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# Bacterial Diversity and Occurrences in Cassava Effluent-Contaminated Soil at Umuoma, Uli Community, Anambra State, Nigeria

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**ABSTRACT**

A good quality soil is characterized by adequate nutrients as a result of abundant nutrients cycling bacteria. Most soil in Nigeria has been subjected to different kinds of pollutants resulting from anthropogenic activities which have become a major threatening factor to the quality of soil. This study was undertaken to determine the effect of cassava effluent on soil bacterial activities. A total of 30 composite soil samples were aseptically collected from cassava effluent disposal site at Uli community using a sterile soil auger. The samples were analyzed for total heterotrophic bacterial count (THBC) and nitrifying bacterial count (NBC) using standard plate technique. The predominant bacterial isolates that aided nutrients cycling were appropriately characterized, thereafter ascertained their diversities in both impacted and non-impacted soil samples. Also, the bacterial isolates were characterized based on morphology, microscopy, and biochemical characteristics. There was a significant reduction in the THBC and NBC in the impacted soil and Gram negative rods such as *Pseudomonas* and *Klebsiella* species were mostly isolated. The study has revealed that cassava effluent affects microbial distribution in the soil.
Keywords: Bacterial, Diversity, Cassava, Effluent-Contaminated and Soil

## INTRODUCTION

The risk to human lives and aquatic organisms constituted by industrial and gaseous effluents cannot be over-stressed. Most industries are responsible of releasing contaminants into the environment [1-4]. Soil and water bodies are particularly polluted with toxicants from food processing and allied industries and inhabitants of the affected areas are exposed to health related risks as a result of this uncontrollable industrial discharge [5-6]. Soil is the uppermost layer of the earth's crust formed as a result of the microbial transformation of weathered rocks [7-8]. Soil is stratified into several layers and the topsoil is the most prolific. The top soil consists of soil microorganisms which are involved in the degradation of organic matter and nutrient cycling. This has an effect on global geochemical nutrient. The topsoil gets the ultimate effect from environmental pollutants. Such pollutants include hydrocarbon pollutants, palm oil mill effluent, human and animal wastes, wood waste, wastewater from agro-allied industries and refineries, mining effluent as well as cassava mill effluent from cassava processing activities [9-10]. Cassava (*Manihot esculenta*) belongs to the family Euporbaceae. It is one of the largest sources of energy-giving foods in the tropics. Cassava is an essential food in Nigeria and other developing countries. Nigeria is the largest producer of cassava

while the greatest exporter of this crop is Thailand. There has been great 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. The unpleasant smell coming from the fermenting effluent calls for the establishment of laws to guide the discharge of cassava waste generated. In Niger Delta region of Nigeria, cassava tubers are processed for eating either as starch, garri, fufu, dried or wet cassava flour. Garri is widespread among all processed cassava products in Nigeria. Garri production is accompanied with the release of water, hydrocyanic acid, organic matter and sieves from the pulp. This research work is aimed at evaluating the bacterial diversity and occurrences in cassava effluent -contaminated soil collected from Umuoma, Uli community, Anambra state.

## MATERIALS AND METHODS

### Study Area

The study was conducted at Umuoma, Uli, Ihiala Local Government Area, Anambra State. Uli is a village located between latitudes 5.47°N and 5.783°N and longitude 6.52°E and 6.87°E on the South eastern part of Nigeria. Uli extends westward to the confluence of the rivers of Atammiri and Eyinja, and across Usham lake down to the lower Niger region. Uli has rainforest vegetation with two seasonal climatic conditions: rainy season and dry season, which is characterized by the harmattan between December and February. Uli is characterized by double maxima of rainfall with a light drop in either July or August known as dry spell or August break. The annual total rainfall is about 1,600 mm with a relative humidity of 80 % at dawn.

### Sample Collection

The soil surface was carefully scrapped out using sterile spoon. The soil auger was derived to a plough depth of 15 cm in the sampling site, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thoroughly mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples used for this study. The samples were carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

### Sample Preparation

This was carried out using the modified method of [2]. One gram of the soil sample was weighed into a 50 mL beaker (Pyrex) using analytical weighing balance (JJJ430BC), little normal saline (0.85% NaCl) was added; this was shake thoroughly and made up to 10 mL using the normal saline. Then ten-fold serial dilution was carried out by transferring one milliliter of the prepared sample into nine milliliters of the diluent (normal saline), and this was serially carried out to form dilution $10^{-6}$.

### Effects of Cassava Effluent on Bacterial Load in Soil Samples

### Estimation of Total Heterotrophic Bacterial Counts (THBC)

The prepared samples were aseptically introduced (1.0 mL) into Petri dishes (90 mmX 15 mm) containing sterile prepared nutrient agar (BIOTECH) as described by Frank and Robert (2015). These were placed in electric incubator in vertical positions at 35±°C for 24 h. THBC were enumerated by counting the number of colonies in each plate after 24 h, and the mean counts were calculated and presented in form of mean ± standard deviation.

### Estimation of Lipolytic Bacterial Counts (LBC)

The prepared samples were aseptically cultured on sterile poured plates (90 mm x 15 mm) containing Tributyrin agar (TA) as described by Ibe *et al.* (2014). The plates were incubated in inverted position in electric incubator (STXB128) at 30±2°C for 24 – 48 h. LBC was enumerated by counting the number of colonies surrounded by the clear zones.

## Characterization of Predominant Bacterial Isolates that Aided Nutrients Cycling from the Studied Samples

### Purification of the Isolates

The plates that showed discrete colonies were selected after 24 h, and aseptically streaked each colony on sterile plates (90mm×15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturer's description. The streaked plates were placed in a bacteriological incubator in inverted positions and incubated at 35±2$^0$C for 24h as described in [2].

### Characterization of the Pure Isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics.

### Morphological characterization of the pure isolates

The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Cheesbrough (2010). The presence or absence of capsule was also carried out. The presence or absence of flagellum was determined by carrying out motility test.

### Gram staining technique

A thin smear was made in a cleaned grease free microscopic slide (75mmX25mm X 1mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens.

### Biochemical characterization of the pure isolates

The capability of the pure isolates to produce catalase, indole, oxidase, acetoin, grow in 6.55 % NaCl and to utilize sugars, sugar alcohols and other substances (ribose, sorbitol, arabinose, sacharose, glucose trehalose, lactose, starch, inulin, salicin, hiparate) and also the haemolytic activity of the isolates were done using the methods described by [2].

### Indole test

Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 ml of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the pure isolates and kept in an incubator at 37°C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

### Sugar fermentation test

The capability of the pure isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham's tubes. The medium was then sterilized by autoclaving. The sugar solution were prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate pure isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

### Hydrogen sulphide production

This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The pure isolates were aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for hydrogen sulphide production.

### Citrate utilization test

The Simmon's Citrate Agar was prepare according to the manufacturer's direction and the pure isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

### Catalase test

The test was carried out as described by [2]. A smear of the pure isolates was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide ($H_2O_2$) was added on the smear. Prompt effervescence indicated catalase production.

### Statistical Analysis

The densities of the bacterial group in the impacted and non-impacted soil were compared using students' T test, and P values greater than 0.05 were considered non-significant ($P > 0.05$).

## RESULTS

### Diversities and Occurrences of the Predominant Bacterial Isolates in the Impacted Sites that Aid Nutrient Cycling

The occurrences of the implicated isolates that aid nutrients cycling are shown in Table 1. The study showed that the number of the isolates were significantly ($P < 0.05$) reduced in the impacted soil samples. Isolate BMW recorded the highest occurrence (15), followed by isolate XAO (2), while isolate MFC recorded the least occurrence (3) in the impacted soil samples. However, there was a higher occurrence of the isolates in the non-impacted soil soils.

**Table 1: Occurrences of the selected bacterial isolates in impacted and non-impacted soil samples (N=30)**

| Isolate | Impacted soil | Non-impacted (Control) |
|---|---|---|
| BMW | 15 | 24 |
| XAO | 2 | 14 |
| MFC | 3 | 13 |
| Total | 20 | 51 |

**Characteristics and Identities of the most Predominant Bacterial Isolates in the population that Aid Nutrient Cycling**

The cultural and morphological characteristics of the implicated bacterial isolates are shown in Table 2. The isolates; X, Y, and Z exhibited varying characteristics culturally and microscopically. Isolates X and Z were Gram negative rods, circular colonies with varied appearance on nutrient agar plates. Isolate X was colorless, while isolate Y had yellow coloration, with entire margin. Isolate Z had pale yellow color with entire margin, and convex elevation. The isolates were catalase positive and utilized glucose. They exhibited varied degree of utilizing sugar molecules as shown in Table 3. All the isolates utilized glucose as their carbon source while other sugars and sugar alcohols such as sucrose, maltose, mannose, lactose, mannitol, and sorbitol were rarely utilized (Table 3). Similarly, all the bacterial species were catalase and citrate positive while hydrogen sulphide and indole were not produced by all the isolates.

**Table 2: Cultural and morphological characteristics of some selected bacterial isolates**

| Parameter | X | Y | Z |
|---|---|---|---|
| Appearance on NA | Colorless | Yellow | Pale yellow, later white |
| Shape of colony | Circular | Circular | Circular |
| Elevation | Raised | Raised | Convex |
| Margin | Smooth | Smooth | Entire |
| Gram Reaction | Negative | Positive | Negative |
| Cell Morphology | Rods | Cocci | Rods |
| Possible Bacterium | *Klebsiella* | *Micrococcus* | *Pseudomonas* |

**Table 3: Biochemical characteristics of the selected bacterial isolates**

| Parameter | X | Y | Z |
|---|---|---|---|
| Catalase | + | + | + |
| Citrate | + | _ | +/_ |
| Indole | _ | _ | _ |
| Hydrogen sulphide | _ | _ | _ |
| Glucose | + | + | + |
| Maltose | + | _ | _ |
| Lactose | + | _ | _ |
| Mannitol | + | _ | _ |
| Mannose | + | _ | _ |
| Sorbitol | + | _ | _ |
| Bacterium | *Klebsiella* | *Micrococcus* | *Pseudomonas* |

## DISCUSSION

The significant decrease in the mean bacterial counts in the cassava effluent contaminated soils could be attributed to toxic nature of the effluent. Research had shown that cassava effluent contains components (cyanide) that interfere with bacterial proliferation [4]. These chemicals are known to resist biodegradation and selection of bacterial diversity had been documented by several researchers [5]. The predominance of Gram negative bacteria in the sampled dumping sites could be ascribed to environmental selection and the ability of the isolates to degrade high organic substances. The Gram negative bacteria such as *Pseudomonas, Bacillus, Micrococcus,* and *Enterobacter* had been reported to be involved in nutrient cycling and in decomposition of complex organic substances in the environment. Nutrient cycling such as nitrogen fixation, nitrification, phosphate solubilizing etc. are vital in the survival of living organisms in the soil and also determine the extent of crop yield. Similar bacteria were isolated by [7] who investigated the effects of heavy metals in microbial biodegradation. However, this observation disagrees with the study documented by [9] who isolated mostly fungal species in heavy metal contaminated soil, which they attributed to high saprophytic activity of fungi. The ability of the bacterial isolates to utilize glucose and sugar alcohols could be attributed to high metabolic activity. This observation corroborates to the study documented by [7] who evaluated the effects of heavy metals in microbial biodegradation. The toxicity of the chemical component (cyanide) interferes with the metabolic processes that occur within the bacterial species that cycle nutrients. This observation disagrees with the finding of [6] whose isolates proliferated in the presence of the toxic heavy metals, which was attributed to the ability of the isolates to synthesize enzyme that converts the heavy metals to non- toxic compounds. The heavy metals block the sites which enzymes bind by attaching to the active protein groups of enzymes. Also, heavy metals reduce soil nutrient and cause damage of cell wall of microorganisms especially copper toxicity. This observation corroborates with the study documented by [7] who investigated microbial activities on heavy metal contaminated soil and discovered a significant reduction in enzymatic activities. Also, a study conducted by [3] showed that lead and cadmium contaminated soil had a reduction in

mobility, availability, and microbial diversities.

## CONCLUSION

This study therefore revealed adverse environmental effects of cassava effluent on soil biological parameters. Again, it also calls for serious rehabilitation, if the soil will be used for agricultural and other purposes as the factors important in soil health are negatively affected. Further observations in the study suggest the need for proper legislation against indiscriminate disposal of industrial wastes into our environment whether organic or inorganic, biodegradable or not.

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