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Microbial and physico-chemical analyses of soil receiving cassava mill waste water in Umudike, Abia State, Nigeria.

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Abstract

The microbiological and physico-chemical characteristics of soil receiving cassava mill waste water in five different cassava processing mill sites in Umudike, Abia State, Nigeria, were assessed. The mean bacterial counts of the assessed soil ranged from 3.72±4.51×10^5 cfu/g to 5.77±6.11 ×10^5 cfu/g for contaminated soil and 6.10±4.36×10^5 cfu/g to 8.79±4.51×10^5 cfu/g for pristine soil. The mean fungal counts ranged from 6.67±1.16×10^3 cfu/g to 11.00±1.00×10^3 cfu/g for the contaminated soil and 11.00±0.21×10^3 cfu/g to 17.33±1.01 x10^3 cfu/g for the pristine soil. The microorganisms isolated include; *Klebsiella* spp., *Proteus* spp., *Bacillus* spp., *Escherichia coli*, *Enterobacter* spp., *Staphylococcus aureus*, *Pseudomonas* spp., *Streptococcus* spp., *Micrococcus* spp., *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., yeast, *Mucor* spp. and *Fusarium* spp. The physicochemical parameters assessed had different ranges for the contaminated soil samples and the control. The study thus reveals that the cassava mill effluent has some deleterious effects on soil structure, soil microbiota, and soil quality, and hence recommends that appropriate measures be put in place to enforce and regulate the treatment of such effluents prior to discharge to receiving soil and/or water bodies.

Keywords: Microbial, Physicochemical, analyses, soil, cassava, waste water.

Introduction

Environmental pollution is considered to be one of the most dangerous hazards affecting both developing and developed countries. Most of our farm land and water bodies have become polluted due to industrial growth, urbanization and man-made problems resulting from population growth (Adewumi et. al., 2016). Effluents from industries cause high level of damage to life especially aquatic (Okafor, 2011). The indiscriminate discharge of contaminants from food processing and allied industries into the receiving environments lead to health related risks (Salami & Egwin, 2007).

The soil is the foremost constituent of the environment, and an important part of the ecosystem affecting directly or indirectly the environmental quality. It is where living things and majorly humans access nutrients for existence (Izah et. al., 2017). The soil is at the topmost layer of the earth's crust and formed through biotransformation of weathered rocks (Kolwan et. al., 2006). It has different layers having the topsoil is the most prolific. Soil microorganisms are found in the uppermost of the soil breaking down organic materials and cycling nutrients affecting global biogeochemical cycling (Bunning and Jimenez, 2003). The uppermost soil is majorly affected by contaminants from the environment consisting of different types of wastes including cassava mill effluents (Arimoro & Osakwe, 2006; Enenje & Ifenkwe, 2012; Izah et. al., 2016). Unfortunately, in developing countries like

Nigeria, the wastes from indstires and agro-based industries are seldomly treated (Izah et. al., 2017).

Cassava (*Manihot esculenta*) belongs to the family *Euphorbiaceae* and is grown almost entirely within the tropics. It is a perennial shrub grown for edible tubers thought to have originated in Tropical America and have been introduced to Africa around the 16th Century. It is widely cultivated, accounting for over half of the root tuber crops grown in African countries and one of the major foods produced in Nigeria. Recently the crop has become tremendously important industrially for production of livestock feed, starch, textile, industrial alcohol, and for the manufacture of cassava flour, macaroni, and a variety of beverages (Omomowo et. al., 2015). In Sub-Saharan Africa, cassava is currently the major staple food for 40% of the population and for an estimated 500 million people in the tropics (Burns et. al., 2010; Okafor, 2004). In Nigeria, 'garri' – a grated, fermented and dehydrated cassava food product is one of the most popular staple foods produced from cassava, which provides a major source of calories for families because of its high starch content. The processing of cassava has received more attention due to its increased importance in agricultural and economic development and also in food security, especially in Africa (Okafor, 2004).

Cassava processing into various food products produces a lot of waste water. The disposal of cassava waste water is of public health concern as it affects adversely the environmental and life generally (Omomowo et. al., 2015). It has been reported that cassava processing produces large discharge of waste water that contain substances that are highly lethal, mobile in soil, affect bio-diversities, destroy microbes, causes the extinction of benthic macro invertebrates, makes marine organisms difficult to survive, and inhibits the germination of cereal seeds (Akani et. al., 2006; Olorunfemi et. al., 2008; Omomowo et.al., 2015). Most cassava processing industries discharge significant quantities of cassava waste water, with cyanide as its major toxic component into rivers, lakes, sewage canals, agricultural fields and the environment. This eventually flows back to streams or downstream surface water locations (Oliveira et. al., 2001).

Currently, there is neither a specific method of disposal or of treating the cyanide-laden waste water resulting from cassava processing in Nigeria, nor any government policy guidelines (Okuande &Adekalu, 2013). Disposal of effluents from cassava processing is becoming an increasing problem, and with the increasing popularity of new, appropriate technologies for cassava processing, which can also be applied on a large scale, the future will see larger cassava processing plants, and waste handling may become a major problem (Omomowo et. al., 2015).

This study was therefore, aimed at evaluating the effect of cassava mill waste water on soil in Umudike, Abia State, Nigeria while analysing its physico-chemical parameters and microbiological characteristics.

Materials & Methods

Sample collection

Soil samples were collected from different cassava processing mill sites in five villages (Amaoba, Amawom, Ndolu, Olokoro, Umuariaga) in Umudike, Abia State, Nigeria. Three soil samples were collected from different sites per village. The collection points were the point of discharge of the cassava effluent and the control collected from a pristine source that has not witnessed any form of cassava effluent contamination. The samples were collected using sterile containers, labeled appropriately, and were transported to the laboratory afterwards in ice cube chest for immediate analysis.

Sample preparation and Enumeration of microorganisms

One gram of each of the soil samples was aseptically measured into sterile test tubes containing 9mls of distilled water to make stock solutions; from which subsequent ten-fold serial dilutions were made. The total aerobic bacterial counts and fungal counts were determined by the standard pour plate method. The Nutrient agar was used for bacteria and the plates incubated at 35±2°C for 24-48 hours. Sabouraud dextrose agar was used for fungi and incubated at 25±2°C for 72 hours. Microbial growth on the plates were counted and expressed as colony forming unit per gram (cfu/g) of the soil sample. Pure cultures of the isolates were obtained by sub-culturing in fresh medium and stored in the refrigerator at 4°C for further studies.

Characterization and Identification of the microbial isolates

The microbial isolates were characterized and identified using standard microbiological, cultural, morphological and biochemical characteristics as described by Cheesbrough (2006). The fungal isolates were further examined macroscopically, while the needle mount technique was used to examine them microscopically. They were identified using methods described by Gots et. al. (2003).

Analyses of physico-chemical parameters

Various physico-chemical parameters of the soil samples were analysed ranging from pH, organic carbon, organic matter, exchangeable bases to cyanide content. The Hach conductivity meter (Model CO 150) was used to measure the pH of the soil samples. The Turbidimetric method (using Barium Chloride) was used to analyse the sulphate content of the soil samples, Cadium reduction method was used to check for nitrate content and the Ascorbic acid method used for analysing phosphate contents of the soil samples as described by Eze & Onyilide (2015) and APHA (2005).

Results and Discussion

Table 1: Bacterial and Fungal counts of soils receiving cassava mill waste water

Location	Sample	Bacterial counts $\times 10^5$ cfu/g	Fungal counts $\times 10^3$ cfu/g
Amaoba	Abs	5.77±6.11	11.00±1.00
Amaoba	Abc	7.87±2.52	15.67±2.52
Olokoro	Ols	5.03±2.52	8.33±1.12
Olokoro	Olc	6.93±3.57	11.67±1.16
Amawom	Ams	4.65±4.04	6.67±1.06
Amawom	Amc	6.10±4.36	17.33±1.01
Ndoru	Nds	3.72±4.51	10.11±2.00
Ndoru	Ndc	7.80±3.61	11.00±0.21
Umuariaga	Ums	4.90±4.36	7.76±1.16
Umuariaga	Umc	8.79±4.51	14.67±1.16

Key: Abs: Amaoba sample; Abc: Amoba control; Ams: Amawom sample; Amc: Amawom control; Nds: Ndoru sample; Ndc: Ndoru control; Ols: Olokoro sample; Olc: Olokoro control; Ums: Umuariaga sample; Umc: Umuariaga control.

Values are means of triplicate analysis ± standard deviation.

Table 3: Physico-chemical properties of the soils receiving cassava mill waste water

	pH	Organic matter (%)	Exchangeable bases Sodium (mol/kg)	Potassium (mol/kg)	Calcium (mol/kg)	Phosphorus (mol/kg)	HCN (mg/kg)
Abs	5.63	1.47± 0.15	1.77±0.15	0.90± 0.17	4.80± 0.46	53.34± 1.17	125.16 ± 4.46
Abc	6.68	2.20± 0.20	2.13± 0.46	1.20 ±0.35	6.37± 2.51	35.12± 2.01	4.63± 7.51
Ols	6.47	1.87± 0.12	2.00± 0.17	0.67±0.04	3.50±1.00	47.43± 2.20	78.73± 8.75
Olc	6.65	2.40± 0.20	2.47± 0.42	1.20 ±0.17	4.30± 2.65	39.63± 1.54	5.47± 1.99
Ams	5.79	1.77± 0.16	1.83± 0.06	1.28 ± 0.12	2.50± 1.00	40.80± 7.03	108.6± 9.45
Amc	6.64	1.97± 0.16	1.93± 0.31	1.33 ± 0.46	3.18± 0.35	33.10± 8.72	3.90± 5.19
Nds	6.67	1.33± 0.02	3.13± 0.46	1.18 ± 0.06	2.33± 1.15	34.27± 1.17	49.47± 2.54
Ndc	6.78	1.47± 0.11	3.35± 0.06	0.73 ± 0.12	2.53± 1.15	28.43± 1.42	3.69± 1.28
Ums	6.32	2.53±0.12	2.53± 0.31	1.73 ± 0.13	3.07± 0.58	55.7± 5.32	59.93 ± 5.82
Umc	6.50	2.73±0.04	2.60± 0.10	1.27 ± 0.12	3.33±2.08	37.00±2.08	4.10±6.92

Values shows mean of duplicate analysis± standard deviation

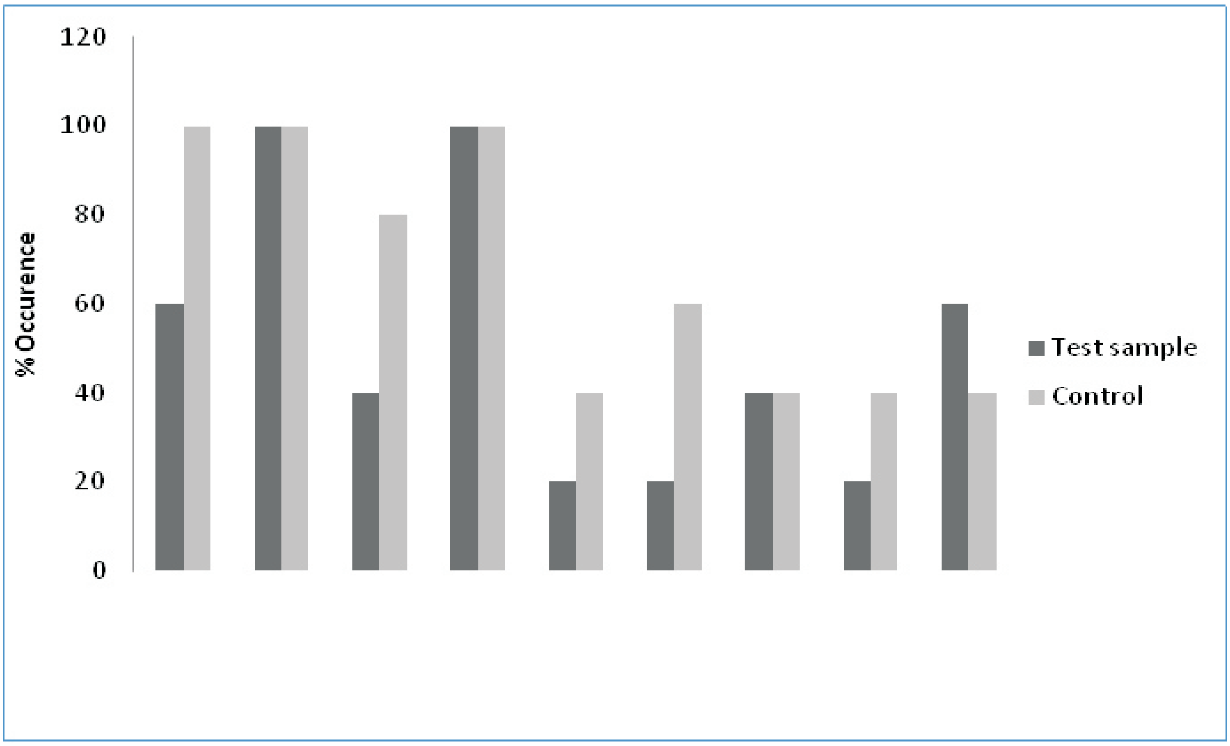

Figure 1: Occurrence of bacteria isolates in soils receiving cassava mill waste water

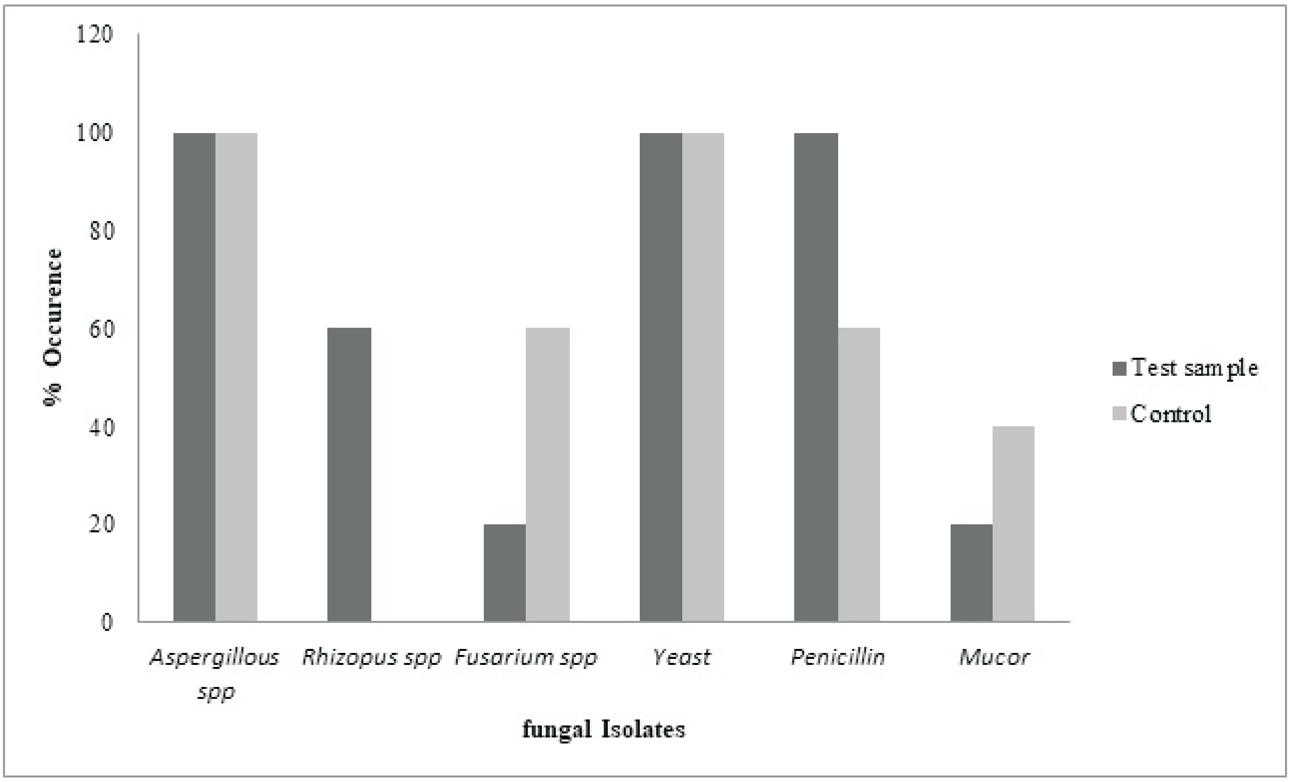

Figure 2: Occurrence of fungi isolates in soils receiving cassava mill waste water.

This study was conducted to assess the microbial and physico-chemical properties of soil receiving cassava mill waste water in five different areas across Umudike, Abia State, Nigeria. From the results obtained, the mean bacterial counts in the contaminated soils ranged from $3.77 \pm 4.51 \times 10^5$cfu/g (Nds) to $5.77 \pm 6.11 \times 10^5$cfu/g (Abs), while that of the control (pristine soil) samples ranged from $6.10 \pm 4.36 \times 10^5$cfu/g (Amc) to $8.79 \pm 4.51 \times 10^5$cfu/g (Umc) (Table 1). The average fungal counts in the contaminated soils were from $6.67 \pm 1.16 \times 10^3$cfu/g (Ams) to $11.00 \pm 1.00 \times 10^3$cfu/g (Abs), while counts of the pristine soil were from $11.00 \pm 0.21 \times 10^3$cfu/g (Ndc) to $17.33 \pm 1.01 \times 10^3$cfu/g cfu/g (Amc) (Table 1). The results of the microbial counts in the pristine soil samples were higher than that obtained from the contaminated soil samples, suggesting that the cassava waste water has some negative effects on the microbial diversity of the polluted soil. Microorganisms in soils are being rapidly used as sensitive indicators of soil health and quality because of the relationships between microbial diversity, soil and ecosystem sustainability (Hill et. al., 2000). The significant low counts of microbes found in the contaminated soil (when compared to the pristine soil samples) could be related to the waste water which makes the soil acidic owing to the presence of cyanide in the cassava waste water released into the soil. The presence of these toxic compounds could lead to the death of the microorganisms found in the soil or inhibit their growth. These results were found to be in consonance with those obtained by Izah and Aigberua (2017), who obtained lower microbial loads in cassava mill effluent contaminated soils in a rural community when compared to the analyzed control samples; Eze and Onyilide (2015), who recorded lower microbial load in cassava effluent contaminated soils when compared to their analyzed control samples, and Igbinosa and Igiehon (2015), who recorded significantly lower fungal counts in cassava effluent contaminated soils when compared to the analyzed control soil samples.

With regards to the microorganisms isolated from the soil samples, the bacterial isolates include; *Klebsiella* spp., *Proteus* spp., *Bacillus* spp., *Escherichia coli, Enterobacter* spp., *Staphylococcus aureus, Pseudomonas* spp., *Streptococcus* spp. and *Micrococcus* spp., while the fungal isolates include; *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., yeast, *Mucor* spp. and *Fusarium* spp. These isolates were similar to those obtained by Eze and Onyilide (2015), Igbinosa and Igiehon (2015), Izah and Aigberua (2017) and Omomowo et. al. (2015) on cassava mill effluents contaminated soil. As a result of some genetic traits acquired or possessed by these microorganisms, they are able to break down cyanide and survive in acidic environments (Okechi et. al., 2012). Bacillus spp. is an organism that is indigenous and constantly found in the soil environment. The aerobic spore formers have been used and have been implicated in the investigation of microbial quality of cassava flour "lafun". The release of toxic compounds into the waste water by the organisms can be harmful. The presence of coliforms usually shows contamination by human fecal matter. This can be as a result of defecation in near-by bushes where the cassava waste water is being released (Eze & Onyilide, 2015), or from domestic animals, that roam about the vicinity in quest for food. There can be contamination of cassava waste water with normal flora of cassava users and handlers; as regards to isolation of *Staphylococcus aureus*. Other microbes such as *Proteus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Micrococcus* spp., *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., yeast, *Mucor* spp. and *Fusarium* spp. have been widely reported in environmental samples and food (Izah & Aigberua, 2017).

Different trends were obtained in the occurrence of bacteria and fungi in the soils (Figures 3 and 4). For the bacterial isolates, *Bacillus* spp. and *Pseudomonas* spp. were found in all the soil samples (with 100% occurrence in both the control and contaminated soil samples), while *Staphylococcus aureus* recorded 100% occurrence only in the control samples, with 60% occurrence in the contaminated soil samples. *Escherichia coli* and *Streptococcus* spp. recorded the least percentage occurrence (with 20% in the polluted samples, and 40% in the control samples respectively). *Proteus* spp. recorded 80% occurrence in the control samples, and 40% in the contaminated soils; *Enterobacter* spp. recorded 60% occurrence in the control samples, and 20% in the contaminated soil samples. *Klebsiella* spp. recorded 40% occurrence in both the control and contaminated soil samples, while *Micrococcus* spp. recorded 60% in the contaminated soil samples, and 40% in the control samples respectively. For the fungal isolates, *Aspergillus* spp. and yeast occurred in all the samples (with 100% occurrence in both the control and contaminated soil samples respectively), while *Penicillium* spp. recorded 100% occurrence in only the contaminated soil samples, and 60% occurrence in the control samples. *Rhizopus* spp. was found only in the contaminated soil samples with 60% occurrence; none was recorded in the control samples. *Mucor* spp. recorded the least percentage occurrence (with 40% in the control samples and 20% in the contaminated soil samples), while *Fusarium* spp. recorded 60% occurrence in the control samples, and 20% occurrence in the contaminated soil samples.

The physico-chemical analyses of the soil samples are depicted in Table 3. The result had various ranges for the contaminated soil and the control. These include; pH that ranged from 5.63-6.67 for the contaminated soil sample; 6.50-6.78 for the control. Organic matter (%) 1.33±0.02-2.53±0.12 for the contaminated soil sample; 1.47±0.11-2.73±0.04 for the control. Sodium (mol/kg) 1.77±0.15–3.35±0.06 for the contaminated soil sample; 1.93±0.31–3.35±0.06 for the control. Potassium (mol/kg) 0.67±0.04 - 1.73±0.13 for the contaminated soil sample; 0.73 ± 0.12 - 1.33±0.46 for the control. Calcium (mol/kg) 2.33±1.15 - 4.80± 0.46 for the contaminated soil sample; 2.53± 1.15 - 6.37±2.51 for the control. Phosphorus (mol/kg) 34.27±1.17 - 55.7±5.32 for the contaminated soil sample; 28.43±1.42-39.63±1.54 for the control, HCN (mg/kg) 49.47±2.54 - 125.16 ± 4.46 for the contaminated soil sample; 3.69± 1.28 - 5.47±1.99 for the control. It is very visible from the result that the cassava mill waste water caused certain changes in the contaminated soil samples. The result indicated lower minerals (exchangeable bases) in the contaminated soils than in the control (apart from that of Phosphorus). Similar results were obtained by Eze & Onyilide (2015). Lower results pertaining to organic matter were obtained in the contaminated soils, when compared to the control samples, which suggests that microbial activity in the affected areas may be low, and this may affect the agricultural productivity of the soil. This also shows the negative impact of cassava mill waste water discharged in the soil.

Expectedly, the cyanide (HCN) levels in the contaminated soils were higher in the contaminated soil samples, when compared to the control samples, in fact much higher than the critical level of 50mg/kg which is toxic to human and livestock, as stipulated by the WHO recommendations (Adewumi et. al., 2016). "Cassava tubers contain cyanogenic glycosides 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. When the contents of the vacuoles come in contact with the cell wall, they allow the hydrolysis of linamarin and louaustralin to occur" (Eze & Onyilide, 2015). "The hydrogen cyanide dissolves in the effluent and remains 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" (Obueh & Odesiri-Eruteyan, 2016). This is harmful to soil health and causes reduction the soil quality resulting in the high acidity of soil (Eze & Onyilide, 2015). The occurrence of cyanide compound in the soil can result to the bacterial growth hinderance (Desse &Taye, 2001).

The pH values of the contaminated soil samples were found to be generally lower (apart from Sample 3, which fell within the WHO permissible values) than the WHO recommended values of 6.5-8.5, when compared to the control samples (which all fell within the permissible standard). "The low pH could be attributed to the presence of cyanogenic glycosides in the cassava waste water contaminated soil" (Etinosa, O. I. and Ozede, N. I. (2015). Cassava waste waters are of concern because of much pollutants they contain (Uzochukwu et. al., 2001). This portends a grievous consequence on the underground water resources in the nearest future if urgent steps are not taken to properly convey the cassava wastewater to aerobic treatment plants. More so, the continuous application of the effluent to the soil without treatment would result into soil withering and alteration in soil parameters.

Nigeria is no doubt one of the leading and largest cassava-producing nations in the world. Abia State is known for producing cassava in large quantities. However, the practice of constant disposal of untreated cassava wastewater directly to the soil, and/or surrounding water bodies has constantly raised public health issues and great concern. Apart from the high cyanide content of the wastewater, which was duly reported by this study, and which can be very detrimental to soil structure, quality, and biota, the untreated wastewater also accords a highly offensive odour to the environment. The study also confirmed the adverse effect of the cassava effluent on the soil microbial diversity, as the microbial load of the contaminated soils was low relative to the control samples. However, some organisms like *Aspergillus, Penicillium, Pseudomonas* and *Bacillus* species were found to thrive well in the effluent contaminated soil. Thus, there is a possibility of using this cassava waste water-tolerant species for bioremediation of such contaminated soils. The waste water can be used for the irrigation of crops after adequate treatment. Since the waste water is rich in nitrate, it will also assist in controlling erosion especially on sandy soils because it may increase the stability of such soils.

In all, the waste water from cassava processing mills, when discharged onto soils causes physico-chemical and microbiological changes in the soil, which calls for serious and urgent concern and intervention, especially if the receiving soils are to be used for agricultural and other related purposes. Therefore, there is the need for an introduction of regulations by National and State Environmental agencies to control the disposal of waste water generated from cassava mill.

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