

EFFECT OF CASSAVA MILL EFFLUENT ON MICROBIAL PROPERTIES OF GARDEN SOIL – EZIOBODO IMO STATE NIGERIA

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ABSTRACT: *An assessment of the Effect of Cassava Effluent on Garden Soil was made. Two soil samples were collected; one from a farmland polluted with cassava effluent and, another as an unpolluted sample – free from cassava effluent pollution. The microbial analyses were carried out to investigate the effects of the cassava effluent on the soil microbial qualities of garden soil. Results showed that unpolluted soil sample was normal, while the results of the polluted soil sample showed extinct or absence of normal garden soil microbial fauna with the presence of *Staphylococcus aureus* which are more harmful than good wherever they are found. However, the presumptive identification of fungi in the polluted soil sample showed presence of *Candida sp.* The results of the bacteriological count showed absence of coliform bacteria, and the Triple sugar iron and various biochemical reactions showed the absence of bacteria such as *Bacillus sp* which possess nitrogenise and is able to fix atmospheric nitrogen. Such genus of bacteria could stimulate plant growth by colonizing plant tissues – external or internal and providing fixed nitrogen to the host plant. Also various species of *Bacillus* have the ability to increase plant nutrients in soil. *Bacillus* forms positive interactions (symbiotic) involving bacteria and fungi to stimulate growth in plants. Many strains are capable of inhibiting pathogenic growth or activity directly and indirectly in soil. Enlightenment campaign, detoxifying cassava effluent in accordance with regulatory Standard, appropriate method(s) of environmental friendly disposal of both solid and cassava wastewater are recommended for safe and healthy environment.*

KEYWORDS: Cassava effluent, soil, contamination, microbes, pollution, environment

INTRODUCTION

Manihot esculenta crantz (cassava) Synonymous with *manihot utilissima* belong to the family “Euphorbiaceae”. It is native to South America and is extensively cultivated in the tropical and subtropical regions of the world for its edible starchy tuberous root and are harvested between 7 to 13 months based on the cultivars planted (Cooke, 1985); Taye, 1994). The tubers are quite rich in carbohydrates (85-90%) with very small amount of protein (1.3%) in addition to cyanogenic glucoside (Nwabueze and Odunsi, 2007; Oyewole and Afolani, 2001). As a major source of carbohydrate in the world with Africa being the largest centre of production (Claude and Denis, 1990). Annual cassava production in Africa is about 84 million tones, with Nigeria being the highest with 30 million tonnes, Democratic Republic of Congo with 16.8 million tones, Tanzania

with 5.7 million tones, Mozambique with 5.3 million tones and Madagascar with 2.4 million tones (Nweke, 1992). Cassava has diverse uses depending on the community. The edible tubers are processed into various forms which include Chips, pellets, cakes, and flour. The flour could be fried to produce gari or steeped in water to ferment to produce foofoo when cooked (Oyewole and Udunfa, 1992). In Nigeria, nearly every community depends on it as a source of food. The reliance on cassava as source of food and exposure to goitrogenic effects of thiocyanate has been identified as being responsible for the endemic goiters in Akoko area of South Western Nigeria (Akindahausi, et al.; 1998). Wastewater from cassava processing is released into the environment probably farm land or even water body without proper or any treatment at all in most rural areas where cassava is processed. This has been identified as a source of pollution.

LITERATURE REVIEW

Cassava processing methods are a combination of various operations, each having a different potential impact on the environment. The impact of cassava processing should be considered at two levels – broad and site-specific. Generally, the maximum impact will be at the site – specific level. Two categories of methods typify cassava processing: methods that require a lot of water and those that do not require much water. Most traditional products, such as farinha, have modest water input requirements. Water consumption in the production of farinha is a relatively low 5m³/tonne product. For starch production, however, water is required at all stages irrespective of processing scale. Therefore, large volumes of water are needed – on average (2-6 times) more than for farinha production. Large factories possess the technological ability to efficiently use water, often incorporating recycling systems. The theoretical maximum water conservation rate is never achieved, as a minimum quantity of water is required for complete washing of starch. If this need cannot be satisfied, starch quality is adversely affected (Rojas et al., 1996).

Wastewater from cassava processing, if released directly into the environment before proper treatment; is a source of pollution. In many areas where traditional processing is practiced, wastewater is normally discharged beyond the “factory” wall into roadside ditches or fields and allowed to flow freely, settling in shallow depressions. Eventually this will percolate into the sub soil or flow into streams. In Colombia, starch processors usually return the effluent directly to streams and other surface water sources.

Besides large quantities of soil, discharged wastewater contains a number of contaminating substances. Normally, wastewater discharged from a cassava starch processing factory is acidic with high organic matter content (soluble carbohydrates and proteins) and suspended solids (lipids and non-soluble carbohydrates -starch or cellulose fibres). Wastewater also contains cyanide as well as sulphur dioxide if this is used during the extraction process. Cassava roots contain cyanogenic glucosides (the precursors of HCN) in various concentrations depending on the variety and growing conditions. Cyanide is released during peeling, slicing and crushing, such that these operations can reduce the level of cyanide to safe limits. The bound cyanide is converted to free cyanide during the milling operation. Forty to seventy percent of the total cyanide appears in the water used to wash the starch from the disintegrated tissue and about 5 – 10% in fibrous residue used in animal feed (Arguedes and Cooke, 1982). Released cyanide, either in expressed juice,

wash waster or water spray, quickly evaporates. Evaporation of cyanide will occur either during processing or after discharge (Cooke and Maduagwu, 1978).

According to a certain analysis which was conducted on cassava tuber, to determine some of the active ingredients and the toxicity of cassava effluent: the photochemical analysis indicated the presence of hydrogen cyanide, oxalate and phytate, while hydrogen cyanide and oxalate concentrations were high in the fermented effluent than the fresh cassava sample, the reverse was the case with phytate.

The terms ecosystem and ecology usually call to mind scenes of lions stalking vast herds of wildbeast on the grassy savannas of East Africa, or the interplay of phytoplankton, fish, and fisherman in some great estuary. Like a savanna or estuary, a soil is an ecosystem in which thousands of different creatures interact and contribute to the global cycles that make all life possible (Nyle and Ray, 2002).

The importance of soil as a natural body derives in large part from its role as an interface between the worlds of rock (the lithosphere), air (the atmosphere), water (the hydrosphere), and living things (the biosphere). Environments where all four of these worlds interact are often the most complex and productive on Earth. An estuary, where shallow waters meet the land and air, is an example of such an environment. Its productivity and ecological complexity far surpass those of a deep ocean trench, for example (where the hydrosphere is rather isolated), or the upper atmosphere where rocks and water have little influence). The soil, or pedosphere, is another example of such an environment.

The concept of the soil as interface means different things at different scales. At the scale of kilometres, soils channel water from rain to rivers and transfer mineral elements from country rocks to the oceans. They also remove and supply vast amounts of atmospheric gases, substantially influencing the global balance of methane and carbon dioxide. At a scale of a few meters, soil forms the transition zone between hard rock and air, holding both liquid water and oxygen gas for use by plants roots. It transfers mineral elements from the Earth's rock crust to its vegetation. It processes or stores the organic remains of terrestrial plants and animals. At scale of a few millimetres, soil provides diverse microhabitats for air breathing and aquatic organisms, channels water and nutrients to plant roots, and provides surfaces and solution vessels for thousands of biochemical reactions. Finally, at the scale of a few micrometers and smaller (less than a millionth of a meter), soil provides ordered and complex surfaces, both mineral and organic, that act as templates for chemical reactions and interact with and water and solutes.

Soil organism numbers are influenced primarily by the amount and quality of food available. Other factors affecting their numbers include physical factor (example, moisture and temperature), biotic factors (example, predation and competition) and chemical characteristics of the soil (example, acidity, dissolved nutrients, and salinity). The species that inhabit the soil in a desert will certainly be different from those in a humid forest, which in turn, will be quite different from those in a cultivated field. Acid soils are populated by species different from those in alkaline soils. Likewise,

species diversification and abundance in a tropical rain forest are different from those in a cool temperate area.

Despite these variations, a few generalizations can be made. For example, forested areas usually support a more diverse soil fauna than grasslands, although the total faunal mass per hectare and level of faunal activity are generally higher in grasslands, cultivated fields are generally lower than undisturbed native lands in numbers and biomass of soil organisms, especially the fauna, partly because tillage destroys much of the soil habitat.

The total soil biomass, the living fraction of soil, is generally related to the amount of organic matter present on a dry weight basis, the living portion is usually between one (1%) and eight (8%) percent of the total soil organic matter. In addition, scientists commonly observe that the ratios of soil organic matter to detritus, to microbial biomass to faunal biomass are approximately 1000: 100: 1.

Organic Matter Requirements: plant detritus and soil organic matter are used as energy sources by the majority of soil micro-organisms, the heterotrophs, but not by the autotrophs. The addition of almost any energy – rich organic substance, including the compounds excreted by plant roots, stimulates microbial growth and activity. Certain bacteria and fungi are stimulated by amino acids and other growth factors found in the rhizosphere or produced by other organisms.

Bacteria tend to respond most rapidly to additions of simple compounds such as starch and sugars, while fungi and actinomycetes overshadow the bacteria if cellulose and more resistant compounds dominate the added organic materials. In addition, if organic materials are left on the soil surface (as in coniferous forest litter), fungi dominate the microbial activity. Bacteria commonly play a larger role if the substrates are mixed into the soil, as by earthworms, root distribution, or tillage.

Oxygen Requirements: While most microorganisms are aerobic and use oxygen as the electron acceptor in their metabolism, some bacteria are anaerobic and use substances other than oxygen (example, nitrate ion (NO_3^-), sulphate ion (SO_4^{2-}), or other electron acceptors). Facultative bacteria can use either aerobic or anaerobic forms of metabolism. All three of these types of metabolism are usually carried out simultaneously in different habitats within a soil (Nyle and Ray, 2002).

Moisture and Temperature: Optimum moisture level for higher plants (moisture potential of -10 to -70kpa) is usually best for most aerobic microbes. Too high water content will limit the oxygen supply. Microbial activity is generally greatest when temperatures are 20 to 40°C. The warmer end of the range tends to favour actinomycetes. Ordinary soil temperature extremes seldom kill bacteria, and commonly only temporarily suppress their activity. However, except for certain cryophilic species, most micro-organisms cease metabolic activity below about 5°C a temperature sometimes referred to as biological zero.

Exchangeable Calcium and PH

Levels of exchangeable calcium and PH help determine which specific organisms thrive in a particular soil. Although in any chemical condition found in soils, some bacteria species will

thrive, high calcium and near neutral PH generally result in the largest, most diverse bacterial populations. Low PH allows fungi to become dominant. The effect of PH and calcium helps explain why fungi tend to dominate in forested soils, while bacteria biomass generally exceeds fungal biomass in most sub-humid to semi-arid prairie and rangeland soils.

The soil fauna and flora are indispensable to the plant productivity and the ecological functioning of soils. Of their many beneficial effects, only the most important can be emphasized here.

Organic Material Decomposition: Perhaps the most significant contribution of the soil fauna and flora to higher plants is that of plant residue decomposition. By this process, dead leaves, roots, and other plant tissues are broken down converting organically held nutrients into mineral forms available for renewed plant uptake. The release of nitrogen is a prime example. Soil organisms also assimilate wastes from animals (including human sewage) and other organic materials added to soils. As a by – product of their metabolism, microbes synthesize new compounds, some of which help to stabilize soil structure and others of which contribute to humus formation.

Breakdown of Toxic Compounds: Many organic compounds toxic to plants or animals find their way into the soil. Some of these toxins are produced by soil organisms as metabolic by – products, some are applied purposefully by humans as agrochemicals to kill pests, and some are deposited in the soil because of unintentional environmental contamination. If these compounds accumulation remains unchanged, they would do enormous ecological damage. Fortunately, most biologically produced toxins do not remain long in the soil, for soil ecosystems include organisms that not only are unharmed by these compounds but can produce enzymes that allow them to use these toxins as food.

Some toxins are xenobiotic (artificial) compounds foreign to biological systems, and these may resist attack by commonly occurring microbial enzymes. Soil bacteria and fungi are especially important in helping maintain a nontoxic soil environment by breaking down toxic compounds. The detoxifying activity of these microorganisms is by far the greatest in the surface layers of soil, where microbial numbers are concentrated in response to the greater availability of organic matter and oxygen.

Inorganic Transformations: The transformation of inorganic compounds is of great significance to the functions of soil systems, including plant growth. Nitrates, sulphates, and to a lesser degree, phosphate ions are present in soils primarily due to the action of microorganisms. Bacteria and fungi assimilate some of the nitrogen, phosphorous, and sulphur in the organic materials they digest. Excess amounts of these nutrients may be excreted into the soil solution in inorganic form either by the bacterial and fungi themselves or by the nematodes and protozoa that feed on them. In this manner, the soil food web converts organically bound forms of nitrogen, phosphorus, and sulphur into mineral forms that can be taken up by higher plants.

Likewise, the availabilities of the other essential elements, such as iron and manganese, are determined largely by microbial action. In well- drained soils, these elements are oxidized by autotrophic organisms to their higher valence states, in which forms they are quite insoluble. This keeps iron and manganese mostly insoluble and non-toxic forms, even under fairly acid conditions.

If such oxidation did not occur, plant growth would be jeopardized because of toxic quantities of these elements in solution. Microbial oxidation also controls the potential for toxicity in soil contaminated with selenium or chromium.

Nitrogen Fixation: The fixation of elemental nitrogen gas, which cannot be used directly by higher plants, into compounds usable by plants is one of the most important microbial processes in soils. Actinomycetes in the genus frankia fix major amounts of nitrogen in forest ecosystem; cyanobacteria are important in flooded rice paddies, wetlands and deserts; and rhizobia bacteria are the most important group for the capture of gaseous nitrogen in agricultural soils. By far the greatest amount of nitrogen fixation by these organisms occurs in root nodules or in other associations with plants. Worldwide, enormous quantities of atmospheric nitrogen are fixed annually into forms usable higher plants.

Cassava processing, especially in areas where the industry is highly concentrated, is regarded as polluting and a burden on natural resources. Some forms of processing, particularly for starch, have developed beyond traditional methods and are now water intensive yet often sited in areas of water scarcity. By its nature, cassava processing for starch extraction produces large amount of effluent high in organic content. If untreated, this may be displayed in the form of stagnant effluent ponds from which strong odours emanate. Other forms of processing, despite not requiring water, generate very visible dust waste. As a consequence of the visual display of pollution, cassava is often perceived by local populations as contributing significantly to environmental damage and water deficit. Yet despite this notion, supported mainly by the visual display of pollution, few systematic impact studies have been conducted.

Annual cassava production in Africa is about 84 million tonnes, mainly in Nigeria (30), the Democratic Republic of Congo (16.8), Ghana (7.1) Tanzania (5.7) Mozambique (5.3) and Madagascar (2.4). Cassava is primarily produced by small scale farmers and processed at the family – or villages – level. Despite small scale operation, cassava production in Africa is highly commercialized, with as much as 45% of the total output marketed (Nweke, 1992). A great diversity of products is derived from cassava. The most representative are gari in West Africa, Chickwangué in Central Africa, and a tap and Ugali in East Africa.

Traditional processing techniques are flexible in their use of the different processing resources. Retting (soaking) is employed in humid regions (Central Africa), while in the dryer regions (Western Africa) a fermentation step is usually included. Cassava granules are commonly produced in areas of high population density, while chips and flour are more widely used in those with low population density (Nweke, 1992). In some regions, techniques for making chips and flour are water intensive; but in other areas, only sunshine is required. A major feature of cassava processing in Africa is that villages in each climatic zone concentrate on making products for which the zone is endowed with the necessary resources (Nweke, 1992). Cassava processing has many technological pathways adapted to the use of locally available processing resources. Where water for fermentation is scarce, heaping or stacking fermentation techniques are used.

MATERIALS AND METHODS

Area of Study

The farm land is located at Eziobodo, Owerri West Local Government Area, Imo State, Nigeria. It is located in globe by 4° 45'N and 7° 15'N latitude and 6° 50'E and 7° 25'E longitude with elevation of 88m above sea level. The area has the following geological characteristic:

Owerri lies entirely within coastal plain sandstones (Benin Formation) which have a thickness of about 800m. The Benin formation extends from the west across the Niger Delta and southward, beyond the present coastline. It is over 90 percent sandstone with minor shale intercalations in some places. It is coarse grained, gravely, locally fine grained, poorly sorted, sub-angular to well-rounded, and bears lignite streaks and wood fragments. The Benin formation is thus partly marine, partly deltaic, partly estuarine and partly lagoonal and fluviolacustrine in origin (Reyment, 1976). Its age ranges from Miocene to recent. The terrain of the area is characterized by two types of land forms: highly undulating ridges and nearly flat topography. Various structural units (point bars, channel fills, natural levees, back swamp deposits and oxbow fills) are identifiable within the formation indicating the variability of the shallow water depositional medium. The otherwise continuous body of the Benin formation is interrupted by the Afam clay member which consists mainly of clay with few intercalated sandstone bodies.

Stratigraphically, the Benin formation is overlain by recent alluvium and recent sediments and underlain by the Agbada formation. Its outcrop lateral equivalent is probably the Ogwash - Uku-Asaba formation.

Sample Collection Technique

Soil samples were collected using the transect survey (straight line) method. Soil samples (polluted and control) were collected at different points though, in the same farmland at depth of 10 to 15 centimeters using soil Auger. The samples were collected into different polythene bags and transported to Imo State Environmental Protection Agency (ISEPA) laboratory for microbial analysis.

Sterilization of Equipment and Materials

Glass wares such as test tubes, pipettes, Petri dishes and so on were sterilized by standard methods as described by Cheesborough (2000) and Pelczar et al., (1986). Forceps, spatulas and slides were also sterilized by hot air oven at a temperature of 105°C for 2 hours. Wire loops were sterilized by flaming to red hot.

Preparation of the Samples

The samples were used fresh for the microbial analysis to avoid loss of some members of microbial flora during drying also, to avoid contamination. Ten grammes (10g) of each sample was measured and blended in a sterile blender (sterilized by UV-irradiation) using 100ml of sterile distilled water. After blending, it was transferred to a sterile conical flask and cooled. An aliquot of each sample was used for microbial analysis.

PREPARATION OF MEDIA

Preparation of Nutrient AGAR (NA)

This was prepared according to the manufacture's instructions. Twenty eight grammes (28g) of dehydrated Nutrient Agar base medium was dissolved in about 800ml of distilled water. The mixture was heated in a water bath until the Agar melted. It was made up to 1 litre and its PH checked to conform to the standard (7.2 to 7.6). The prepared medium was used for both plate and slant preparation. Before sterilization of the medium, a part of it was dispensed in 15ml volume into McCartney bottles. These and the other remaining portion were sterilized in an autoclave at 121°C and 15Psi for 15minutes. After autoclave, the McCartney bottles with sterilized medium were arranged in racks to cool and gel in a slanted position to form sterile Agar slants used for sub cultures. The bulk of the medium was allowed to cool to 45°C before it was aseptically poured into sterile Petri dishes to form agar gel used for bacterial propagation.

Preparation of Macconkey Agar

This was also prepared according to the manufacturer's instructions. 51.5g of the dehydrated MacConkey Agar was dissolved in 1 litre of distilled water. The mixture was heated to melt completely. A portion of the medium was transformed in 15ml portion to McCartney bottles for slants. The entire medium was sterilized in an autoclave at 121°C and 15Psi for 15 minutes. Thereafter, slant bottles were allowed to cool and gel on racks in slanted positions while the rest was aseptically poured into sterile Petri dishes to form agar gel used for propagation of bacteria.

Preparation of Potato Dextrose Agar (PDA)

This medium was prepared according to the manufacturer's instructions too. Thirty nine grammes (39g) of the dehydrated base powder was dissolved in 600ml of distilled water and mixed vigorously. The mixture was heated to melt the agar and then diluted to 1 litre and its PH checked to conform to standard (5.6 + 0.2). A portion of the medium was transformed in 15ml portion to McCartney bottles for slants. The entire medium was sterilized in an autoclave at 121°C and 15Psi for minutes. After, the slant bottles were allowed to cool and gel on racks in slanted positions while the rest was aseptically poured into sterile Petri dishes to form agar gel used for propagation of fungi.

Determination of Microbiological Load (Bacteria and Fungi)

The method of the International Commission on Microbiological Specification for Foods, ICMSF (1978) was adopted and used. One millilitre (1ml) of the prepared samples (polluted and control) were diluted in 9ml of sterile distilled water (diluent) and mixed vigorously by shaking 1ml of the resultant mixture was aseptically transferred to 9ml of sterile water in a test tube. This action was carried out under sterile aseptic conditions. The dilution was continued serially until the 6th dilution was attained (10^{-6}). One – tenth millilitre (0.1ml) of the 6th dilution was inoculated into a sterile Potato Dextrose Agar (PDA) and Nutrient Agar (NA) plates respectively. The spread plate technique as illustrated by Pelczar and Cham (1977) was employed. A flamed glass hockey stick shaped rod was used to spread the inoculums evenly over the surface of the agar in the plate. The arrangement was done for both polluted and (unpolluted) soil samples. The Potato Dextrose Agar

(PDA) culture plate was incubated at room temperature for four days, while the Nutrient Agar (NA) culture plate was incubated at 37°C for 24 hours in the incubator.

All the plates were observed daily. On establishment of growth, the number of colonies formed in each culture plate was counted using the Galle Kamp electronic colony counter. A mean of the counts from each plate was obtained and multiplied with the appropriate dilution factor to obtain the microbial loads as the total viable microbial colonies per unit weight of the sample expressed as the colony forming unit (cfu) per gramme of the sample. It was calculated using the formula below:

$$\text{Cfu/g} = 1/W \times N \times D$$

Where W = weight of sample analysed in gramme

N = Average number of colonies per plate

D = Dilution factor.

In all cases, the microbial counts were taken from plate not more than 300 colonies in accordance with (Ogbulie, Uwaezuoke and Ogbulie, 1998).

Preparation of Pure Cultures of Isolates (Bacteria and Fungi)

After incubation and establishment of growth, Nutrient Agar culture plates were observed for discrete colonies. Inoculums from such discrete colonies were aseptically taken and sub cultured into sterile nutrient agar slants. Similarly, inoculums for such discrete colonies in Potato Dextrose Agar plates were also inoculated into sterile PDA slants.

Potato Dextrose Agar slants were incubated at room temperature for three days (72hours) and the Nutrient Agar slants were incubated at 37°C for 24 hours. This pure culture of bacteria and fungi obtained were used for identification.

Identification of Bacteria Isolates

Each bacteria isolate to be identified was subjected to systematic step by step analysis and examination as described by Cheesbrough (2000). At the end, the characteristic of individual isolate were matched against those in Buchanan and Gibbons (1974).

Colonial or Cultural Characteristics

Each colony of bacteria isolates were examined for specific features which include: extent of growth, colony, shape, colour, elevation, consistency and so on. And findings were recorded.

Microscopic Examination

The isolates were examined microscopically to determine their motility and cellular arrangement, including shapes and sizes of cell reaction to specific dyes such as gram stain, spore stain, flagella and capsule stain were also observed microscopically. Results of these tests with dyes gave indication of the presence or absence of spores, capsules, flagella and so on. Gram's reaction reveals the cell morphology as well as grouping isolates into two major categories: gram +ve and gram -ve.

Gram Staining

The gram stain is one of the most useful staining procedures in bacteriology developed by a Danish bacteriologist (Hans Christian Grams). It separates bacteria into large groups – Gram positive and Gram negative. The distinction is based on whether or not the bacteria resist decolourization with acetone or alcohol after staining with a primary stain such as crystal violet and subsequent treatment with iodine (which acts as a mordant). Gram positive bacteria resist decolourization and remain dark purple in colour. The Gram negative bacteria are decolourized, losing the purple colour of crystal violet.

Triple Sugar Iron Agar (TSIA)

This test is used to determine the ability of certain bacteria to utilize a specific sugar incorporated into a basal growth medium with or without the production of gas along with a possible hydrogen sulphide (H₂S) production.

Triple Sugar Iron Agar was prepared in slants; the medium was inoculated by stabbing the butt and streaking the slant with a loop full of the appropriate isolate, inoculated at 37°C for 48 hours. The production of gas is marked by cracks in the Agar as well as air gap at the bottom of the test tube while hydrogen sulphide production is indicated by the presence of black precipitate which indicates the reduction of sodium thiosulphate to hydrogen sulphide. After incubation period of 37°C for 48 hours, the results obtained were recorded.

Biochemical Examination

Isolate that cannot be identified by colonial and microscopic examination were identified base on few biochemical reactions such as enzyme production Catalase test, Motility test, Citrate test, Indole test, Gram staining test, Urease test, and Triple sugar iron test were carried out as described by Bakers and Sliverton (1998) and results were recorded.

Identification of Fungal Isolates

Fungal isolates were identified primarily based on their colonial features and microscopic examination. The slide culture technique adopted by Pelczar and Cham (1977) was employed. The isolates were cultured in a semi- solid agar medium and grown on a microscopic slide between two ridges of wax covered with a cover slip. After incubation, the culture was examined directly under the microscope and its cultural features observed were recorded. Portions of the culture were also made on a slide in lacto phenol cotton blue, and on examination determined features of each fungal isolate was observed and recorded. Findings were cross checked with features present in Barnett and Hunter (1987).

RESULTS AND DISCUSSION**Results**

Below, are the results of the microbial count?

Table 4.1

Dilution factor	Polluted Sample		Unpolluted Sample	
	Colony Count (cfu/g)	Total Coliform (cfu/g)	Colony Count (cfu/g)	Total Coliform (cfu/g)
10 ⁻¹	Discarded	Nil	Discarded	0.33 x 10 ¹
10 ⁻²	0.05 x 10 ¹	Nil	0.15 x 10 ¹	0.1 x 10 ²
10 ⁻³	0.18 x 10 ²	Nil	0.42 x 10 ²	0.1 x 10 ³
10 ⁻⁴	0.77 x 10 ³	Nil	0.56 x 10 ³	Nil
10 ⁻⁵	0.71 x 10 ⁴	Nil	0.59 x 10 ⁴	Nil
10 ⁻⁶	0.91 x 10 ⁵	Nil	0.77 x 10 ⁵	Nil

Table 4.2: CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

Colour	Diameter	Elevation	Age (shape)	Probable Organisms
Golden yellow	2m	Raised surface	Round (entire)	<i>Staphylococcus aureus</i>
Creamy	2 – 4cm	Flat	Irregular	<i>Bacillus sp</i>
Brown	2 – 4mm	Raised	Entire	<i>Shigella sp</i>

4.1 Biochemical Reactions of Isolates**Table 4.3: Triple sugar iron agar**

Butt colour	Slant colour	Results
Yellow	Red	Glucose only fermented
Yellow	Yellow	Glucose, lactose or sucrose
Red	Red	No action on sugar

Table 4.4 Characterization and identification of isolates from Nutrient Agar media

Isolates	Triple Sugar Iron (TSI)						Biochemical Reactions						Suspected Genus
	Suc or Lac	H ₂ S Pro	Gas Pro	Slant	Butt	Glu	Cit	Ind	Ur	Cat	Gram Staining	Mot	
NAC1	+ve	+ve	+ve	AK	AC	+ve	+ve	-ve	-ve	+ve	+Cocci (chains)	-ve	<i>Staphylococcus aureus</i>
NAC11	+ve	+ve	+ve	AK(AC)	AC	+ve	+ve	-ve	-ve	+ve	+Cocci (single)	-ve	<i>Staphylococcus aureus</i>
NAC5A	-ve	+ve	+ve	AK	AC	+ve	+ve	-ve	+ve	+ve	+Slender Rods	-ve	<i>Bacillus sp.</i>
NAC5B	-ve	+ve	-ve	AK	AC	+ve	+ve	-ve	+ve	+ve	-Long Rod	-ve	<i>Shigella sp.</i>

KEY

AC=Acid

AK=Alkaline

NAC1=First polluted soil sample

NAC11=Second polluted soil sample

NAC5A=First control soil sample

NAC5B=Second control soil sample

Suc is Sucrose, Lac is Lactose, H₂S Pro is Acid Production, Gas Pro is Gas Production, Glu is Glucose, Cit is Citrate, Ind is Indole, Ur is Urease, Cat is Catalase and Mot is Motility.

The table 4.5 below shows Presumptive Identification of Fungi

Isolates	Fungal characteristics	Organisms identified (presumptive)
P (PDA)	Hyphae, distinct points of constriction simulating link sausages (Pseudohyphae) with budding yeast forms (blastospores) often seen.	<u><i>Candida sp</i></u>
C (PDA)	Pale brownish – gray colony with brownish hyphae opposite very short – rhizoid. Hyphae irregular in size and devoid of septa.	<u><i>Rhizopus sp</i></u>

KEY

P (PDA) = Potato Dextrose Agar for polluted soil sample

C (PDA) = Potato Dextrose Agar for control soil sample.

DISCUSSION

Table 4.1 showed absence of coliform bacteria on the Total Coliform Count column for the polluted soil sample. Such bacteria are autotrophic in nature – taking energy from the environment in the form of sunlight or inorganic chemicals and use it to create energy rich molecules. During such process, the organism breaks down both organic and inorganic materials to derive energy for growth and cell building thereby creating soil rich in nutrients for plant growth. The essence of the unpolluted soil sample analysis is for comparison. According to the same table, the column for the Total Coliform Count of the unpolluted soil sample indicates presence of coliform bacteria.

Table 4.4 showed Triple Sugar Iron Test (Triple Sugar Iron Agar).

On the slant, all the isolates from MacConkey Agar showed alkalinity. On the Butt, all the isolates from MacConkey Agar showed acid production and characterized by yellow colouration of the Butt. Three isolates (NAC1, NAC11 and NAC5A) from MacConkey Agar showed gas production (hydrogen sulphide) and one (NAC5B) did not show hydrogen sulphide gas production and hence negative.

The same table 4.4 under Citrate column showed that all the isolates have the ability to utilize citrate as their sole carbon source and ammonia as only source of nitrogen, hence positive. The same table for Indole showed that all the isolates lack the ability to break down amino acid, tryptophan with the enzyme tryptophanase and hence negative. The table also showed under Urease column that two isolates (NAC1 and NAC11) lack the ability to hydrolyse urea – a common source of nitrogen to ammonia and carbon dioxide (negative), while two isolates (NAC5A and NAC5B) have the ability and hence positive. Similarly, the table under Catalase reaction column showed that all the isolates produced catalase enzymes which can break down hydrogen peroxide to water and oxygen, hence positive. Gram staining reaction showed that three isolates (NAC1, NAC11, and NAC5A) are gram positive and NAC5B (isolate) negative. While NAC1 under microscopic identification showed to be positive Cocci in chains, NAC11 positive Cocci (single), NAC5A positive slender Rods and NAC5B gram negative long Rod. Similarly, Motility Test showed that all the isolates lack the ability (flagella) to move from one place to another and hence negative.

Thereafter, the results of the biochemical analysis of the isolates were matched with chart to get the suspected genus of bacteria. The row for NAC1 and NAC11 isolates were suspected to be *Staphylococcus aureus*, NAC5A *Bacillus sp.* and *Shigella sp.*

From table 4.5 for Presumptive Identification of fungi under microscope, the isolate P (PDA) showed presence of hyphae, distinct points of constriction simulating link sausages (Pseudohyphae) with budding yeast forms (blastospores) and hence *Candida sp.* The isolate C (PDA) showed brownish – gray colony with brownish hyphae opposite very short – rhizoid. Hyphae irregular in size and devoid of septa.

Additionally, the microbial analyses showed that the polluted soil sample contains bacteria such as *Staphylococcus aureus* and no coliform. In the same, the control sample contains *Bacillus sp.*,

Shigella sp. and presence of coliform. *Staphylococcus aureus* is a facultative anaerobic gram positive organism that grows by aerobic respiration or by fermentation that yields principally lactic acid. *Bacillus* has some strains that possess nitrogenase that are able to fix atmospheric nitrogen. Theoretically, such *Bacillus* strains could stimulate plant growth by colonizing plant tissues – external or internal and providing fixed nitrogen to the host plant. In reality, nitrogen fixation by *Bacillus* has been detected in the rhizosphere of many plants and in case was attributed to a significant growth response in wheat (Rennie and Larson, 1979).

Various *Bacillus species* have proven able to increase nutrients availability in the rhizosphere for example; phosphorous is often abundant in soils but in forms mostly unavailable to plants, as part of insoluble or poor soluble inorganic or organic phosphate pools (Anderson, 1976). Other indirect ways by which *Bacillus* can stimulate plant growth are positive interactions with symbiotic bacteria and fungi for example, *Bacillus* has been shown capable of increasing legume root nodule number and size (Chanway et al., 1990; Peterson et al., 1996), accelerating formation of endomycorrhizae (Von Alten et al., 1993; Budi et al., 1999) and increasing the number of ectomycorrhizal root tips (Garbaye, 1994).

Many *Bacillus* strains are capable of inhibiting pathogen growth or activity directly, inoculation of plants with certain strains can lead to Induced Systemic Resistance (ISR), in which certain plant defence mechanisms are turned on so that when challenged by a disease – causing microorganism, the plant must be able to resist infection by the pathogen. Because bacteria are able of biocontrol and are able to suppress pathogens either directly, through an antagonistic interaction or indirectly by inducing systemic resistance between the host plant.

Shigella boydii is a bacillary (rod shaped) gram negative bacterium that does not form spores and usually not mobile. It inhabits in the intestine and rectum of humans and other primates. It can survive in faeces and soil contaminated with fecal matter.

The tentative fungal isolates are *Candida sp* in polluted soil sample and *Rhizopus sp.* in unpolluted soil sample. *Rhizopus* is a genus of common saprobic fungi on plants. They enhance decomposition of organic substances in the soil thereby availing nutrients in absorbable form to plants. *Candida sp* is a genus of fungi that is usually found in a contaminated soil and *Staphylococcus aureus* for bacteria. Such organisms do more harm than good. They do not actively involve in decomposition processes in soil.

CONCLUSION AND RECOMMENDATION

Conclusion

Through the assessment of soil viability of a farm land polluted with effluent from cassava mills, though during the collection of the soil samples; we observed that maize was dying and others their leaves are yellow. Therefore the soil sample was assessed along with an unpolluted sample of the same farm. Rather, the results showed presence of *Staphylococcus aureus* which more harms than good wherever it is found and the presumptive identification of fungi in the polluted soil sample showed presence of *Candida sp* instead of *Rhizopus sp.* present in the unpolluted sample. *Rhizopus sp.* is a spore fungus that forms a symbiotic association with plants to enhance the root

structures of plants which makes absorption of nutrients in soil easy. Such association is called mycorrhizae whereby the hyphae form mycelium which can be absorbed by plants and most importantly degradation of organic and inorganic substances in soil. *Candida sp.* just like *Staphylococcus aureus* in bacteria does not contribute positively or viably to agricultural lands (soil) hence a skim flora.

Therefore, I conclude that the death of the maize crops was caused by the displacement of *Bacillus sp* by *Staphylococcus aureus* and *Rhizopus sp.* by *Candida sp.*

Recommendation

- I strongly recommend that cassava effluents should be treated first to reduce the microbial load before discharging either to natural drains or farm land.
- Run-off from such farms should be checkmated to avoid the pollution of a nearby river which could lead to loss of aquatic life, aesthetic value and other physical parameters of such river.
- I also recommend that cassava effluent discharge from the mills should be highly controlled and regulated.
- Environmental awareness seminar is recommended to the public over the significant impact of cassava effluent on soil and surface water.

Suggestions for Further Studies

I suggest that subsequent studies should focus on the following:

- The effect of cassava effluent on ground water and
- The effect of cassava effluent on surface water.

REFERENCES

- Akindahausi, A. A., Grissom F.E., Adewisusi SR, Afolabi D.A., Torimiro S. E., Oke O. L. (1998). Parameters of thyvoid function in the endemic goiter of Akingba and Oke-Agbe Villages of Akoko are of South Western Nigeria. *Afr. J. Med. Med. Sci.* 27 (3-4): 239-42 PMID 10497657.
- AOAC, 2002. *Official Methods of Analysis*. 13th edition. Association Analytical Chemists. Washington D.C.
- Arguedes, P., Cooke R. D. (1982). Residual Cynaide concentrations during extraction of cassava starch. *Food Technology* 17:251.262.
- Barnett, H., Hunter B. B. (1987). *Illustrated Genera of Imperfecti Fungi*, 4thedn. Macmillan Publishing Company, New York, USA. pp. 106, 130.
- Bengtsson, B. E., Trient T. (1994). Tapiocal-starch waste water toxicity characterized by Microtox and duck weed tests. *Ambio.* 23:473-477.
- Chanway, C. P. (1995). Differential Response of Western hemlock from low and high elevations to inoculation with plant growth – promoting *Bacillus Polymyxa*. *Soil Biology and Biochemistry.* PP. 27, 767-775.
- Cheesbrough, M. (2000). *District Laboratory Practice in Tropical. Countries, Part 2*, Cambridge University Press, UK. pp. 35-38, 62-69.

- Claude, F., Denis F. (1990). African cassava mosaic virus: Etiology, Epidemiology and control plant disease. 64(6): 404-411.
- Doran, J.W., M. Sarrantonio, and M.A. Liebig (1996). Soil health and sustainability. *Advances in Agronomy*. 56:1-54.
- F.J.Baker; R.E. Silvertown; Chris Pallister (1998). *Introduction to laboratory technology*. 7th edition. Oxford: Woburn, MA: Butterworth-Heinemann publisher, UK.
- Garbaye, J. (1994). Helper Bacteria: a new dimension to the Mycorrhizal Symbiosis. *New Phytologist*. PP. 126, 197-210.
- Henry, G. and Westby, A. (2000). Global Cassava Starch Markets: Current Situation and Outlook. In: Howeler, R. H. Oates, C. G. and O. Brien, G. M. (eds) *Proceedings of the International Symposium on Cassava, Starch and Starch Derivatives*, 7-12 November, Nanning, Guangxi, China, pp. 79-100. International Commission of Microbiological Specifications for Foods (ICMSF) 1978. *Microorganisms in Foods 1. Second Edition. Their Significance and Methods of Enumeration*. University of Toronto Press. Toronto, Canada.
- Nwabueze, T. U., Odunsi F. O. (2007). Optimization of process conditions for cassava (*Manihot esculenta*) lafun production. *Afr. J. Biotechnol.* 6: 603-611.
- Nweke, F. I. (1992). Traditional Cassava Processing in sub-Saharan Africa and research implication In: Scott GJ, Fergusson P. I, Herrera J. E. (Ed) *production development for root and tuber crops. Volume III – Africa* International Potatato centre (CIP), Peru. pp. 147-164.
- Nyle, C. Brady and Ray, R. Well (2002). *The Nature and Properties of Soil*. 13th edition. Pearson Education, Inc. and Dorling Kindersley publishing, New Delhi, India. PP. 18-23 and 465-477.
- Ogbulie, J. N., Uwaezuoke, J.C., and Ogiehor, S. I (1998). *Introductory Microbiology Practical*. First Edition. Springfield publishers, Nigeria. PP. 28-28 and 68-80.
- Oladele, A. O. (2007). Top Ten Environmental Priorities for Africa. *Afr. J. Environ. Sci. Technol.* 1 (2): 1.
- Oyewole IO, Magaji ZJ, Awoyinka OA (2007). Biochemical and toxicological studies of aqueous extract of *Tithonia diversifolia* (Hemsl.) leaves in Wister albino rats. *J. Med. Plant. Res.* 1(2): 030-033.
- OYEWOL, E.O.B., ODUNFA, S.A., (1992). Effects of processing variables on cassava fermentation for "fufu" production *Tropical Science*, 3 2 : 231-240.
- Pelczar, M. J., Chan ECS, Krieg N. R., (1993). *Microbiology: Concepts and Applications*. 1st edn. McGraw-Hill Inc., New York, USA, pp. 80-100, 158-161, 370.
- Peterson, D.J., Srinivasan, M.S., and Chanway, C.P. (1996). *Bacillus Polymyxa* Stimulates increased *Rhizobium eti* Populations and Nodulation when co-resident in the rhizosphere of *Phaeolus Vulgaris*. *FEMS Microbial Letters*. PP. 142, 271-276.
- P. Narayanan (2007). *Environmental Pollution Principles, Analysis and Control*. First Edition. Satish Kumar Jain publishers, New Delhi, India. PP. 189-191.
- P. Purushothama Raj (2008). *Soil Mechanics and Foundation Engineering*. First impression. Dorling Kindersley, India. PP. 33, 38, 52-54 and 302.

- Rojas Mattinez, A., and A. V alienate- Banuct (1996). Analysis Comparativo de la Quiroptero fauna de Valle de Tehuacan. Cuicarlán puebla – Oaxaca, Acta Zoologica Mexicana. PP. 1-22 and 67.
- Taye Mulugeta (1994). Cassava in Southern and South West Ethiopia. Cassava News Letter, Vol 18, pp 6-7, CIAT, Columbia.
- Von Alten, H., Lindemann, A. and Schonbeck, F. (1993). Stimulation of Vesicular – arbuscular Mycorrhiza. PP. 2,167-173.