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Microbiological and Physicochemical Characteristics of Soil receiving Cassava Effluent in Elele, Rivers State, Nigeria

Eze V. C.[1,*], Onyilide D. M.[2]

[1]Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Umuahia, Abia State, Nigeria
[2]Department of Microbiology, Faculty of Science, Madonna University Elele, Rivers State, Nigeria
*Corresponding author: mekus2020@gmail.com

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Abstract The microbiological and physicochemical characteristics of soil receiving cassava effluent in Elele Rivers State, Nigeria were carried out. A total of twenty four (24) samples were collected and analysed microbiologically for total aerobic bacterial plate count, coliform count, *Escherichia coli* count and fungal count using pour plate technique. The media used were nutrient agar, MacConkey agar, eosin methylene blue agar, Sabouraud dextrose agar. The T-test was used to test for significant difference. The mean total aerobic plate count of contaminated soil ranged from 5.76 ± 0.05 log_{10}cfu/g to 5.60 ± 0.11 log_{10}cfu/g, coliform count of contaminated soil ranged from 4.71 ± 0.07 log_{10}cfu/g to 4.56 ± 0.08 log_{10}cfu/g, *Escherichia coli* count of contaminated soil ranged from 2.56 ± 0.06 log_{10}cfu/g to 2.39 ± 0.11 log_{10}cfu/g, fungal count of contaminated soil ranged from 3.65 ± 0.09 log_{10}cfu/g to 3.47 ± 0.09 log_{10}cfu/g. The control sample has the following values for total aerobic plate count 5.84 ± 0.07 log_{10}cfu/g, Coliform count of 4.28 ± 0.11 log_{10}cfu/g, fungal count of 3.19 ± 0.16 log_{10}cfu/g and *Escherichia coli* count of 2.14 ± 0.12 log_{10}cfu/g. Microorganisms isolated were: *Klebsiella* species, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* species, *Enterobacter* species, *Bacillus* species, *Proteus* species, *Aspergillus* species, *Rhizopus* species and *Penicillium* species. Soil contaminated with cassava effluent caused some physicochemical changes in the samples collected which were: Cyanide content 3.0 mg/kg, conductivity 33.4uS/cm, phosphate 0.52 mg/kg, nitrate 0.35 mg/kg, sulphate 13.0 mg/kg, calcium 167.0 mg/kg, pH 6.3 mg/kg, magnesium 89.0 mg/kg, potassium 4.0 mg/kg and sodium 92.0 mg/kg. The cassava effluent should therefore be treated before discharge into the environment to prevent possible pollution.

Keywords: *cassava, characteristics, effluent, microbiological, physicochemical, Elele*

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1. Introduction

Cassava (*Manihot esculenta* Crantz, synonymous with *Manihot utilissima* Rhol) belongs to the family Euphorbiaceae. The tubers are quite rich in carbohydrates (85-90%) with a very small amount of protein (1.3%) in addition to cyanogenic glucoside (*Linamarin* and *Lotaustiallin*) which are present in cassava [1,2]. This high carbohydrate content makes cassava a major food item especially for the lower income earners in most tropical countries especially Africa and Asia [3]. Cassava is a starchy food for more than 300 million people in many tropical countries of the world. Cassava food products are the most important staples of rural and urban household in Southern Nigeria. In Nigeria, traditional foods processed at home/ in small scale cottage operation constitute the principal mode of utilization of cassava [4].

It is generally believed to have originated from Brazil in South America. Cassava has spread to many other tropical countries like West Indians, South East Asia, and other West Africa, especially in Nigeria, Sierra Leone and Liberia. In Nigeria, cassava is extensively cultured and classified into two kinds: namely Sweet cassava (*Manihot esculenta)* and Bitter cassava (*Manihot utilisssima)*. Bitter cassava contains glucoside which forms hydrocyanic acid during processing. Hydrocyanic acid can be removed by cooking or fermenting in water for specific period. There are varieties of cassava which differ significantly in their colour, stem and period of maturity [5].

Cassava processing plant also known as cassava mill was invented in 1919 and planted in 1934 and is extensively used in Nigeria, especially in the southern part where cassava is a major agricultural produce [6,7]. It is used to grind peeled cassava tubers which are drained for 2-4 days and then baked over fire in pans to produce Garri- a major staple food [8]. The edible tubers are processed into various forms which include chips, pellets, cakes and flour. The flour could be fried to produce Garri or steeped in water to ferment and produce fufu when

cooked [2]. The production and consequent consumption of cassava have increased extensively in recent times.

The increased utilization of processed cassava products has increased the environmental pollution associated with the disposal of effluents. The highly offensive odour emanating from the fermenting effluent calls for regulation in the discharge of waste generated [9,10]. In most areas, cassava mills are mainly on small scale basis, owned and managed by individuals who have no basic knowledge of environmental protection. Though on small scale basis, there are many of them, which when put together, create enormous impact on the environment. Cassava also contains much pollutant such as disease-causing pathogens e.g. bacteria and fungi. Disposal of agricultural by-products such as cassava waste from processing activities is a concern in Nigeria. There is an appreciable high level of contamination arising from the discharge of effluents on agricultural soil hence the need for proper treatment before discharge and conversion of these cassava wastes into biosorbent that can remove toxic and valuable metals from the effluent.

Effluent is a liquid or solid waste, especially chemicals produced by factories or from agricultural products or domestic waste. Effluents usually contain a wide variety of chemicals, debris and various microorganisms which are mostly emptied on soil or carried away through special underground pipes called Sewers. Types of effluents include industrial effluent, agricultural effluents, domestic effluent and storm effluent [9,11].

This work therefore, is aimed at assessing the impact of cassava effluents on soil with respect to its physicochemical parameters and microbiological characteristics in Elele, Rivers State, Nigeria.

2. Materials and Methods

2.1. Collection of Samples

A total of 24 samples were collected using sterile containers and were transported to the laboratory which was analyzed immediately. A sterile glass bottle was used for physicochemical analysis.

2.2. Chemical Reagents

The chemical reagents used in the study were of analytical grade and were products of BDH Chemicals, Poole's, England and Sigma Chemical Company, St. Louis Missouri, USA. The microbiological media used were products of Oxoid and Difco Laboratories, England. They were nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification and for stock culture, Sabouraud dextrose agar used for the isolation of fungi, eosin methylene blue agar for the isolation of *Escherichia coli* and MacConkey agar for coliform counts.

2.3. Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the polluted soils were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined by plating in duplicate using pour plate technique. Then the molten nutrient agar, Sabouraud dextrose agar, *Salmonella-Shigella* agar, thiosulphate citrate bile sucrose agar, MacConkey agar and eosin methylene blue agar at 45^0C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi, coliforms and *Escherichia coli* respectively. They were swirled to mix and colony counts were taken after incubating the plates at 30^0C for 48 hrs and preserved by sub culturing the bacterial isolates into nutrient agar slants which were used for biochemical tests.

2.4. Characterization and Identification of Bacterial and Fungal Isolates

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, and catalase production. Citrate utilization, oxidative/fermentative utilization of glucose, indole production, methyl red - Voges Proskauer reaction, urease and coagulase production, starch hydrolysis, production of H_2S from triple sugar iron (TSI) agar and sugar fermentation. The tests were carried out according to the methods described by [11,12,13] Microbial identification was performed using the keys provided in the scheme of [14].

Fungal isolates were examined macroscopically and microscopically using the needle mounts technique. Their identification was performed according to the scheme of [15] and [16].

2.5. Determination of the Physiochemical Parameters

A number of physicochemical parameters of the contaminated soil samples were determined. They included pH, conductivity, nitrate, phosphate, sulphate, oil content and exchangeable cations. The pH was measured using Hach pH meter (Model EC1O); conductivity was measured using Hach conductivity meter (Model CO150). Sulphate, nitrate and phosphate were determined using Barium chloride (Turbidimetric), Cadmium reduction and Ascorbic acid methods respectively. All analyses were in accordance with APHA [17].

2.6 Determination of Oil Content

The method was adopted from ASTM [18]. The soil samples were air dried and sieved. Ten grams of the air dried sieved samples were weighed into 60ml glass bottles and 20ml of tetrachloroethylene was poured into the glass bottles. These bottles were placed into a shaker maintained at room temperature. The system was allowed to into a 20ml glass bottle using a glass funnel stuffed with cotton wool on which anhydrous sodium sulphate was placed. Analysis of the samples was done using Hach DR4000 spectrophotometer. The TPH was determined by treating the extracts with silica gel before analyzing with the spectrophotometer.

2.7. Determination of Exchangeable Cations

The method for the determination was adopted from APHA [17]. The soil samples were first extracted using IN ammonium acetate solution. This was done by weighing 5g of sieved air dried samples and adding to 30ml of the

extracting solution in a tube. This was shaken on a mechanical shaker for two hours. They were then centrifuged for five minutes and the supernatant carefully decanted into a 100ml volumetric flask. This was then made up to the mark with the extracting solution. The exchangeable cations (Na, K, Ca^{2+}, and $Mg2^{+}$) of the extract were determined using Unicam Atomic Absorption Spectrophotometer, Model 969.

3. Results

The results of the microbiological and physicochemical characteristics of soil receiving cassava effluent in Elele Rivers State, Nigeria are recorded in Table 1-Table 3.

Table 1 shows the mean counts of microorganism isolated from contaminated and control soil. The mean microbial counts of the contaminated soil ranged as follows: total aerobic plate count, 5.60 ± 0.11 Log_{10} CFU/g to 5.76 ± 0.05 Log_{10}CFU/g; coliform count, 4.56 ± 0.08 Log_{10}CFU/g to 4.71 ± 0.07 Log_{10}CFU/g; *Escherichia coli* count, 2.39 ± 0.11Log_{10}CFU/g to 2.56 ± 0.06 Log_{10}CFU/g and fungal count, 3.47 ± 0.09 Log_{10}CFU/g to 3.65 ± 0.09 Log_{10}CFU/g. The ANOVA, P > 0.05 shows that there was no significant difference in the mean microbial counts of contaminated soil samples within the different weeks. The mean microbial counts of the control soil were total aerobic plate count, 5.84 ± 0.07 Log_{10}CFU/g; coliform count, 4.88 ± 0.01 Log_{10}CFU/g; *Escherichia coli* count, 2.74 ± 0.04 Log_{10}CFU/g and fungal count, 3.89 ± 0.06 Log_{10}CFU/g. The t-test showed that there was significant difference between the microbial counts of the contaminated soil and the control soil samples.

Table 1. The mean counts of microorganisms isolated from the soil contaminated with cassava effluent

	Log_{10}cfu/g			
Week	TABPC	CC	EC	FC
1	5.76 ± 0.05	4.71 ± 0.07	2.56 ± 0.06	3.65 ± 0.09
2	5.75 ± 0.04	4.69 ± 0.05	2.54 ± 0.04	3.64 ± 0.08
3	5.73 ± 0.02	4.65 ± 0.01	2.53 ± 0.03	3.57 ± 0.01
4	5.74 ± 0.03	4.63 ± 0.01	2.51 ± 0.01	3.59 ± 0.03
5	5.72 ± 0.01	4.60 ± 0.04	2.47 ± 0.03	3.49 ± 0.07
6	5.60 ± 0.01	4.56 ± 0.08	2.39 ± 0.01	3.47 ± 0.09
Control	5.84 ± 0.07	4.28 ± 0.01	2.14 ± 0.04	3.19 ± 0.06

Key: TAPC – Total aerobic plate count; CC – Coliform count, EC – *Escherichia coli*
FC – Fungal count.

Table 2. The microorganisms isolated from the contaminated and control soil samples and their percentage occurrence

Isolate	Control (%)	Contaminated (%)	Total (%)
Staphylococcus aureus	2(14.29)	2(14.29)	4(28.58)
Pseudomonas aeruginosa	2(14.29)	1(7.14)	3(21.43)
Escherichia coli	1(7.14)	1(7.14)	2(14.28)
Enterobacter species	2(14.29)	2(14.29)	4(28.58)
Klebsiella species	2(14.29)	2(14.29)	4(28.58)
Proteus species	1(7.14)	1(7.14)	2(14.28)
Bacillus species	1(7.14)	1(7.14)	2(14.28)
Aspergillus species	1(7.14)	2(14.28)	3(21.43)
Rhizopus species	1(7.14)	1(7.14)	2(14.28)
Penicillium species	1(7.14)	1(7.14)	2(14.28)
Total	14(100)	14(100)	28(200)

Table 2: Shows percentage occurrence of total microorganisms isolated 28(100%). Bacteria isolated are: *Klebsiella* species 4(28.58%), *Staphylococcus aureus* 4(28.58%), *Pseudomonas* species, 3(21.43%), *Enterobacter* species 4(28.58%), *Escherichia coli* 2(14.28%), *Bacillus* species 2(14.28%) and *Proteus* species 2(14.28%). Fungi isolated are: *Aspergillus* species 3(21.43%), *Rhizopus* species 2(14.28%) and *Penicillium* species 2(14.28%). P-value is greater than 0.05 (1.000), hence there was no significant difference in the amount of microorganisms isolated from both contaminated and uncontaminated sample.

The mean values of the physicochemical parameters of the contaminated soil and the control soil sample are shown in Table 3. The contaminated soil had the following mean values, pH 6.3 ± 0.02; conductivity, 33.4 ± 1.0 µS/cm; oil content, 1.0 ± 0.03 mg/Kg; cyanide content, 3.0 ± 0.05 mg/Kg; phosphate, 0.52 ± 0.01 mg/Kg; nitrate, 0.35 ± 0.01 mg/Kg; sulphate, 13.0 ± 0.50 mg/Kg; calcium, 167.0 ± 5.0 mg/Kg; magnesium, 85.0 ± 2.0 mg/Kg; potassium, 4.0 ± 0.2 mg/Kg and sodium, 92.0 ± 3.0 mg/Kg. The mean values for the control soil sample were observed as follows: pH, 7.1 ± 0.2; conductivity, 16.6 ± 0.5µS/cm; oil content, 1.0 ± 0.01 mg/Kg; cyanide content, <0.01 ± 0.00 mg/Kg; phosphate, 0.36 ± 0.01 mg/Kg; nitrate, 0.32 ± 0.01 mg/Kg; sulphate, 11.0 ± 0.5 mg/Kg; calcium, 203.0 ± 8.0 mg/Kg; magnesium, 89.0 ± 3.0 mg/Kg; potassium, 5.0 ± 0.5 mg/Kg and sodium, 106.0 ± 4.0 mg/Kg. The t-test showed that there was significant difference in the mean values of the physicochemical parameters of contaminated and control soil samples for conductivity, cyanide content, phosphate, sulphate, calcium, magnesium and sodium while there is no significant difference for pH, oil content, nitrate and potassium.

Table 3. The mean values of the physicochemical parameters of the contaminated and control soil samples

Parameter	Contaminated	Control
pH	6.3	7.1
Conductivity (µS/cm)	33.4	16.6
Oil Content (mg/kg)	1.0	1.0
Cyanide Content (mg/kg)	3.0	<0.01
Phosphate (mg/kg)	0.52	0.36
Nitrate (mg/kg)	0.35	0.32
Sulphate (mg/kg)	13.0	11.0
Calcium (mg/kg)	167.0	203.0
Magnesium (mg/kg)	85.0	89.0
Potassium (mg/kg)	4.0	5.0
Sodium (mg/kg)	92.0	106.0

4. Discussion

This research has shown the microbiological and physicochemical characteristics of soil receiving cassava effluent. There was an observed increase and decrease in most of the physicochemical parameters.

The microbial load of the contaminated soil was lower than the control sample. The relatively low count of contaminated soil could be attributed to the effluent making the soil acidic due to the presence of cyanide. The presence of the cyanide in the soil could lead to inhibition of bacteria growth [3].

The bacteria isolated from cassava effluent included *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter* spp., *Klebsiella* spp., *Proteus* spp. and *Bacillus* spp. while the fungi isolated were *Aspergillus* spp., *Rhizopus* spp. and *Penicillium* spp. [9,19]. *Bacillus* spp. is well known indigenous and persistent bacteria in the soil environment [20,21]. The microorganisms isolated from the effluent contaminated soil were in line with the works of [9] and [19], who isolated similar organisms. The organisms may release toxins in the effluent which can be very harmful. Fungal infection acquired through the environment causes allergies and only a few are transmissible from infected humans [19].

The presence of coliforms is indication of human faecal contamination. This may result from inhabitants who defaecate near the bushes where the cassava effluent is being produced. The faecal materials might enter the stream through runoff as rainfall. *Escherichia coli* is not specifically confined to the human intestine. It is also present in the faeces of many domestic animals and birds and can be source of contamination [22,23,24].

Cassava effluent can also be contaminated with normal flora of cassava users and handlers as well as some pathogenic microorganism, which are not visible but can be detected by careful isolation procedures.

Cassava effluents are of concern because of much pollutant they contain [25]. The liquid residue, technically called *Manipueira* contain mineral such as nitrogen, carbon, phosphorus, calcium, magnesium, sulfur, manganese, sodium, which after anaerobic biodigestion can still be used for irrigation, since the digestion process does not substantially decrease the mineral content [26]. Continuous application of effluent to soil result into withering [27].

The physiochemical parameters investigated showed variations among samples. Soil contaminated with cassava effluent caused some changes in soil samples collected. The cyanide content, conductivity, phosphate, nitrate and sulphate were higher in the contaminated soil samples than the control soil. The mean values of pH, magnesium, potassium, calcium and sodium of the contaminated samples were lower than those of the control soil due to high content of hydrogen cyanide present in the contaminated soil [25]. Cassava tubers contain cyanogenic gylcosides namely: *Linamarin* and *Louaustralin* which are formed from amino acids. They are stored in the vacuoles of plant cells and are converted into hydrogen cyanide (HCN). When the contents of the vacuoles come in contact with the cell wall, they allow the hydrolysis of *linamarin* and *louaustralin* to occur. The hydrogen cyanide dissolves in the effluent and remain in solution, when it enters the soil part of the cyanogenic glycosides remain unconverted by microorganisms because of the few enzymes present in the cassava fibre which are not enough for complete conversion. This is detrimental to soil health and reduces quality of the soil and can result in the decrease of soil pH (increased acidity), magnesium, potassium, calcium and sodium while cyanide content, conductivity, phosphate, nitrate and sulphate were on the increase. This cations present in these metals are dangerous and poisonous to humans when consumed.

A cassava mill should be situated far from residential homes. Cassava mills must be owned and managed by individuals who have basic knowledge of environmental protection. Effluents from cassava processing plants must be regarded as harmful waste water and should not be allowed to spread over farm lands without thought of possible negative side effects. Effluent can also be used as herbicide or for weed control.

5. Conclusion

The effluent from cassava plant when discharged on soil causes physiochemical and microbiological changes in the soil which calls for serious concern if the soil will be used for agricultural and other purposes. It is clear that most of these cassava mills are sited near residential areas. There is therefore the need for an introduction of regulations to control the disposal of effluent generated from cassava.

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