15]. The complete identification of fungal isolates was done by comparing the result of their cultural and morphological characteristics with those of known Taxa [16, 17].

Glycerol solution (10%) was prepared and dispensed in McCartney bottles and autoclaved at 1210C for 15minutes, allowed to cool, then the pure cultures were inoculated into each McCartney bottle, till the clear colourless solution turns turbid and stored in the refrigerator. This serves as pure stock cultures for subsequent characterization [18]. The following standard characterization tests were performed: Gram's staining reaction, Motility test, Catalase test, Coagulase test, Starch hydrolysis, Indole, Methyl red, Voges Proskauer, and Carbohydrate fermentation test (Glucose, Fructose, Sucrose, and Lactose). The organisms were tentatively identified

to the genera level using identification manuals [19].

Microscopic Examination of Fungi Using Wet Mount

The wet mount method as described by Cheesebrough was used [20]. A small portion of the isolate was picked with a sterile needle and teased out in a drop of water on a clean microscopic slide using wire loop to emulsify the smear. A drop of lactophenol cotton blue was added to the smear and emulsified, covered with a cover slip and was examined under microscope, starting with ×10 objective and highest power objective, ×40 for better field and magnification. The microscopic examination included sexual and asexual reproductive structures like conidia, conidiophores reliable characters for specie recognition.

Analysis of the Physicochemical Parameters of the Cassava Effluent Polluted Soils Samples

The soil samples were analyzed for temperature, pH (hydrogen ion concentration), conductivity, nitrate, phosphate, sulphate by Spectrophotometer/Turbidiometry and organic carbon according to [21, 22].

Results

Total Heterotrophic Count of Bacteria and Fungi in Cassava Effluent Polluted Soils and Control Soil in the Different Locations

The total heterotrophic count of bacteria and total fungal count of the cassava effluent polluted soils and control soil in the different locations are as shown in Figure 1 and Figure 2 respectively. The total heterotrophic count of bacteria in locations A, B, C, and D of the cassava effluent polluted soils ranged from 1.79×107CFU/g to 1.58×10^7 CFU/g, from 1.58×10^7CFU/g to 1.40×10^7CFU/g, from 1.35×10^7CFU/g to 1.18×10^7CFU/g, and from 1.90×10^7CFU/g to 1.77×10^7CFU/g respectively. While bacterial count of the control ranged from 3.5×10^6 CFU/g to 3.0×10^6 CFU/g soil. The mean value of the total heterotrophic bacterial count in locations A, B, C, and D for the duration of the study was1.70×10^7 CFU/g, 1.49×10^7 CFU/g, 1.24×10^7 CFU/g and 1.82×10^7 CFU/g respectively. While the mean value for the Control soil was 0.35×10^7 CFU/g. The calculated F-value (141.9) for the bacterial counts is greater than F critical (F0.05 (1)4, 10) (3.48) the null hypothesis is rejected. Hence there is significant difference.

The total fungal count in locations A, B, C, and D of the cassava effluent polluted soils ranged from 6.2×106SFU/g to 4.6×10^6SFU/g, from 3.5×10^6SFU/g to 2.0×10^6SFU/g, from 1.6×10^6SFU/g to 1.4×10^6SFU/g and from 7.0×10^6SFU/g to 5.5×106SFU/g respectively while fungal count of the control ranged from 3.0×106SFU/g to 1.0×10^6SFU/g. The mean value of the total fungal count in locations A, B, C, and D for the duration of the study was 5.4×10^6 SFU/g, 2.8×10^6 SFU/g, 1.5×10^6 SFU/g and 6.2×10^6 SFU/g respectively. While the mean value of fungi for the Control soil was 2.0×10^6 SFU/g. The calculated F-value (141.9) for the fungal counts is greater than F critical (F0.05 (1)4, 10) (3.48) the null hypothesis is rejected. Hence there is significant difference.

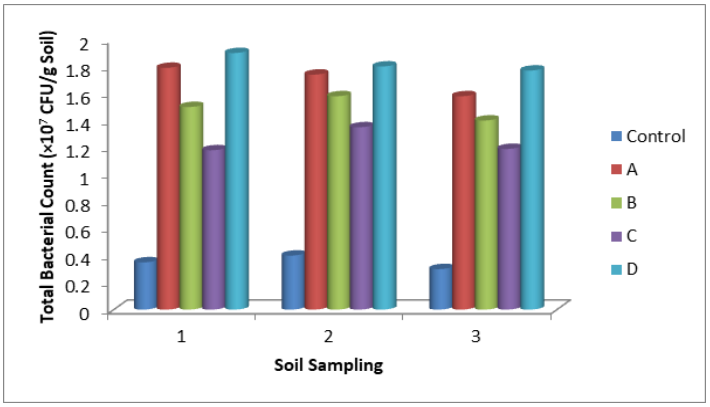

Figure 1: Total Heterotrophic Bacteria Count (CFU/g soil) of Cassava Effluent Polluted Soils

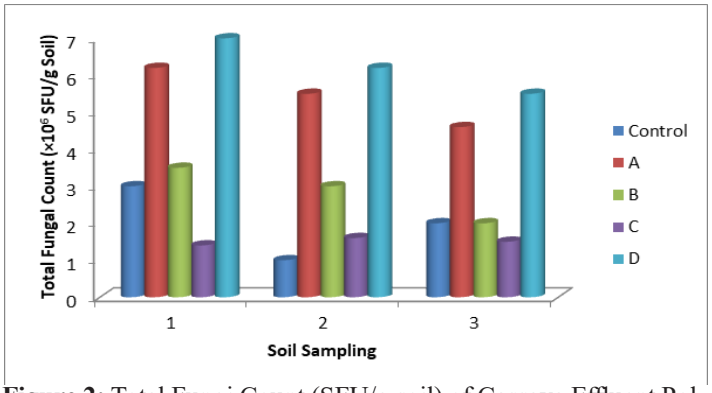

Figure 2: Total Fungi Count (SFU/g soil) of Cassava Effluent Polluted Soils

[Table 1] shows the bacteria and fungi that were isolated, identified, characterized and their percentage frequency of occurrence during the study. The bacteria were Staphylococcus aureus (20.58%), Bacillus spp (17.64%), Escherichia coli (14.7%), Corynebacterium spp (11.8%), Lactobacillus spp (8.82%), Alcaligenes faecalis (8.82%), Pseudomonas spp (8.82%), klebsiella spp (5.88%) while the least was Kurthia spp (2.94%). The fungi isolates were Aspergillus niger (30%), Penicillium spp (20%), Microsporium canis (15%), Fusarium spp (10%), Mucor spp (10%), Saccharomyces cerevisiae (10%), while the least was Epidermophyton floccosum (5%).

Table 1: Frequency of Isolation of Bacteria and Fungi from cassava effluent polluted soils

Bacteria	Frequency (%)	Fungi	Frequency (%)
Staphylococcus aureus	20.58	Aspergillus niger	30
Bacillus spp	17,64	Penicillium spp	20
Escherichia coli	14.7	Microsporium canis	15
Corynebacterium spp	11.8	Fusarium spp	10
Pseudomonas spp	8.82	Mucor spp	10
Lactobacillus spp	8.82	Saccharomyces cerevisiae	10
Alcaligenes faecalis	8.82	Epidermophyton floccosum	5
Klebsiella spp	5.88	-	-
Kurthia spp	2.94	-	-

The mean values of physicochemical constituents of cassava effluent polluted soils and control soil is shown in [Table 2]. The physicochemical parameters of the effluent polluted soil of the each location and control were determined, the result are as follows; pH value ranged from 7.37-7.84; Conductivity value ranged from 149-172µs/cm; Sulphate value ranged from 168.0-184.0mg/kg; phosphate values ranged from 9.4-10.5mg/kg; Nitrate value ranged from 31.10-61.77mg/kg; Total Organic Carbon values ranged from 0.047-0.51%; Temperature values ranged from 25.4-27.80C.While the control values were pH 6.99; Conductivity 60µs/cm, Sulphate 34.5mg/kg, Phosphate 2,1mg/kg, Nitrate 21,77mg/kg, Total Organic Carbon 0.045%, and Temperature 27.80C.

Table 2: Mean values of physicochemical constituents of cassava effluent polluted soils and control soil

Parameter	Control	A	B	C	D (Pit)
Temperature (ºC)	27.8	27.8	25.4	26.2	27.5
pH	6.99	7.59	7.37	7.48	7.84
Conductivity µs/cm	60	160	149	157	172
Sulphate mg/kg	34.5	172.0	168.0	170.0	184.0
Phosphate mg/kg	2.1	10.3	10.1	9.4	10.5
Nitrate mg/kg	21.77	35.30	33.59	31.10	61.77
Total organic carbon %	0.045	0.054	0.047	0.51	0.49

Discussion

This present study has revealed the presence and population of various bacteria and fungi associated with soils polluted with cassava processing effluent or wastewater. It was observed throughout the study that, counts of both total heterotrophic bacteria and fungi decreased from the point of discharge which is location A through B to location C. Ehiagbonare et al., (2009) reported that fresh cassava effluent is known to be high in cyanogenic content which led to the greatest loss of microbes in the soil thus causing soil infertility for agricultural production. However, location D being the effluent receiving pit recorded the highest count for both heterotrophic bacteria and fungi while the control soil recorded the lowest count except for the fungal counts of location C [23]. Location D being the effluent receiving pit recorded the highest microbial counts as a result of the accumulation of the wastewater and hence nutrients in the pit. This can also be attributed to the fact that, the cassava effluent in the pit from where soil sample D was collected had undergone fermentation which must have reduced the cyanogenic content of the soil (Akingbala et al., 2014), thereby allowing the proliferation of both bacterial and fungal population. Analysis of variance of total heterotrophic bacteria and fungi count (ANOVA) using F-test showed that there was significant difference in the heterotrophic bacteria and fungi count of various samples from the different locations at $p \leq 0.05$. Degradation of cyanide during fermentation has been reported widely to be distributed in natural ecosystems and have enzymatic systems that can be broadly described as oxidative, hydrolytic, and substitution transfer in nature [24, 25].

The bacteria isolated from the cassava effluent polluted soils in this study and their frequencies were Staphylococcus aureus (20.58%), Bacillus spp (17.64%), Escherichia coli (14.7%), Corynebacterium spp (11.8%), Lactobacillus spp (8.82%), Alcaligenes faecalis (8.82%), Pseudomonas spp (8.82%), Klebsiella spp (5.88%) while the least was Kurthia spp (2.94%). The fungi isolates were Aspergillus niger (30%), Penicillium spp (20%), Microsporium canis (15%), Fusarium spp (10%), Mucor spp (10%), Saccharomyces cerevisiae (10%), while the least was Epidermophyton floccosum (5%). These microorganisms have been reported to be associated with fermentation of cassava and cassava processing effluent [26, 27]. Okafor and Uzuegbu (1987) also isolated Lactic acid bacteria and showed their association with cassava fermentation. Oyewole and Odunfa (1988) reported Bacillus spp, Corynebacterium spp, Lactobacillus spp, Klebsiella spp [28, 29]. Bacillus spp, Corynebacterium spp, Lactobacillus spp, Klebsiella spp and Aspergillus niger were also reported by [24, 28]. Aspergillus spp, Penicillium

spp, Mucor spp, and Saccharomyces cerevisiae have been reported by [24, 28].

On the other hand, Akpan et al., (2011) also isolated Lactococcus lactic, Lactobacillus lactis, Bacillus subtilis, Pseudomonas aeruginosa while the fungi isolated were also Aspergillius niger, Penicillium spp., and and Rhizopus spp., from cassava effluent in their study [9]. Cassava waste water constitutes one of the barriers between disease, organism and man. Every city, town and village is faced with the problem of disposing of watery waste from cassava processing which usually bring about serious nuisance and health problems [9]. Most of the bacteria and fungi isolated in this study are known potential pathogens of man and domestic animals. Several diseases are reported to be associated with the bacteria (Prescott et al., 2002) and fungi (Sykes and Outerbridge, 2014) isolated from the cassava processing effluent polluted soils of this present study [29, 30].

In this study, the cassava effluent increased pH, sulphate, phosphate, nitrate, total organic carbon, electrical conductivity, and counts of bacteria and fungi of polluted soils. Akpan et al., (2011) also reported an increase in pH, N, organic carbon, exchangeable acidity, microorganisms, but they however reported a decreased CEC, available P, Mg and K [9]. The pH of the soil samples in this present study move towards alkaline, with a pH value ranging from 6.99 - 7.84 which simply means that fungi are generally aciduric growing between pH 4.0-6.5 and some can withstand lower acidity, it therefore means that the soil samples encourage the proliferation of bacteria than fungi. The conductivity value for control was 60 µs/cm and that of the polluted soils ranged from 149-172µs/cm. The electrical conductivity is a measure of the salinity of the soil and saline soils are found to contain large amount of soluble salts such as sodium, calcium, magnesium with chlorides, and bicarbonates [31]. According to the Federal ministry of Agriculture and Natural Resources (1990), a good soil electrical conductivity level is above 100 µs/cm while poor level rate has values below 100 µs/cm, therefore from this result, the electrical conductivity of the polluted soils are found to be higher than the control soil which means that there is not enough nutrient available in the soil and could perhaps show a sterile soil thereby resulting in the decline in the activity of soil microorganisms [32]. This impact important soil processes such nitrification, denitrification and decomposition. The nitrate levels of the polluted soil were found in the decreasing order of 61.77-31.10mg/kg, while that of the control soil is 21.77mg/kg, in high cases the rate is above 40 and in low cases it is less than 40 [32]. From this result, the nitrate levels in the control are normal while that of the polluted soil are high, this agree with the work of (Martin, 1977) that the production of nitrate is greater in neutral to alkaline pH than in acidic environment. The available phosphate was also found in the decreasing order, which is 10.5-9.4mg/kg and 2.1mg/kg for control soil. The values obtained for total phosphorous suggest that the cassava effluent in polluted soil can increase the phosphate level in soil. The total organic carbon ranges from 0.047-0.51% and control is 0.045%. From this result, the high organic carbon content of the effluent polluted soil may have contributed to the proliferation of these aerobic microorganisms as reported by previous workers [33, 34]. This explains the higher counts of bacteria and fungi reported in the polluted soils in contrast to the control soil. This explains that the microorganisms were able to utilize the starch and other mineral in the cassava processing effluent or wastewater as nutrients and they also make a large portion soluble releasing quantities in excess of their own nutritional demands.

Generally, physicochemical constituents were higher in the cassava effluent polluted soils than in the control. The cassava effluent however impacted negatively on the soil microbial populations and diversity which will sure affect the soil ecology and fertility. The presence of potential pathogens poses serious health hazard by disease associated with these organisms. Considering the high levels of phosphorous and nitrates and other constituents of cassava effluent polluted soils, the effluent should be allowed to undergo some form of treatment before it is discharged into the environment. Such treatments could convert cassava processing effluent into stable manure considering which would improve agricultural yields [1-40].

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