International Journal of
Health Systems and Medical Science

ISSN: 2833-7433
Volume 1 | No 4 | Oct-2022

Microbial and Physicochemical Assessment of Soil Contaminated with Cassava Waste Water in Makurdi Metropolis

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Abstract: Environment pollution is a burning topic of the day. Air, water and soil are being polluted alike. Soil being a "universal sink" bears the greatest burden of environmental pollution. It is getting polluted in a number of ways. There is urgency in controlling the soil pollution in order to preserve the soil fertility and increase the productivity. In this research work, the microbial and physiochemical assessment of soil contaminated with cassava waste water were studied using standard-based method and standard analytical methods. A total of 6 soil samples were obtained from Naka road, North bank and Gboko road. Three of the soil samples were contaminated with cassava waste water and the remaining three soil samples were used as control. The isolation and enumeration of microbial population was carried out using standard-based methods. Standard analytical methods were used to assay for physicochemical properties. The highest bacterial count of $3.40x10^3$, $2.85x10^3$ and $2.70x10^3$ CFU/g for Naka road, Gboko road and North bank respectively while for uncontaminated soil were $4.70x10^4$, $2.90x10^4$ and $2.70x10^4$ CFU/g for North bank Naka road, and Gboko road respectively. There is significant difference in the total viable count between contaminated and uncontaminated ($P<0.05$). The fungal counts for the polluted and control soil ranged from fungi count $1.16 \times 10^3 \pm 5.70 \times 10^1$ to $1.4\times10^3\pm2.82\times10^3$ CFU/g, respectively. The fungal counts were significantly lower than the bacterial counts ($p < 0.05$). The bacteria isolates were *pseudomonas* spp, *Bacillus* spp, *Micrococcus* spp, *Klebsiella* spp, *Escherichia* coli, *Staphylococcus* spp, *and Proteus* spp and for the fungi isolates were *Aspergillus* spp, *Geotrichum* spp, *Mucor* spp and *Rhizopus* spp. The present study shows that the cassava effluent can have an increasing or limiting effect on the microbial diversity of the polluted soil which could also be attributed to the simultaneous impact on the physicochemical parameters of the soil. Therefore the release of Cassava waste water into the environment should be discouraged; processor should be trained on simple treatment technique on effluents that will make it less harmful to the environment. And there need for public awareness on the danger of releasing effluents into the environment.

Keywords: Soil, Cassava, waste water, Organisms.

INTRODUCTION

Environment pollution is a burning topic of the day. Air, water and soil are being polluted alike. Soil being a "universal sink" bears the greatest burden of environmental pollution. It is getting polluted in a number of ways. There is urgency in controlling the soil pollution in order to preserve the soil fertility and increase the productivity. Pollution may be defined as an undesirable change in the physical, chemical and biological characteristics of air, water and soil which affect human life, lives of other useful living plants and animals, industrial progress, living conditions and cultural assets. A pollutant is something which adversely interfers with health, comfort, property or environment of the people. In case most pollutants and wastes are introduced into the environment through sewage, accidental discharge, by-products or residues from the production of things that are useful. Through these process natural resources such as soil, water and air are polluted (Ashraf *et al.*, 2014).

Microorganisms are very small forms of life that can sometimes live as single cells, although many also form colonies of cells. A microscope is usually needed to see individual cells of these organisms. Many more microorganisms exist in topsoil, where food sources are plentiful, than in subsoil. They are especially abundant in the area immediately next to plant roots called the (rhizosphere), where sloughed-off cells and chemicals released by roots provide ready food sources. These organisms are primary decomposers of organic matter, but they do other things, such as provide nitrogen through fixation to help growing plants, detoxify harmful chemicals (toxins), suppress disease organisms, and produce products that might stimulate plant growth. Soil microorganisms have had another direct importance for humans—they are the source of most of the antibiotic medicines we use to fight diseases (8).

Soil microorganisms can be grouped into bacteria, actinomycetes, fungi, algae, protozoa, and nematodes. Apart from the dead plant or animal residues in soils, Soil Organic Manure is composed of a significant content of living microorganisms and their dead fractions. The humus fraction is resistant to microbial decomposition and persists for thousands of years contributing to the long-lived carbon pool in soils. Soil microorganisms are involved in the decomposition of soil organic matter, and the rate of decomposition depends both on the nature of microorganisms in soil and the nature of organic matter sources. Enhancing the activities of soil fungi has been recognized as one of the potential options for reducing Soil Organic Carbon turnover, thereby increasing carbon sequestration. Melanin, chitin, and glomalin are examples of fungal-derived recalcitrant residues that tend to exist for a long time in soils. Apart from the humification process, soil microorganisms are involved in mineralization of Soil Organic Manure, thereby resulting in the loss of carbon from soils (16).

Cassava (*ManihotesculentaCrantz*, synonymous with *ManihotutilissimaRhol*) 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 cyanogenicglucoside (*Linamarin* and *Lotaustiallin*) which are present in cassava (12). This high carbohydrate content makes cassava a major food item especially for the lower income earners in most tropical countries especially Africa and Asia (5). 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 (Eze and Onyilide 2015).

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 (*Manihotesculenta*) and Bitter cassava (*Manihotutilisssima*). 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 (10).

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. 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 (7). 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 (15). 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 (Ferronato and Torretta 2019). 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 (4). The aim of this study is to determine the Microbial and Physiochemical assessment of soil contaminated with cassava waste water in Makurdi Metropolis, Benue State.

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Makurdi Local Government Area. Makurdi local Government Area has a population of 300,000 persons (NPC, 2006), and lies between latitudes 7º40¹N and 7º53¹ N of the equator, and between longitudes 8º22¹E and 8º35¹E of Greenwich Meridian. It is a163km radius circle, covering 804km² land mass. Climatically, Makurdi falls within the tropical, sub-humid, wet and dry climate which has two distinct seasons, namely wet and season and dry season. The wet season starts from April and lasts till October, while the dry season starts in November and lasts till March. Rainfall ranges from 775 millimeters to1792 millimeters, with a mean annual value of 1190 millimeters.

3.2. Sampling Techniques

A total of 6 soil samples were obtained from Naka road, North bank and Gboko road. Three of the soil samples were contaminated with cassava waste water and the remaining three soil samples were used as control. The samples were collected using sterile containers and were transported to the laboratory for analysis.

3.3 Chemicals and reagents.

Nutrient agar, Macconkey agar, Potato dextrose agar (PDA), distilled water, Acetone, Simon's citrate agar, Urea agar, peptone water, hydrogen peroxide, lacto phenol cotton blue, and picric acid.

3.4 Equipments, Apparatus and instruments

Weighing balance, Test tubes and test tube rack, wire loop, Heating mantle, conical flask, Petri dish, pH meter, Sprayer, measuring Cylinder, Aluminum Foil, Spectrophotometer, Syringe, Incubator, pressure pot, Sample Bottle, Spirit Lamp, Cotton wool, Microscope and microscope slide.

3.5 Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the polluted soil 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, eosin methylene blue agar at 45^0C and was potato dextrose agar was poured into the petri dishes containing 1mL of the appropriate dilution for isolation of the total heterotrophic bacteria and fungi, coliforms and Escherichia coli respectively. They were swirled to mix and colony count was taken after incubating the plates at 30^0C for 48hrs and culture growth was preserved by sub culturing the bacterial isolates into nutrient agar slant which was used for biochemical tests.

3.6 Characterization and Identification of Bacterial and Fungal Isolates

Bacterial isolates were characterized and identify after studying their Gram reaction as well as cell micro morphology. Other tests like 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 H2S from triple sugar iron (TSI) agar and sugar fermentation were carried out according to the methods described by Ochei and Kolhatkar (2008). Fungal isolates were examined macroscopically and

microscopically using the needle mounts technique. Their identification was performed according to the scheme of APHA (2005).

3.7. Determination of the Physicochemical Parameters

A number of physicochemical parameters of the contaminated soil samples were determined. These include pH, conductivity, nitrate, phosphate, sulphate, oil content and exchangeable cations. The pH was measured using pH meter; conductivity was measured using conductivity meter. Sulphate, nitrate and phosphate were determined using Barium chloride (Turbid metric), Cadmium reduction and Ascorbic acid methods respectively. All analyses were in accordance with (2).

3.8 Biochemical tests

3.8.1 Catalase test. This test was carried out to determine the ability of the test organism to produce enzyme that breaks down hydrogen peroxide to oxygen and water. A drop of hydrogen peroxide was added to the growths isolated on the subculture plate and observation was made after 10-20 seconds. Observation of white bubbles confirms positive, while no bubbles with original color gives negative result (4).

3.8.2 Urease test. This test was done to determine the ability of the test organism to produce enzyme urease, which breaks down urea to ammonia and carbon dioxide.

2ml of urea agar was measured in to a sample bottle, slanted and allow cooling and jelling to occur. The test organism was collected, inoculated on the medium and incubated for 24 hour, after which a pink color was observed for positive and no color change for negative (4).

3.8.3 Indole test: This test was done to differentiate Gram negative bacilli. 2ml of peptone water was dispensed in to a sterilized sample bottle and the test organism was inoculated. This was incubated for 24-48 hours at 35-37^{0}c after which 0.2ml of Kovac's reagent was added and mixed. A positive test gives pink coloration at the top of the medium, while no color change is an indicative of negative test.

3.8.4 Citrate test: This test was carried out to determine the ability of the test organism to utilize citrate as its sole source of carbon. Simon's citrate agar medium was dispensed in a sample bottle and sterilize for 15 minutes at 121^{0}C. The organism was collected and inoculated incubated for 24 hours at 37^{0}C.

3.8.5 Microscopy: After 48 hours of incubation the suspended organisms were seen and were used to prepared smears on clean slides. The slides was cleaned with alcohol, the test organism was placed on each of the clean slides using a sterilized wire loop and each slide were stained with lactophenol for about 1 minutes. The slide was subjected to the observation of the suspected organism under oil immersion objective lens (x100) of a bright field microscope (3).

RESULTS

The mean viable, coliform and fungi count of soil samples contaminated with cassava waste water as presented in Table 1: the total heterotrophic bacteria and fungi count range from 2.70 x $10^3 \pm 8.49$ x 10^2 to $3.4\times10^3 \pm 8.49\times10^3$ CFU/g and fungi count 1.16 x $10^3 \pm 5.70$ x 10^1 to $1.4\times10^3 \pm 2.82\times10^3$ CFU/g. Control soil on the other hand ranges from 2.70 x $10^4 \pm 1.56$ x 10^3 for Gboko road to $4.0\times10^4 \pm 2.83\times10^3$ CFU/g for Naka road samples and $2.5\times10^4 \pm 7.49\times10^3$ CFU/g typically there is significant variation($p<0.05$). Table2: presents the prevalence of isolates across locations.

Table3: presents the cultural morphology and biochemical characteristics of bacteria isolates. Seven genera of bacteria were identified in this study *Pseudomonas* spp, *Klebsiella* spp, *Bacillus* spp, *Escherichia coli*, *Staphylococcus* spp, and *Proteus*spp: Table4: presents the morphology and characteristics of fungi isolates. Four (4) genera of fungi were identified in this study: *Aspergillus* spp, *Geotrichum* spp, *Mucor* spp and *Rhizopus* spp. Table 5: presents the physicochemical parameter of soil samples which were; Temperature, Soil pH, Colour and Texture.

Table 1: Total Viable, Coliform and Fungi Count of Samples from the Study Locations

Location	TVC	TCC	TFC
Naka Road	$3.40 \times 10^{3} \pm 8.49 \times 10^{2b}$	$1.60 \times 10^{3} \pm 8.49 \times 10^{2b}$	$1.16 \times 10^{3} \pm 5.70 \times 10^{1c}$
North bank	$2.70 \times 10^{3} \pm 8.49 \times 10^{2b}$	$2.00 \times 10^{3} \pm 1.13 \times 10^{3b}$	$3.00 \times 10^{3} \pm 1.69 \times 10^{3c}$
Gboko Road	$2.85 \times 10^{3} \pm 3.54 \times 10^{2b}$	$2.15 \times 10^{3} \pm 4.94 \times 10^{3b}$	$1.40 \times 10^{4} \pm 2.82 \times 10^{3b}$
Control(Naka Road)	$2.90 \times 10^{4} \pm 9.90 \times 10^{3b}$	$4.90 \times 10^{4} \pm 1.41 \times 10^{3a}$	$1.90 \times 10^{3} \pm 4.29 \times 10^{2c}$
Control(Naka Road)	$4.00 \times 10^{4} \pm 2.83 \times 10^{3a}$	$5.95 \times 10^{4} \pm 1.20 \times 10^{4a}$	$2.50 \times 10^{4} \pm 7.49 \times 10^{3a}$
Control(Gboko Road)	$2.70 \times 10^{4} \pm 1.56 \times 10^{3a}$	$5.40 \times 10^{4} \pm 2.82 \times 10^{4a}$	$1.22 \times 10^{4} \pm 1.13 \times 10^{3a}$
P- Value	0.008	0.001	0.002

Table 2: Prevalence of Isolates across Locations

Isolates	L1	L2	L3	C1	C2	C3	Total
Pseudomonas spp	0(0.00)	0(0.00)	0(0.00)	1(1.56)	2(3.13)	1(1.56)	4(6.35)
Bacillus spp	1(1.56)	1(1.56)	2(3.13)	3(4.69)	1(1.56)	2(3.13)	10(15.63)
Micrococcus	0(0.00)	1(1.56)	0(0.00)	1(1.56)	2(3.13)	1(1.56)	5(7.81)
Klebsiella spp	0(0.00)	1(1.56)	0(0. 00)	2(3.13)	3(4.69)	2(3.13)	8(12.50)
Escherichia coli	1(1.56)	0(0.00)	1(1.56)	1(1.56)	1(1.56)	0(0.00)	4(6.35)
Staphylococcus specie	1(2.86)	1(2.86)	0(0.00)	2(5.71)	0(0.00)	1(2.86)	5(7.81)
Proteus spp	0(0.00)	0(0.00)	1(1.56)	1(1.56)	2(5.71)	1(1.56)	5(7.81)
Aspergillus spp	1(1.56)	0(0.00)	1(1.56)	1(1.56)	1(1.56)	1(1.56)	5(7.81)
Geotrichum spp	0(0.00)	1(1.56)	0(0.00)	2(5.71)	1(1.56)	0(0.00)	5(7.81)
Mucor spp	0(0.00)	0(0.00)	0(0.00)	1(1.56)	0(0.00)	1(1.56)	4(6.35)
Rhizopus	2(5.71)	1(1.56)	2(5.71)	2(5.71)	2(5.71)	3(4.69)	2 (3.13)
Total	6(9.38)	6(9.38)	7(10.94)	17(26.56)	15(23.44)	13(20.31)	64(100)

Key: L1 – Naka Road L3 – Gboko Road C2- Control 2 spp-species

L2 - North Bank C1- Control 1 C3- Control 3

Table 3: Morphology and Biochemical Characteristics of Bacteria Isolates.

Colony colour	Colony shape	Morphology	Gram's reaction	Cat	Cit	Urease	Indole	Oxidase	H_2S	Suspected organisms
Cream	Circular	Cocci	+	+	+	-	-	-	-	*Staphylococcus* spp
Green Metallic Sheen	Circular	Rod	-	+	-	-	+	-	-	*Escherichia* coli
Yellow	Circular	Rod	+	+	+	-	-	-	-	*Micrococcus* spp
Mucoid	Circular	Rod	-	+	-	+	-	-	-	*Klebsiella* spp
Green	Circular	Rod	-	+	+	-	-	+	-	*Pseudomonas* spp
Pale	Circular	Rod	-	+	+	+	-	-	+	*Proteus* spp
White	Irregular	Rod	+	+	+	-	-	-	-	*Bacillus* spp

Key: H_2S- Hydrogen Sulphide

Cit- Citrate utilization

Cat- Catalase production

Rxn- Reaction

Table 4: Macroscopic and Microscopic Characteristics of Fungi

Macroscopic	Microscopic	Fungi isolates
Velvety filamentous white growth that sporulates black powdery spores	Long septate with conidiophores bearing brown spores and phialide at its apex	*Aspergillus* spp

Whitish smooth circular and raised colony or growth	Presence of arthrospore spores with rounded end	*Geotrichum* spp
White and wooly aerial growth that darkens as its sporulates	Non-septate hyphae with straight sporangiophore with many spherical spores.	*mucor* spp
Long hyphael growth which sporulated within two days to turn to black spore	Non-septate, branched mycelium with round shaped sporangia	*Rhizopus* spp

Key: spp – species

Table 5: Physicochemical Parameters

Sample	Temperature(°c)	Soil pH	Colour	Texture
Naka road	28	8.0	Brown	Silt
North bank	29	7.0	Dark-brown	Coarse
Gboko Road	25	7.5	Dark	fine
Control soil 1	30	8.2	Brown	Silt
Control soil 2	32	8.5	Dark-brown x	Coarse
Control soil 3	31	8.4	Dark	Fine

Discussion

Environment pollution is a burning topic of the day. Air, water and soil are being polluted alike. Soil being a "universal sink" bears the greatest burden of environmental pollution. (Ashraf *et al.*, 2014).The impact of Cassava waste water on the physiochemical and microbial quality of the soil around Cassava processing zone constitute great concern as it alters the natural environment. This effluent is released indiscriminately into the environment without any form of treatment. This activity of Cassava processor has serious impact on the soil as these effluents contain chemical that may affect the biotic components of the soil.

The result of this study reveal that the Microbial population (Total viable count, total coliform count and total fungi count) reduced significantly in soil contaminated with Cassava effluent when compared with control soil from the same area, although this contradict the findings of Igbinosa and Igiehon (2015) whose findings indicated that there is significant increase observed in the microbial density of the polluted soil.Total viable count for contaminated soil where $3.40x10^3$, $2.70x10^3$ and $2.85x10^3$ CFU/g for Naka road, North bank and Gboko Road respectively while for uncontaminated soil were $2.90x10^4$, $4.70x10^4$ and $2.70x10^4$ CFU/g respectively. There is significant difference in the total viable count between contaminated and uncontaminated .Although Igbinosa and Igiehon (2015) observed that the fungal counts of the polluted soil were significantly lower than of bacterial counts generally. This results shows that Cassava waste effluent negatively affect the microbial population. This may be attributed to the negative impact of harsh chemicals like cyanide that is present in the effluent and other chemical by-products of cassava fermentation. This finding agrees with (14 and 9).

Also the Study identified Pseudomonas specie, Bacillus micrococcus, *Klebsiella*, *Escherichia*coli, *Staphylococcus* and *Proteus* as bacteria genera found in the study area while *Aspergillus*Specie were fungi flora identified in this study. These bacteria and fungi species were isolated by previous authors (11 and 6). However, not all these soil microbial where found in the contaminated soil. *Pseudomonas*, *KlebisiellaProteus* specie and *Trichodema* were not found at all in the three area studied but were found in the control soil thereby suggesting that these microbial genera could not withstand the negative impact of the effluent. These findings of concern as disruption of the microbial constitute serious threat to the soil.

The fungal counts for the polluted and control soil ranged from fungi count $1.16 \times 10^3 \pm 5.70 \times 10^1$ to $1.4\times10^3\pm2.82\times10^3$ CFU/g, respectively. This suggests that the cassava effluent has effects on the fungal diversity of the polluted soil. The fungal counts were much lower than the bacterial counts and this is in agreement with the report from (1).

Conclusion

Based on the result of this study the following conclusions are reached.

1. That release of Cassava waste effluents to the soil affects the microbial population as well as the microbial diversities of the soil.
2. This waste contains pollutants that also affect the physicochemical composition of the soil.

Recommendations

1. The release of Cassava waste water into the environment should be discouraged.
2. Cassava processor should be trained on simple treatment technique on effluents that will make it less harmful to the environment.
3. There is the need for public awareness on the danger of releasing effluents into the environment.
4. Further research is recommended to find out ways for simple and affordable means of treatment and also ways of converting the effluent into useful substances (waste to wealth) that will benefit mankind.

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