Effect of cassava processing wastewater on the bacteriological quality of soil in Aba, Abia State, Nigeria

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Cassava wastewater from cassava mills can have negative impacts on farmlands despite the fact that cassava when milled are processed into different food products. Its possible deleterious effect to farmlands and human health has led to the study on the effects of cassava processing wastewater on the bacteriological quality of soil in Aba, Abia State, Nigeria. A total of fifteen samples were collected which constituted of five samples each from cassava wastewater, soil polluted with cassava wastewater and soil free of cassava wastewater (control). The total bacterial counts, total coliform counts and faecal coliform counts were determined using spread plate technique. The bacteriological load of the contaminated soil was lower than the control sample. The relatively low count of contaminated soil could be attributed to the acidity imparted to the soil by the effluent due to the presence of cyanide. The presence of the cyanide in the soil and fermented cassava could lead to inhibition of bacterial growth. Nine bacterial species of both fecal and non-fecal origin were isolated from the samples from untreated cassava mill wastewater, untreated cassava mill wastewater polluted soil and unpolluted soil. They were Klebsiella aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Micrococcus luteus and Bacillus cereus. The occurrence of some of the bacteria is associated with processing environment and equipment used in processing including water used in washing, knife/cutlass used in peeling, bag used in storing prior to pressing and hygienic status of the processors. Generally, wastewater from the cassava mill processing site could pose some detrimental problems on the soil. Therefore these wastewater from cassava processing plants must be regarded as harmful wastewater and should not be discharged into the environment without prior treatment.

Keywords: wastewater, farmlands, cassava and soil microbes
1. Introduction

Soil is the top layer of the earth’s lithosphere, formed from withered rock that has been transformed by living organisms [1]. Its formation is the result of the combined action of weathering and colonization of the geological materials by microbes [2]. Soil has many layers, with the soil being the most productive. The biological components of the top soil consist mainly of soil organisms, especially microorganisms which are key players in the circling of nitrogen, sulphur, phosphorous and the decomposition of organic residues. These affect nutrients and carbon cycling on the global scale [3]. The top soil receives the greater impact from pollutants.

Cassava is the third major source of carbohydrate in the world with diverse uses depending on the community, and it serves as food security for millions of people in the developing world [4]. All communities in Nigeria depend so much on cassava because of its wide usage when processed into garri, tapioca, akpu, fufu and starch.

In Nigeria, cassava is extensively cultivated and classified into two kinds: namely sweet cassava (Manihot esculenta) and bitter cassava (Manihot utilissima) which form 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 color, stem and period of maturity [5]. Cassava (Manihot esculenta crantz) is a root tuber crop that is widely cultivated in the tropical region of the world [6, 7]. As a shrubby perennial that grows to a height of 6.8ft, it is often propagated by planting short section of the stem [8, 9].

Cassava is mainly a food crop whose tubers are harvested between 7-12 months based on the cultivars planted. The tubers are quite rich in carbohydrate (85.9%) with very small amount of protein (1.3%), in addition to cyanogenic glucoside [10]. This high carbohydrate content makes cassava a major food item especially for the low income earners in most tropical countries especially Africa and Asia [11]. 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 variety of beverages [12]. Cassava is processed traditionally according to local customs and preferences [13]. There has been an upsurge in the production and utilization of cassava in the past few years. This has led to the establishment of cassava milling engines in most environments with the consequence of an extensive ecological pollution associated with the effluent discharge. Currently, effluent from cassava processing plants is regarded as harmless wastewater. It is allowed to spread over farm lands without thought of possible negative impacts, but it generates objectionable odor and constitutes breeding grounds for flies and insects, which are carriers of diseases. Therefore, it is crucial to assess the bacteriological quality of the cassava wastewater and its impacts on soil in Aba, Abia State, Nigeria so as to safeguard the health of the residents from any possible diseases associated with its effects.

2. MATERIALS AND METHODS

Study area

This study was carried out at five cassava-processing sites in Aba, Abia State, in southeast Nigeria. The study area is located between latitude 7°21’30”E and longitude 5°3’30”N as shown in Figure 1. Sample locations in the figure represents Iheorji, Iheorji Avenue, Umnogele, 16 Dike and Owerri Aba respectively. The area is popularly known for cassava production all year round, which is readily processed into garri flour. The cassava waste water (effluent) from the milling sites is allowed to flow into farmlands close to the processing sites.
Collection of samples

Five samples each of fresh cassava wastewater, soil polluted with cassava wastewater and soil free of cassava wastewater (control) making a total of fifteen samples were collected from cassava processing sites in Iheorji, Iheorji Avenue, Umuogeli, 16 Dike Road, Owerri Aba in Aba South Local Government area, Aba, Abia State and analyzed bacteriologically. The samples of cassava wastewater were aseptically collected using sterile syringes and sterile two-liter rubber containers with screw caps while the soil samples were collected using a sterile soil auger into sterile screw cap containers from a depth of 15cm and the soil free of cassava wastewater (control) were also collected at a distance of 100m away from the milling site. These samples were transported to the laboratory in an ice box within one hour of collection to maintain the temperature after which they were analyzed within 24 hours of collection.

Bacterial isolation

Total bacterial count
This was carried out as described by Cheesbrough [14]. Nutrient agar was weighed and prepared based on the manufacturer’s instruction. It was sterilized in an autoclave at 15 psi (121°C) for 15 minutes, allowed to cool and then aseptically dispensed into Petri dishes. 0.1ml of appropriate ten-fold serial dilution ($10^5$) of the samples was inoculated into each of the nutrient agar plates using spread plate method. Duplicate plates were prepared and labeled for the fifteen samples. The inoculated plates were incubated at 28°C for 24 hours after which the bacterial colonies that developed were counted using colony counter and the result recorded. Each colony was sub-cultured and stored on a sterile nutrient agar slant for characterization and identification.

**Total Coliform Count**

The method described by Chessbrough [14] was used. MacConkey agar was weighed and prepared based on the manufacturer’s instruction. It was sterilized in an autoclave at 15 psi (121°C) for 15 minutes, allowed to cool and then aseptically dispensed into Petri dishes. 0.1ml of appropriate ten-fold serial dilution ($10^5$) of the samples was inoculated into each of the MacConkey agar using spread plate method. Duplicate plates were prepared and labeled for the fifteen samples. The inoculated plates were incubated at 28°C for 24 hours after which the bacterial colonies that developed were counted and the result recorded. Each colony was sub-cultured and stored on a sterile nutrient agar slant for identification.

**Faecal Coliform Count**

The method described by Chessbrough [14] was adopted. Eosin methylene blue (EMB) agar was weighed and prepared based on the manufacturer’s instruction. It was sterilized in an autoclave at 15 psi (121°C) for 15 minutes, allowed to cool and then aseptically dispensed into Petri dishes. 0.1ml of appropriate ten-fold serial dilution ($10^5$) of the samples was inoculated into each of the Eosin methylene blue (EMB) agar using spread plate method. Duplicate plates were prepared and labeled for the fifteen samples. The inoculated plates were incubated at 28°C for 24 hours after which the bacterial colonies that developed were counted and the result recorded. Each colony was sub-cultured and stored on a sterile nutrient agar slant for further studies.

**Characterization and identification of isolates**

The bacterial isolates were characterized on the basis of their morphological, biochemical and molecular characteristics. Gram staining, motility test, catalase test, coagulase test, sugar fermentation test, indole test, oxidase test, methyl red test, citrate utilization test, Voges-Prostauer test, urease test and spore test were carried out as done by Cheesbrough [14]. They were identified according to the scheme of Krieg and Holt [15].
Gram staining

A smear of an isolate from each of the sample of the effluent, polluted and unpolluted soil were made on a clean slide and allowed to dry. It was heat fixed by passing the smear through the Bunsen burner. This was done to enhance the sticking of the organism to the microscope slide. The smear was flooded with crystal violet and left for 60 seconds before washing off with clean water. Lugol’s iodine was added and allowed to stand for 60 seconds before being washed off and decolorized with alcohol for 10 seconds. The slide was then washed off, stained with safranin for one minute, washed off and allowed to air dry. A drop of immersion oil was added to the slide which was then viewed under the microscope using 100× objective lens.

Motility test

Each bacterial isolate was separately inoculated into a semi-solid nutrient agar using a sterile straight wire and incubated at 37°C for 24 hours. Migration of the isolates away from the line of inoculation was recorded as a positive result while lack of migration away from the line of inoculation indicated a negative result.

Catalase test

This test is used to detect the enzyme catalase which protects the bacteria from hydrogen peroxide accumulation which can occur during aerobic metabolism. Catalase breaks the hydrogen peroxide into oxygen and water. It is used to differentiate those bacteria that produce the enzyme catalase, such as Staphylococci from non-catalase producing bacteria such as Streptococci. The organism was picked and emulsified on a clean slide placed inside a Petri dish. A drop of 3% hydrogen peroxide was added to the slide and the Petri dish was covered immediately. The preparation was observed for active bubbling through the lid. The presence of sustained active bubbles indicated a positive result while the absence indicated a negative result.

Coagulase test

Coagulase causes serum to clot by converting fibrinogen to fibrin. A drop of distilled water was placed on a clean slide and a colony of the test organism was emulsified in it. One milliliter of plasma was added to it and it was mixed gently. The clumping of the organism within 10 seconds indicated a positive result [14].

Sugar fermentation test
This test is used to detect organism which utilize different sugars as source of energy with the production of acid and/or gas. The sugars used were glucose, sucrose, lactose and mannitol. Peptone water broth was prepared based on manufacturer’s instruction. Bromothymol blue indicator was added to the broth. In four separate conical flasks containing glucose, sucrose, lactose and mannitol, the above solution (peptone water broth + indicator) was added at equal proportions. Five milliliters of the mixture were then dispensed into test tubes. Durham tubes were added in an inverted manner and the test tubes were sterilized in an autoclave at 121°C for 15 minutes and upon cooling, they were inoculated with the isolates individually and incubated at 37°C for 24 hours. A change in color of the mixture indicated acid production while gas production was indicated by void in the Durham tubes.

**Indole test**

This test was carried out to determine the organism that breakdowns the amino acid tryptophan into indole. This test is important in the identification of Enterobacteria. The organism was inoculated into a sterile test tube containing 3ml of peptone water and incubated for 48 hours at room temperature. 0.5ml of Kovac’s reagent was thereafter added and the mixture allowed to stand for 10 minutes. The development of a red color in the surface layer indicated a positive result.

**Oxidase test**

This test is used to detect the ability of bacteria to produce the enzyme cytochrome oxidase. This test is used to assist in the identification of *Pseudomonas*, *Neisseria* and *Vibrio*. The presence of this enzyme was tested by mixing two drops of 1% aqueous solution of tetramethyl-p-phenylene diamine dihydrochloride with the organism on the filter paper. The development of a blue-purple color within 10 seconds indicated a positive result.

**Methyl red test**

The test organism was introduced into glucose phosphate peptone water and incubated at 37°C for 48 hours. Five drops of methyl red reagent were added, mixed and result read. A red coloration indicated a positive result while yellow coloration indicated a negative result.

**Citrate utilization test**

The medium used was Simmon citrate agar. The test is used to identify which organisms can utilize citrate as the sole carbon for metabolism. It is used in the differentiation of the organism in the Enterobacteriaceae and other genera. A colony of the organism was streaked onto the medium and incubated at 37°C for 24 hours. A change in color from green to blue indicated a positive result.
Voges proskaeur test

The test organism was introduced into glucose phosphate peptone water and was incubated at 37°C for 48 hours. 3ml of Baritts A (alpha napthol) and 1ml Baritts B (potassium hydroxide) reagents were added mixed and the result read. A pink burgundy coloration indicated a positive result.

Urease test

The medium used was Urea broth. This test is used in differentiating Enterobacteria. The test tubes were sterilized and 2ml of the broth were dispensed into the test tubes. The test organism was heavily inoculated into the test tube and incubated at 28°C for 48 hours. The development of a pink color indicated a positive result.

Spore test

A smear of an isolate from each of the sample of the effluent, polluted and unpolluted soil were made on a clean slide and allowed to dry. It was heat fixed by passing the smear through the Bunsen burner. The smear was flooded with 3drops of malachite green and was placed on top of a beaker of water placed on a warm hot plate. This preparation was allowed to steam for 3 minutes without allowing it to evaporate. The hot plate temperature was adjusted to prevent the stain from boiling and the stain replenished when needed. The slides were thereafter removed from the hot plate, cooled and washed under running tap water so as to wash the malachite green from both sides of the microscopic slide. The smear was counterstained with safranin for 30 seconds. Then washed with tap water to remove secondary stain. It was allowed to air dry. A drop of immersion oil was added to the slide which was then viewed under the microscope using 100× objective lens.

Data Analysis

The data were subjected to one way anova using IBM SPSS Statistics 23 to determine the level of significance between the bacteriological parameters.
1. Results

The bacteriological quality (total bacterial count, total coliform count and faecal coliform count) of the untreated cassava mill wastewater are shown in Table 1. The total bacterial counts were 101-154 cfu/ml; total coliform count, 58-111 cfu/ml and faecal coliform count, 5-18 cfu/ml.

Table 1: Bacteriological quality of the untreated cassava mill wastewater.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cassava mill location</th>
<th>Total bacterial count (cfu/ml)</th>
<th>Total coliform count (cfu/ml)</th>
<th>Faecal coliform count (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iheorji</td>
<td>130</td>
<td>101</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Iheorji Avenue</td>
<td>154</td>
<td>111</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Umuogele</td>
<td>101</td>
<td>98</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>16 Dike Road</td>
<td>109</td>
<td>58</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Owerri Aba</td>
<td>127</td>
<td>88</td>
<td>18</td>
</tr>
</tbody>
</table>

The bacterial (total bacterial count, total coliform count and faecal coliform count) of the untreated cassava mill wastewater-polluted soil are shown in Table 2. The total bacterial counts were 81-111 cfu/g; total coliform counts, 34-99 cfu/g and faecal coliform count, 2-10 cfu/g.

Table 2: Bacteriological quality of the untreated cassava mill wastewater-polluted soil.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cassava mill location</th>
<th>Total bacterial count (cfu/g)</th>
<th>Total coliform count (cfu/g)</th>
<th>Faecal coliform count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iheorji</td>
<td>100</td>
<td>99</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Iheorji Avenue</td>
<td>111</td>
<td>66</td>
<td>2</td>
</tr>
</tbody>
</table>
The bacteriological quality (total bacterial count, total coliform count and faecal coliform count) of the unpolluted soil are shown in Table 3. The total bacterial counts were 150-289 cfu/g; total coliform count, 150-190 cfu/g and faecal coliform count, 10-19 cfu/g.

**Table 3:** Bacteriological quality of the unpolluted soil.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cassava mill location</th>
<th>Total bacterial count (cfu/g)</th>
<th>Total coliform count (cfu/g)</th>
<th>Faecal coliform count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iheorji</td>
<td>180</td>
<td>190</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Iheorji Avenue</td>
<td>289</td>
<td>189</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Umuogele</td>
<td>170</td>
<td>166</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>16 Dike Road</td>
<td>150</td>
<td>150</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Owerri Aba</td>
<td>191</td>
<td>170</td>
<td>19</td>
</tr>
</tbody>
</table>
The morphological and biochemical characteristics of the bacterial isolates from untreated cassava mill wastewater, untreated cassava mill wastewater-polluted soil and unpolluted soil are presented in Table 4. The isolates were *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus mirabilis*, *Micrococcus luteus* and *Bacillus cereus*.

**Table 4:** Morphological and biochemical characteristics of the bacterial isolates from untreated cassava mill wastewater, untreated wastewater-polluted soil and unpolluted soil.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Gram stain</th>
<th>Form</th>
<th>Catalase</th>
<th>Coagulase</th>
<th>Motility</th>
<th>Oxidase</th>
<th>Citrate</th>
<th>Indole</th>
<th>Methyl Red</th>
<th>Vogues Proskaeur</th>
<th>Urease</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Mannitol</th>
<th>Sucrose</th>
<th>Spore</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td><em>Klebsiella aerogenes</em></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td><em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>Cocccus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>Rods</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td><em>Bacillus subtilis</em></td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td><em>Proteus mirabilis</em></td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>Cocccus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>Micrococcus luteus</em></td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>Rods</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td><em>Bacillus cereus</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella aerogenes</em></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
</tr>
</tbody>
</table>
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+ = Positive reaction  
- = Negative reaction

**Table 5:** Number of untreated cassava mill wastewater, untreated cassava mill wastewater-polluted soil and unpolluted soil with bacterial isolates.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Untreated cassava mill wastewater</th>
<th>Untreated cassava mill wastewater-polluted soil</th>
<th>Unpolluted soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella aerogenes</td>
<td>2 (40.00%)</td>
<td>0 (0.00%)</td>
<td>4 (80.00%)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2 (40.00%)</td>
<td>4 (80.00%)</td>
<td>3 (60.00%)</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>1 (20.00%)</td>
<td>4 (80.00%)</td>
<td>3 (60.00%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0 (0.00%)</td>
<td>2 (40.00%)</td>
<td>2 (40.00%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2 (40.00%)</td>
<td>3 (60.00%)</td>
<td>2 (40.00%)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2 (40.00%)</td>
<td>0 (0.00%)</td>
<td>3 (60.00%)</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>4 (80.00%)</td>
<td>0 (0.00%)</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0 (0.00%)</td>
<td>4 (80.00%)</td>
<td>5 (100.00%)</td>
</tr>
<tr>
<td>Microccous luteus</td>
<td>0 (0.00%)</td>
<td>1 (20.00%)</td>
<td>0 (0.00%)</td>
</tr>
</tbody>
</table>

Number of untreated cassava mill wastewater, untreated cassava mill wastewater-polluted soil and unpolluted soil with bacterial isolates are shown in Table 5. **Klebsiella aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli and Staphylococcus aureus** were detected in 2 (40.00%), 2 (40.00%), 1 (20.00%), 2 (40.00%), 2 (40.00%) and 4 (80.00%) of the untreated cassava mill effluent; **Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Escherichia coli, Bacillus subtilis and Microccous luteus** were detected in 4 (80.00%), 4 (80.00%), 2 (40.00%), 3 (60.00%), 4 (80.00%) and 1 (20.00%) of the untreated cassava mill effluent.
wastewater-polluted soil while *Klebsiella aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis* Escherichia coli, *Staphylococcus aureus* and *Bacillus subtilis* were detected in 4 (80.00%), 3 (60.00%), 3 (60.00%), 2 (40.00%), 2 (40.00%), 3 (60.00%) and 5 (100.00%) of the unpolluted soil.
Discussion

Higher bacterial counts were observed in the unpolluted soil samples than those in the polluted soil and the cassava mill wastewater (Table 1). This is similar to the findings of Okoli et al. [16], who reported that cassava mill effluent negatively affected total heterotrophic bacterial count as a lower count was observed in the polluted soils compared to the control soils. This effect is at a higher range of $5.72 \times 10^5$ CFUg$^{-1}$ and $2.29 \times 10^5$ CFUg$^{-1}$ for the control and polluted sites, respectively. In a related study, Ibe et al. [17], as well as Obueh and Odesiri-Eruteyan [18], made a similar observation and attributed their findings to the acidic nature of the effluent due to the presence of cyanogenic glucoside in cassava mill effluent. This result also agreed with the report of Nwaugo et al., [19] that the bioload of all bacterial groups increased with distance away from the waste pit suggesting adverse growth conditions towards the pit. This observation suggests that the high content of cassava mill effluent suppressed bacterial growth but at a very low concentrations could support bacterial growth. High level of cyanogenic glycosides in the effluent is not metabolized by many microorganisms but when in low concentration, could be easily metabolized by the few organisms capable of doing so, while other bacteria species depends on the non-toxic intermediates produced [19].
Higher coliform counts were observed in the unpolluted soil samples than those in the polluted soil and the untreated wastewater (Table 2). A higher coliform count was obtained by Enerijiofi et al. [20], who carried out an assessment of the impact of cassava mill effluent (CME) on the microbial diversity, physicochemical parameters and heavy metals concentrations in the receiving soil and reported coliform count at the range of $1.20 \pm 6.1 \times 10^8$ CFU/g to $9.40 \pm 5.6 \times 10^8$ CFU/g.
The presence of coliforms is an indication of faecal contamination. The faecal coliform organisms were more in the unpolluted soil than the polluted soil and the untreated wastewater (Table 3). This may result from inhabitants who defecate near the bushes where the cassava effluent is being produced or from animals. The faecal materials might enter the stream through runoff as rainfall.

These bacterial counts may be due to lack of efficient control measures in the discharge of the waste water into the environment [21]. Uzochukwu et al. [22], reported that high level of cassava wastewater are produced daily and drained into roads, streets, rivers and agricultural lands in garri processing communities in Nigeria. These singular activities tend to expose the wastewater to bacterial contamination.

The decline in bacterial population due to the effect of CME could also affect the soil environment receiving the effluents [23]. The bacterial 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 and fermented cassava could lead to inhibition of bacteria growth [24].

Microbes are often described as ubiquitous organisms due to the ability to thrive in nearly all environments under different conditions. Some of the environments could be stressful to the microbes. For instance, CME is acidic in nature and contain high cyanide content. This makes it toxic to some certain group of life. However, some still survive under this environment. These microbes are also transferred to the environment (soil or surface water) receiving the CME via discharge [25].

The organisms that were isolated from the untreated wastewater, untreated wastewater-polluted soil and unpolluted soil were Klebsiella aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Micrococcus luteus, and Bacillus cereus (Table 4). The microorganisms isolated in this work were in line with those isolated by Eze and Onyilide [26], from the soil receiving cassava effluent in Elele, Rivers State, Nigeria as well as those of Okoli et al. [16] from long-term impact of cassava mill effluent on some chemical and biological properties of soils Staphylococcus aureus, Escherichia coli, Baccillus spp, Pseudomonas spp, Streptococcus spp and Conyne bacterium spp. were the bacteria species that were identified in the soil samples investigated. The organisms may release toxins in the effluent which can be very harmful [26]. These microbes may possess or have acquired the genetic attributes that enable them to survive in such acidic environment. This ability to degrade cyanide has been reported to be widely distributed in natural ecosystems and have enzymatic systems that can be broadly described as oxidative, hydrolytic and substitution/transfer in nature [27, 28, 29].

Pseudomonas aeruginosa, Proteus vulgaris and Bacillus subtilis were more prominent in the untreated cassava mill wastewater-polluted soil. These findings suggest that cassava mill effluent promotes the proliferation of these bacteria. Bacillus subtilis was also more prominent in the unpolluted soil, while Bacillus cereus was more prominent in the untreated wastewater (Table 5).
The presence of Proteus species was an indication that the contaminated soils were enriched with protein substances [30].

*Escherichia coli* was isolated from the untreated cassava mill wastewater-polluted soil. This is an indication that cassava mill wastewater enhances the proliferation of the bacterial species which could be due to significant glucose level in cassava effluent [22] and the higher pH of the polluted site which enhances faster growth of the bacterium [31]. This is in agreement with the findings of Okoli [16], who studied long-term impact of cassava mill wastewater on some chemical and biological properties of soils and reported that *Escherichia coli* was observed in the polluted site only.

*Bacillus* spp. are well known indigenous and persistent bacteria in the soil environment [32, 33]. These Bacillus spp at a high rate of occurrence in the sampled environment indicates a possible health hazard associated with these organisms as the species is a notable indicator of microbial pollution [34, 35]. *Bacillus cereus* was more prominent and only found in the untreated cassava mill wastewater. *B. cereus* is a spore forming organism. Aerobic spore forming organisms have been implicated in the spoilage of starch based foods and in food intoxication, thus they constitute health hazard and are potential spoilage agents. *B. cereus* is widely distributed in nature and in foods. Its spores have ability to withstand high temperature up to 100 °C and they produce enterotoxins which may cause food poisoning [35,34,36]. The aerobic spore formers have been used and have been implicated in the investigation of microbial quality of cassava flour “lafun” [37]. The pathogenic potential of Bacillus has been reported as it is a known cause of food borne illness when contaminated food is ingested [38]. Odetunde *et al*. [36], reported that these organisms are introduced into food from soil and water used during processing through which they find their passage into man.

*Bacillus subtilis* is a spore forming organism that is mostly found in soil and vegetation with an optimal growth temperature from 25-35 degrees Celsius. It is non-pathogenic but can contaminate food and be considered an opportunistic pathogen among the immuno-compromised. They are used on seeds, vegetables, and plants as a fungicide because of their ability to produce antibiotics. *B.subtilis* inhabits the root system of the plant competing with disease causing organisms. Some *B. subtilis* strains are capable of producing toxins for insects. These strains are used by farms to protect their crops [39].

*Pseudomonas aeruginosa* and *Bacillus subtilis* were the most dominant organisms found in the samples examined from the untreated wastewater, wastewater-polluted soil and unpolluted soil (Figure 1) *Bacillus subtilis, Pseudomonas aeruginosa* and *Proteus vulgaris* were dominant in the untreated wastewater-polluted soil, *Bacillus cereus* were dominant in the untreated wastewater while *Bacillus subtilis* were dominant in the unpolluted soil. Statistical analysis showed that cassava wastewater had significant (P < 0.05) effects on total bacterial counts, total coliform counts and faecal coliform counts.
These bacteriological isolates may have originated from soil, water and materials used during the processing of cassava while the variation of the isolates may be due to the handling process and the prevailing environmental conditions. Therefore, the isolates can be said to be transient microorganisms surviving only in the absence or low cyanide and/or other inhibitory substances in cassava (Olowoyo et al., 2001). Cassava wastewater 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 [26].

**CONCLUSION**

It can be concluded from the result of this study that cassava wastewater alters the bacteriological characteristics of the contaminated soils. The bacteriological load of the contaminated soil was lower than that of the unpolluted sample. These bacterial counts may be due to lack of efficient control measures in the discharge of the waste water into the environment. Also, the relatively low bacterial count of contaminated soil could be attributed to the acidity of the wastewater due to the presence of cyanide. This implied that cassava mill effluent reduces the number and performance of bacteria in the soil due to acidic nature of the soil caused by the cassava mill wastewater, therefore cassava wastewater must be treated before its discharge into the soil environment.

**REFERENCES**


