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BACTERIAL DENSITY IN CASSAVA EFFLUENT-CONTAMINATED SOIL AT UMUAKU, ULI COMMUNITY, ANAMBRA STATE

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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 bacterial density in cassava effluent contaminated soil at Umuaku, Uli community, Anambra State. A total of 30 composite soil samples were aseptically collected from cassava effluent disposal site 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, and their diversities in both impacted and non-impacted soil samples were enumerated. 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 *Enterobacter, Burkholderia,* and *Bacillus* species (Gram positive) were mostly isolated. The study has revealed that cassava effluent affects microbial distribution in the soil.**

Keywords: **Bacteria, Waste, Cassava Effluent.**

1. INTRODUCTION

Cassava is a single species crop, (*Manihot esculenta*) though with several varieties. It is a dicotyledonous plant belonging to the botanical family *Euphorbiaceae*. It contains laticifers and produces latex (Nwakiby *et al.*, 2021). The cassava plant is said to originate in Northeast Brazil with an additional Centre of origin in Central America, from these Centers, the crop spread to several parts of the world including Africa, Asia and America especially in the tropical zones. There are several varieties and cultures but generally cassava is said to be of two main varieties based on the characteristics and contents of its Cyanogenic glycoside of its root/tubers. These are bitter and sweet; the bitter variety has its Cyanogenic glucoside distributed throughout the tuber and in high concentration while the sweet variety has low Cyanogenic glucoside, mainly in the peel of the tuber (Ejimofor *et al.,* 2022). The fresh/pulp of the sweet variety therefore has low Cyanogenic glucoside. However, the growing environmental conditions could influence the Cyanogenic glucoside concentrations of each variety. Cassava is grown in tropical lowland under warm, moist climate with a temperature range of 25-30 °C and rainfall of 100-150cm per year well distributed. It can however, grow under lower rainfall levels too but not doing very well. The best soil type for it is a light sandy loam and well- drained soil of medium fertility as high fertility encourages more vegetative growth than tube formation. Cassava is a perennial plant, usually harvested within 12-18months naturally (Bhattari *et al.*, 2015).

Generally, cassava crop is cultivated for its tubers/roots, but cases of other parts of the plant being put into effective uses abound especially the leaves. Cassava leaves has been used as vegetable for human consumption in a few East African countries like Tanzania, Angola, and Malawi. The root tubers produced over 20-30% of the total harvest has been used in animal feed supplements. In animal feed, the leaves serve as forage material. The leaves are harvested and feed to domestic animals like goats, sheep and cattle while the cassava tubers peels are served to pigs as feed supplements (Ejimofor *et al*., 2022). Before utilization of the tuber it is inevitably peeled; about 0.5-2.0% of the tuber is the peel while the edible part is 80-90%.This edible fleshly tuber is composed of 60-70% water, 30-35% carbohydrate, 1-2% protein while fiber, fat and mineral matter make up the remaining. Two important factors that influence the use of cassava tuber are the high content of Cyanogenic glucosides and lack of good storage or keeping quality of the fresh tuber. This implies that the tubers will only be consumed after elaborate processing to reduce the Cyanogenic glucoside content and improve the keeping quality.[2] Processing of cassava tubers for human consumption gives four types of products; these include the meal, flour, chips and starch production. The processing of these cassava tubers result in generation of several types of waste which include not only the peel but the effluent inclusive. The effluent include the milky colloid pressed out of the fresh tuber paste, the latex, the wash water, etc. These wastes are automatically discharged into the surrounding environment causing pollution. The above mentioned pollution effects from cassava wastes becomes more pronounced as specific mills are now established to process cassava tuber to obtain garri and starch, the two forms in which the tubers are mainly consumed (Ejimofor and Oledibe, 2022). In most cassava tuber preparations, the processing mills are established near water bodies or in free land spaces. The discharge of the cassava mill waste results in offensive odour emanating from the biodegraded products of the waste. In this case, the cassava effluent affects the soil or water microbiological properties which in turn affect the general productivity of the particular ecosystem. Since cassava contains some minerals that affect the soil or water quality, assessment of the impacted habitat therefore involves the factors to obtain an appropriate view of the situation. Having established that human activities for economic, food or industrial objectives impact on the environment; the impacted environment should be assessed in order to determine the premeditative approach for a safer, greener and healthier habitat. This research work is aimed at evaluating the bacterial density in cassava effluent contaminated soil at Uli community, Anambra state.

2. MATERIALS AND METHODS

Study Area

The study was conducted at Chukwuemeka Odumegwu Ojukwu University (COOU), 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 Chabukdhara *et al*. (2017). 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 Nitrifying Bacteria Counts (NBC)

The prepared samples were aseptically cultured on sterile poured plates (90 mm X 15 mm) containing Glucose Nitrogen Free Mineral Medium (GNFMM) which comprises 1.0 g K_2HPO_4, 1.0 g $CaCl_2$, 0.5 g NaCl, 0.25 g $MgSO_4$.7H2O, 0.01 g $FeSO_4$.7H2O, 0.1 g $Na_2MoO4.2H_2O$, 0.01 g $MnSO_4.5H_2O$ and 7.0 g glucose in 1000 mL distilled water as described by Wu *et al*. (2015). These were incubated in vertical positions at room temperature (30±2°C). The NBC were enumerated after 48 h.

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 ± 2^0C for 24h as described in Fan *et al.* (2015).

Morphological characterization of the pure isolates

The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out as described in Gupta *et al.* (2016). The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Grujic *et al*. (2017). The presence or absence of capsule was also carried out as described by (Soleimani *et al*., 2017). The presence or absence of flagellum was determined by carrying out motility test as described by Kumar *et al.* (2018).

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 10seconds 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 Siddiquee *et al*. (2015).

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.

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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 Le *et al*. (2017). 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 ($P > 0.05$).

3. RESULTS

Effects of Cassava Effluenton Bacterial Density and Quality of the Implicated Soil Samples

The study revealed the enumeration of total heterotrophic bacterial counts (THBC) and nitrifying bacterial counts (NBC) as presented in Table 1. From the result, the impacted soil recorded the lowest THBC (4.10±0.10) and NBC (0.70±0.01) when compared to the normal soil (control) which had 14.10±0.10 and 2.20±0.10, respectively.

Table 1: Enumeration of nitrogen fixing bacterial count from cassava effluent sites

Bacterial Group	Impacted site	Control site
THBC ($X10^6$CFU/g)	4.10±0.10	14.10±0.10
NBC($X10^4$ CFU/g)	0.70±0.01	2.20±0.10

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; A, B, and C exhibited varying characteristics culturally and microscopically. Isolates B and C were Gram negative rods, circular colonies with varied appearance on nutrient agar plates. Isolate A was milkish white, while isolate B had yellow coloration, with entire margin. Isolate B was colorless with smooth margin, and raised 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	A	B	C
Appearance on NA	Milkish white	Yellow	Colorless
Shape of colony	Irregular	Circular	Circular
Elevation	Flat/Concave	Convex	Raised
Margin	Filamentous	Entire	Smooth
Gram Reaction	Positive	Negative	Negative
Cell Morphology	Rods	Rods	Rods
Possible Bacterium	*Bacillus*	*Burkholderia*	*Enterobacter*

Table 3: Biochemical characteristics of the selected bacterial isolates

Parameter	A	B	C
Catalase	+	+	+
Citrate	+	+	+
Indole	_	_	_
Hydrogen sulphide	_	_	_
Glucose	+	+	+
Maltose	+	_	+
Lactose	+	_	+
Mannitol	+	+	+
Mannose	+/_	+	+
Sorbitol	+/_	_	+
Bacterium	*Bacillus*	*Burkholderia*	*Enterobacter*

4. DISCUSSION

The quality of soil can be altered by introducing chemical agents that influence the activities of beneficial microorganisms. 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 (cyanides) that interfere with bacterial proliferation (Santana *et al*., 2016). These chemicals are known to resist biodegradation and selection of bacterial diversity had been documented by several researchers. 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. 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 Satyaprakash *et al*. (2017) who evaluated the effects of chemical compounds on microbial biodegradation.

5. CONCLUSION

This study therefore has 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 the environment.

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