

**EFFECTS OF SPENT CRUDE OIL ON THE GROWTH AND DEVELOPMENT OF
TANNIA (*Xanthosoma sagittifolium* (L.) SCHOTT) AND TARO (*Colocasia esculenta* (L.)
SCHOTT)**

BY

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SR/1719/RPR/20/156**

**DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
BENIN CITY**

MAY, 2021

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND
BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN,
BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc. HONS) IN PLANT BIOLOGY
AND BIOTECHNOLOGY.**

MAY, 2021

CERTIFICATION

This is to certify that the project work was carried out by **Aisosa Peace OMOFOMWAN** in the Department of Plant Biology and Biotechnology, University of Benin, Benin City under the supervisor of

DR. A. O. CHIME
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DATE

PROF F. I. OKUNGBOWA
(HEAD OF DEPARTMENT)

DATE

EXTERNAL EXAMINER

DATE

DEDICATION

I wish to dedicate this work to God Almighty and my parents.

ACKNOWLEDGEMENTS

The list of people I wish to acknowledge is too long for this page but I will try to capture everyone who contributed in no small measure to the success of this work

Without God, this work would have been impossible to complete and I am grateful for His blessings, guidance, providence, mercy and love upon my life.

To my invaluable parents and family, I am honoured to partake in the fellowship of love that envelopes each and every member of our family. You are all highly appreciated.

To my supervisor, Dr. A. O. Chime, thank you for the inspiration, guidance, patience and understanding. To Dr. Matthew C. Ogwu, I thank you for everything.

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ABSTRACT

This study evaluated the effects of spent crude oil on the growth and development of two cocoyam species (*Colocasia esculenta* [L.] Schott. and *Xanthosoma sagittifolium* [L.] Schott.) as well as the microbiological, proximate and phytochemical composition of soil and cocoyam species. Cocoyam corms from both species were collected from different markets in Benin City while the spent crude oil was obtained from mechanic workshop. 250kg of loamy soil was mixed with 10 litres of spent oil, the same treatment was carried out for the other respective treatment 7 litres, 3 litres and the control which was without oil. Then 25kg of the various soil treatment was transferred into the perforated bowls. After which the corm was planted into the bowl, then the plant was monitored for eight months. After eight month the plant that was given control treatment had the highest plant height (75.5 cm). The plants grown on spent oil contaminated soil had better body mass compared to those grown on soil without any contaminants. The microbiological and phytochemical analysis of the control and contaminated soil and cocoyam species was done using standard laboratory methods. The result of the fungi associated with cocoyam revealed a colony-forming unit of between 64×10^5 and 127×10^5 . The group of fungi mainly associated with the different cocoyam samples obtained from the different markets in Benin City was mainly *Aspergillus* and *Penicillium* species. The *Aspergillus* sp. was more diverse based on their morphological description on PDA when compared to the *Penicillium* sp. The percentage occurrence of the *Penicillium* sp. was found to be higher than those of the *Aspergillus* sp. in all the cocoyam samples analyzed. The results of the proximate values of the Taro samples revealed the presence of carbohydrates, protein, fat, fibre and ash in varying amounts while the results of the phytochemicals revealed the presence of saponin, tannins, alkaloids, flavonoids and polyphenol. Cocoyam contains food compounds that are beneficial and also of economic importance to man. However, there is the need to conserve this food substance against fungal infestation so that its full economic value can be harnessed. Most importantly, the results from the study revealed that the growth and development of cocoyam are affected by spent crude oil pollution of soil. More study is required to elucidate the potential effects and bioremediation potentials of the crops.

CHAPTER ONE

INTRODUCTION

Cocoyams (taro: *Colocasia esculenta* [L.] Schott. and tannia: *Xanthosoma sagittifolium* [L.] Schott.) are root crops produced in tropical and subtropical regions of developing countries. In certain countries such as Ghana, there are surpluses in production but deficits in cereals. Cocoyams are used in a range of indigenous foods. Post-harvest losses are high due to mechanical damage of corms during harvest and microbial attacks on such damaged corms during storage. Cocoyams contain, on average, 25% starch (wet weight basis) with A-type structures characterized by small granule size (<1.5 μm). Non-starch polysaccharides in cocoyams confer gummy properties to the starch. However, mechanical effects of raphides crystals of calcium oxalate and other components produce irritation when raw corm tissue is ingested resulting in several levels of discomfort. With appropriate processing, cocoyams could be a rich source of starch for food and industrial applications and corms have the potential for new product development. Stabilizing cocoyam crops and adding value could greatly improve their utilization in cocoyam producing countries (Owusu-Darko et al. 2014). Africa is the major producer with West and Central Africa, notably Nigeria, Ghana and Cameroon contributing to over 60% of the total Africa production (Onyeka, 2014). Thus, the importance of cocoyam to regional food security can't be overstated.

HISTORY AND CENTER OF ORIGIN OF COCOYAM

Xanthosoma sp. originated from tropical America (Crop trust, 2010); most probably, central and South America (Ramanatha et al., 2010), where the species are believed to have been domesticated from the wild (Bermajo and Leon,1994). *Xanthosoma* sp. are listed as an invasive species in many areas of the world (French Polynesia, Florida, the Galapagos Islands, Puerto

Rico, and costa) in addition to being intentionally introduced to several other regions including Africa and Asia (Crop Trust, 2010). It is a more recent introduction to Ghana and West Africa compared with the other prominent genus, colocasia, which is speculated as being native to the subregion (Doku, 1966) since there is no known period of introduction of the crop. *Xanthosoma* is therefore referred to as "new cocoyam" in West Africa. Brown 2000 traced its introduction to West Africa as far as the 16th and 17th reported it's an introduction to Ghana in 1843 (19th century) by the West Indian missionaries. There is a paucity of information on the species in the region between the 17th and 19th centuries.

BOTANY OF COCOYAM

Tannia and taro are both edible aroids that are herbaceous, monocotyledonous perennial plant grown for their corms. They are lowland crops. They are temperature sensitive and has a high demand for moisture for their large transpiring surfaces. They can grow up to 2meters in height (Alfred et al., 2016) their growth habit is acaulescent; that is they possess short stem are called corms. They produce flowers when they mature. Their leaves are large. They are heart-shaped or saggitate it has a smooth margin both tannia and taro produces single margin both tannia and taro produces single leaves from their petiole. Their cormels are large and fibrous, tannia more than taro. Their adventitious and shallow roots arise from the corms of the plant.

TAXONOMY OF COCOYAM

Tannia (*X.sagittifolium*) and taro (*C. esculenta*) are both edible aroids from the family; Araceae. The family of Araceae has more than 1500 species but most of these are inedible due to the high concentration of calcium oxalate crystals. These are present in tannia and taro but in low quantities, so it can be cooked. (Rashmi et al., 2018). Below are the hierarchy or botanical hierarchy of tannia and taro;

Taro:

Kingdom: Plantae

Division : Magnoliophyta

Class: Liliopsida

Order: Arales

Family: Araceae

Genus: *Colocasia*

Species: *C. esculenta* [L.] Schott.

Tannia:

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Arales

Family: Araceae

Genus: *Xanthosoma*

Species: *X. saggitifolium* [L.] Schott.

CULTIVATION, YIELD AND DISTRIBUTION OF COCOYAM

Cocoyam, *Xanthosoma sagittifolium*, is cultivated in the tropical region for human nutrition, animal feed, and cash income for both farmers and traders. Cocoyam is vegetatively propagated using the corms and to a lesser extent on the variety but may be the region of 4-10t/ha. Nigeria is currently the world's largest producer of Cocoyam, producing about 1800000 tons of cocoyam per annum, according to about 30% of the world's total and 48% of Africa total production (Ogbona et al., 2012).

Cocoyam (*Xanthosoma sagittifolium*) is among the world's six most important root and tuber crops (FAO, 2012). It is a tropical crop and has domesticated in most communities in Oceania, Africa and Asia (Ramanatha et al., 2010) providing sustenance for over a million people (Onokpise et al. 1999; Vaneker and Slaats, 2013). Africa is the major producer with and central Africa, notably, Nigeria, Ghana and Cameroon contributing over 60 % of the total production (Onseka, 2014). Thus the importance of Cocoyam to regional food security can't be overstated. Cultivation spread across Africa taro is widely produced in Africa even though the exact time of its spread to the region is unknown. Nowadays it is cultivated in Cameroon, Nigeria, Ghana and Burkina Faso which has gained high importance (Rashm et al., 2018).

ECONOMIC IMPORTANCE OF COCOYAM

Cocoyam is an important food security crop I. Ghana because it stores better than all other root and tuber crop. Despite its socio-economic relevance, production has declined persistently since the last decade. In Nigeria, cocoyam is mostly popular among the Southeasterners and is eaten in various forms. It is boiled and eaten with red oil; made into a paste and cooked as ekpang nkwukwor, used as thickener In soup such as bitterleaf, oha and several other dishes. Cocoyam leaf is also used as a vegetable in soups. Boiled cocoyam corms and cormels can be peeled, cut

up, dried and Stored or milled into flour, which can be used for soups, biscuits, bread and puddings for beverages. The peels can also be fed to ruminants.

Some health benefits of eating cocoyam include cancer prevention. Taro root plays an important part in the antioxidants activity in our bodies. High level of vitamin A, vitamin C and various other phenolic antioxidants found in taro root help to boost the immune system and help eliminate dangerous free radicals from our system. Free radicals are the dangerous byproduct of cellular metabolism that may result in healthy cells to mutate and turn into cancerous cells. By eliminating these free radicals, our general health is almost guaranteed. Cryptoxanthin, which is found in taro root, is direct to a lowered chance of developing both lung and oral cancers. Cocoyam is extremely beneficial for rheumatoid arthritis.

CRUDE OIL CONTAMINATION

Crude oil is simply unprocessed oil found deep beneath the earth's surface. It can range in colour from clear to black and can be found as a liquid or solid. Overall properties of crude oils are dependent upon their chemical composition and structure. Crude oil is pumped and stored in barrels for future refinement. The refinement process may involve filtering, the addition of additives, and specialized separation techniques to create specific crude oils and crude oil products. Generally, all crude oils are made up of hydrocarbon compounds. The main hydrocarbons found in crude oil are Aliphatics, Alicyclics, and Polycyclic Aromatic Hydrocarbons (Ryder et al., 2004).

The term bioremediation has been made of two parts: "bios" means life and refers to living organisms and "to remediate" means to solve a problem. "Bioremediate" means to use biological organisms to solve an environmental problem such as contaminated soil or groundwater. Bioremediation is the use of living microorganisms to degrade environmental pollutants or to

prevent pollution. In other words, it is a technology for removing pollutants from the environment thus restoring the original natural surroundings and preventing further pollution(Sasikumar and Papinazath, 2003). Bioremediation could simply be defined as a biological process of the decontamination of contaminated environment. The environment may be either terrestrial, aqueous, or both. However, a more comprehensive definition is presented below: bioremediation is a means of cleaning up contaminated environments by exploiting the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (IV) oxide and water, or by conversion into microbial biomass (Baggott, 1993; Mentzer and Ebere, 1996). A point to emphasize here is that bioremediation and biodegradation should not be confused with each other. Bioremediation is a technique that may include biodegradation as only one of the mechanisms involved or applied in the process of bioremediation. Only some contaminants are biodegradable, and only some microorganisms can degrade a fraction of contaminants (Walsh, 1999). Therefore, it would be worth studying the biodegrading potential of microorganisms. Even though microorganisms have been used for the treatment and transformation of waste products for at least a century, bioremediation is considered a new technology for eco-friendly decontamination of polluted environment. As a popular case of the application of this technology, municipal wastewater is microbiologically decontaminated under controlled conditions so that dependent upon the metabolic activities of microorganisms, different systems of activated sludge and fixed films are applied in wastewater treatment facilities (King et al., 1992). Wastes and pollutions can be permanently eliminated. Also, lasting liabilities are eliminated taking advantage of less expensive but in the meantime more long-standing biological systems. Furthermore, the bioremediation methods could be applied in an integrated manner together and coupled with

other treatment approaches. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.

AIMS AND OBJECTIVES

This study was carried out with the following aims and objectives:

1. To study the effects of spent crude oil on the growth and development of the species.
2. To discover if cocoyam has bioremediation properties.
3. To find out the control of spent crude oil in different concentration to the species
4. To ascertain if there are differences in how tannia and taro bioremediate spent crude oil.

CHAPTER TWO

MATERIALS AND METHODS

2.1 STUDY AREA

The cultivation of cocoyam for the morphological characterization was carried out in a plot of land in freedom street, off Enogie palace road, Benin City (6.32N, 5.67E), located within the tropical rain forest zone. It is situated in Edo state which is characterized by two distinct conditions of wet and dry seasons. It experiences high rainfall and humidity for most of the year, with an annual average rainfall of 150 – 250 cm.

2.2 COLLECTION OF COCOYAM Cultivars

The cultivars used were cormels gotten from the market. Both tannia and taro cormels were gotten. The length of the cormels was between 9cm to 21cm. The weight of the cormels was between 100 grams and 600 grams. The length was taken by tape and the weight taken by a weighing scale. The cormels skin was mostly fibrous. They were of varying shapes; round, conical and elongated shapes were obtained from various cormels. Large corms were also gotten, weighing over 7kg. It was cut into smaller sizes weighing an average of 450 grams and then planted. The shape of these smaller pieces was semi-circular.

2.3 source of cultivars

The cultivars were all collected in Benin City. It was done across ten markets. Eleven markets were visited in total. In *Abiko* market no germplasm was found. In *Ugbekun* market tannia alone was obtained. In the remaining nine markets both germplasms were obtained. That is tannia and taro was obtained from the remaining nine markets. These markets are *Uwa*, *Uselu*, New Benin, *Oliha*, *Ikpoba* hill, *Ekiosa*, *Oka*, *Oba* and New market.

The germplasms obtained from the markets were from different places in Edo state and few from Ogun state, *Elegbeka* to be precise. Corms were gotten from *Uhumwode*, *Ovia* northeast, *Oredo*, *Ikpoba Okha*, *Ovia* southwest, *Esan* west and *Owan* west local government area. To know the viability of the germplasms collected, the sellers were asked the exact date or period it was gotten or harvested and also the exact location or area in general from where it was collected. With their answers, it could be told the exact local government area it was gotten from. The market germplasm collection was done on February 29, March 7 and March 9, 2020. The large corm which was cut in pieces was collected on February 17, 2020, from a home garden close to the study area. Table 1 below shows the summary of the germplasm collection.

Table 1: Passport data of collected cocoyam cultivars

Market or Area of collection	LGA	Exact collection location	Genera	Longitude and latitude	Collected date	Corms Harvested date
Ugbekun	Uhunmwonde	Erua	<i>Xanthosoma</i>	6.55N and 5.89E	February 29 2020	February 23 2020
Uwa	Ovia north east	Uhen	<i>Colocasia</i>	6.76N and 5.53E	February 29 2020	February 2020
Uwa	Ovia north east	Uhen	<i>Xanthosoma</i>	6.76N and 5.53E	February 29 2020	February 2020
Uselu	Ovia north east	Ugbogiobo	<i>Xanthosoma</i>	6.54N and 5.65E	February 29 2020	February 2020
Uselu	Ovia north east	Osasimwino ba	<i>Colocasia</i>	6.41N and 5.75E	February 29 2020	December 2019
New Benin	Oredo	Okpenede	<i>Colocasia</i>	6.35 N and 5.83E	February 29 2020	December 2019
New Benin	Ikpoba okha	Aduwawa	<i>Xanthosoma</i>	6.29 N and 5.64E	February 29 2020	January 2020
New market	Uhunmwonde	Ehor	<i>Colocasia</i>	6.46 N and 6.08E	February 29 2020	December 2019
New market	Uhunmwonde	Ehor	<i>Xanthosoma</i>	6.46 N and 6.08E	February 29 2020	January 2020

Oliha	Ose, Ogun state	Elegbeka	<i>Colocasia</i>	6.96N and 5.72E	February 29 2020	February 2020
Oliha	Ovia south west	Iguobazuwa	<i>Xanthosoma</i>	6.54N and 5.35E	February 29 2020	February 2020
Ikpoba hill	Esan west	Ekpoma	<i>Xanthosoma</i>	6.47 N and 5.92E	March 7 2020	February 2020
Ikpoba hill	Uhunmwonde	Ehor	<i>Colocasia</i>	6.46 N and 6.08E	March 7 2020	February 2020
Ekiosa	Ose, Ogun state	Elegbeka	<i>Colocasia</i>	6.96N and 5.72E	March 7 2020	February 2020
Ekiosa	Uhunmwonde	Ehor	<i>Xanthosoma</i>	6.46 N and 6.08E	March 7 2020	January 2020
Oka	Owan west	Ora, Oke	<i>Colocasia</i>	6.50N and 5.41E	March 9 2020	March 7 2020
Oka	Owan west	Ora, Oke	<i>Xanthosoma</i>	6.50N and 5.41E	March 9 2020	March 7 2020
Oba	Uhunmwonde	Ehor	<i>Xanthosoma</i>	6.46 N and 6.08E	March 9 2020	January 2020
Oba	Uhunmwonde	Ehor	<i>Colocasia</i>	6.46 N and 6.08E	March 9 2020	February 2020
Enogie palace road	Ikpoba okha	Ihinmwinrin	<i>Xanthosoma</i>	6.32N and 5.67E	February 17 2020	February 17 2020

2. 4 field layout

The study area is a lowland. The plot is flat and well-drained such that it doesn't get waterlogged during rainfall. The length of the plot is 9.4m and the breadth 8.1m, making the area 76.14m² or 76m². The land was cleared and prepared for planting on the 9th and 10th of March 2020.

2. 5 CRUDE OIL CONTAMINATION AND PLANTING

Spent crude oil were gotten from the garage where vehicle are been repaired and also packed. The spent crude oil were gotten in opposite hundred feet road, Ikpoba Okha Benin city, Edo State. The crude oil was mixed with the trial soil before planting. Planting was done with rubber buckets that were all perforated to allow the control of water. In these, there were 40 buckets in total. Ten buckets each for each treatment levels, which are control (that is without oil), with 3 litres of spent crude oil, with 7 litres of spent crude oil and with 10 litres of crude oil. Before planting 250 Kg of soil was mixed with each were measured and divided into 10 rubber buckets. rubber, another 250g of soil were scale and properly mixed with 10 litres of spent crude oil by putting the soil in 10 bucket rubber after diving the 250g of soil into 10 buckets; 250g of soil were scale and properly mixed with 7 litres of spent crude oil by putting the soil in 10 bucket rubber after diving the 250g of soil into 10 buckets. 250g of soil were scale and properly mixed with 3 litres of spent crude oil by putting the soil in 10 bucket rubber after diving the 250g of soil into 10 buckets.

Corms were not planted immediately after the soil was mixed with spent Crude oil, after waiting for 7 days planting were conducted. The soil was mixed with the soil on March 7 2020. The corms were planted on March 15 2020. Between 4-5, it was cool weather. It was planted in the bucket rubber, in some holes multiple corms were used when the corm was not large. In other

single corms were used in planting. Weeding was done regularly to improve yield, also watering was done twice daily when the rainfall wasn't consistent. Watering was often done twice daily. The germplasms were planted on March 11 2020, from 8am to 10am. It was planted after the yearly rain began. Heaps were used in planting as could be seen in plate 9. The heaps had a depth of 20cm. The height of the heaps from the level ground was 25cm and width 40cm apart. The spacing used was 90cm horizontally and 110cm vertically. It was done so to give the plants enough room for growth and to limit competition. It also gave enough room to easily pass through and take records.

Non-randomized system of planting was used; meaning cultivation wasn't done at random. Codes were given for easy identification of each germplasm. The codes were used in recording of data and it includes letters and numbers. The numbers are to specify just how many from a particular market or location was planted. It was three from each market and 14 from the corms gotten from the nearby garden. The numbers are also to enable treating of each plant individually. The letters also have their importance. Tn means tannia and Ta means taro. Nmk means new market, Oka means oka market, Oba means oba market, Nbn means new Benin market, Ugb means ugbekun market, Ikp means ikpoba hill market, Eks means ekiosa market, Uwa means uwa market, Olh means oliha market, Usl means uselu market and Epr means Enogie palace road. For instance, Ikp Tn 03 means, germplasm 3, tannia, from Ikpoba hill market.

2. 6 characterization of tannia and taro

Characterization was done following the guidelines provided by IPGRI taro and IBPGR *Xanthosoma* descriptor.

Qualitative parameters and quantitative parameters were taken during characterization.

The qualitative parameters are;

- Plant habit; acaulescent(1), erect above ground stem(2), reclining above ground stem(3).
- Leaf base shape in regards to petiole attachment; peltate(1), sub-peltate(2) non-peltate(3).
- Lamina orientation; Drooping(1), Horizontal(2), Cup-shaped(3), Erect - apex up(4), Erect - apex down(5).
- Leaf blade margin; Entire(1), Undulate(2), Sinuate(3).
- Leaf shape; No basal lobes(1), Hastate(2), Sagittate, basal lobes < 1/8th length of leaf(3), Sagittate, basal lobes 1/8th - 1/4th length of leaf(4), Sagittate, basal lobes >1/4th length of leaf(5).
- Shape of cormels; Conical(1), Round(2), Cylindrical(3), Elliptical(4), Dumb-bell(5), Elongated(6), Flat and multifaced(7), Clustered(8).
- Exterior colour of cormels; light brown(1), dark brown(2).
- Exterior surface of cormels; Smooth(1), Fibrous(2), Scales present(3), Fibrous and scales present(4).
- Root colour; White(1), Red(2), Brown(3).
- Uniformity of root colour; no (0), yes(1).
- Petiole colour; colour of top third, middle third and bottom third; Whitish(1), Yellow(2), Orange(3), Light green(4), Green(5), Red(6), Brown(7), Purple(8).
- Leaf blade colour; Whitish (1), Yellow or yellow green (2), Green (3), Dark green (4), Pink(5), Red(6), Purple(7), Blackish(8).
- Type of leaf variegation; absent (0), present (1).
- Leaf sinus denuding; not denuded (0), denuded (1).

- Leaf main vein colour; Whitish (1), Yellow (2), Orange (3), Green (4), Pink (5), Red (6), Brownish (7), Purple (8).
- Vein pattern; V pattern (1), I pattern (2), Y pattern (3), Y pattern and extending to secondary veins(4).

The quantitative parameters are;

- Plant height; measured from base of plant to tip of tallest leaves.
- Number of suckers; the numbers were counted.
- Number of cormels; the numbers were counted.
- Length of cormels; this was measured by a tape.
- Diameter of cormels; this was measured by a tape
- Weight of cormels; this measured by a scale.
- Size of cormels(at maturity), Scored on a scale of 1-9; Small(3), Medium(5), Large(7).
- Length and breadth ratio of cormels; lengths were measured by tape then ratio was calculated.
- Length/breadth ratio of lamina; lengths were measured by tape then ratio was calculated.
- Length of root; this was measured by a tape.
- Petiole length; this was measured by a tape.
- Lamina length; this was measured by a tape.

28 parameters in total.

The readings dealing with length were taken with a tape while that of weight was taken with a weighing scale. The qualitative parameters were taken by sight alone.

The data collected, measured and observed were written and also documented in an excel sheet for statistical analysis.

2.7 SOIL ANALYSIS

2.7.1 MICROBIAL ANALYSIS

2.7.1.1 FUNGI

2.7.1.1.1 PREPARATION OF POTATO DEXTROSE AGAR

Thirty-nine grams of potato dextrose agar (PDA, Oxoid, England) was measured into a conical flask, 500mls of water will be added. The conical flask was then heated. After heating, additional 500mls of water will be added to make it up to be 1 litre. The medium was then sterilized by autoclaving at 15psi (121⁰C) for 15minutes. chloramphenicol at 10gm per 200ml of the medium was introduced at pouring to inhibit the growth of bacteria Inoculation and transfer of culture were carried out on sterile inoculating bench CRC model HSB 60*180, after wiping with methylated spirit.

2.7.1.1.2 STERILIZATION OF GLASSWARE

All glassware and slides were washed in OMO detergent, rinsed in several changes of tap water and finally with distilled water and allowed to dry. They were sterilized in an electric oven at a temperature of 60⁰C for 24 hours. Coverslips were flame sterilized just before use. The droppers, pipettes, cotton wool and plugs were covered with aluminium foil to prevent the entry of condensed water vapour into the media.

2.7.1.1.3 PREPARATION OF STERILE WATER BLANK

Ten millilitres of distilled water was pipetted into McCartney bottles representing each sample collected from the market. McCartney bottles representing each stock solution will be labelled according to the samples.

2.7.1.1.4 PREPARATION OF STOCK SOLUTION

The stock solution will be prepared by weighing one gram of the collected locust bean sample in a glass Petri dish. The weighed samples will be teased into smaller beats and then transferred into the already sterilized McCartney bottles representing the different stock solution mentioned above.

2.7.1.1.5 SERIAL DILUTION

McCartney bottles will be divided into five groups and already sterilized McCartney bottles labelling 10^1 to 10^4 with the stock bottle representing each sample. With a sterile pipette, 1ml each was transferred from the stock bottles into the bottles labelled 10^1 to 10^4 containing nine millilitres of sterilized distilled water for serial dilution preparation.

2.7.1.1.6 METHOD OF INOCULATION

The pour plate method of inoculation was used in the isolation of the microorganisms associated with the samples. One ml each of the serial dilution prepared samples was pipette with the aid of a syringe and was transferred into the corresponding labeled Petri dishes. Nine ml of molten prepared potato dextrose agar (PDA) was dispensed into the Petri dishes respectively. The Petri dishes were inoculated under room temperature for 72hrs.

2.7.1.1.7 DETERMINATION OF MICROBIAL LOAD

The microbial load of the samples was determined by visibly counting the colony forming unit after 72hrs for fungi

The microbial load/ml was then determined by the formula stated below

$$\text{Count/ml} = \frac{\text{No of colonies on plate}}{\text{Amount plated}} \times \frac{1}{\text{dilution factor}}$$

2.7.1.1.8 DETERMINATION OF PERCENTAGE OCCURRENCE

This was done using the formula stated below

$$\% \text{ occurrence} = \frac{\text{No of a particular isolate on plate}}{\text{Total number isolates on same}} \times 100$$

2.7.1.1.9 SUB-CULTURING OF MICROORGANISMS

Freshly prepared PDA was poured into already sterilized Petri dishes, it was allowed to solidify. From the old plates containing the microorganism, the streaking method will be used to isolate the individual bacteria cultures into a fresh plate for further studies. The fungal cultures was subcultured by carefully taking a fresh growing portion with a sterilized needle into a fresh PDA plate.

2.7.1.1.10 MACROSCOPIC AND MICROSCOPIC IDENTIFICATION OF THE ISOLATED FUNGI

Fungi isolated from the groundnut samples were analyzed for morphological using cultural characteristics such as the colony colour. Microscopic features such as shape and texture of conidia were observed under a microscope fitted with a camera (Motic B1 Digital camera) using the cover-slip method in which a little quantity of each culture was transferred onto the base of coverslips buried in PDA.

2.7.1.2 BACTERIA

2.7.1.2.1 PREPARATION OF NUTRIENT AGAR

Twenty-eight grams of nutrient agar (NA, Oxoid, England) will be measured into a conical flask, 500mls of water will be added. The conical flask was then heated. After heating, additional 500mls of water will be added to make it up to be 1 litre. The medium was then sterilized by autoclaving at 15psi (121⁰C) for 15minutes. Fulscin at 10gm per 200ml of medium was introduced at pouring to inhibit the growth of fungi. Inoculation and transfer of culture were

carried out on sterile inoculating bench CRC model HSB 60*180, after wiping with methylated spirit.

2.7.1.2.2 STERILIZATION OF GLASSWARE

All glasswares and slides were washed in OMO detergent, rinsed in several changes of tap water and finally with distilled water and allowed to dry. They were sterilized in an electric oven at a temperature of 60°C for 24 hours. Cover slips were flame sterilized just before use. The droppers, pipettes, cotton wool and plugs were covered with aluminium foil to prevent entry of condensed water vapour into the media.

2.7.1.2.3 PREPARATION OF STERILE WATER BLANK

Ten milliliters of distilled water was pipetted into McCartney bottles representing each samples collected from the market. McCartney bottles representing each stock solution will be labeled according to the samples.

2.7.1.2.4 PREPARATION OF STOCK SOLUTION

Stock solution will be prepared by weighing one gram of the collected locust bean sample in a glass Petri dish. The weighed samples will be teased into smaller beats and then transferred into the already sterilized McCartney bottles representing the different stock solution mentioned above.

2.7.1.2.4 SERIAL DILUTION

McCartney bottles will be divided into five groups and already sterilized McCartney bottles labeling 10^1 to 10^4 with the stock bottle representing each samples. With a sterile pipette, 1ml

each was transferred from the stock bottles into the bottles labeled 10^1 to 10^4 containing nine milliliters of sterilized distilled water for serial dilution preparation.

2.7.1.2.5 METHOD OF INOCULATION

The pour plate method of inoculation was used in the isolation of the microorganisms associated with the samples. One ml each of the serial dilution prepared samples was pipette with the aid of a syringe and was transferred into the corresponding labeled Petri dishes. Nine ml of molten prepared potato dextrose agar (PDA) was dispensed into the Petri dishes respectively. The Petri dishes were inoculated under room temperature for 72hrs.

2.7.1.2.6 DETERMINATION OF MICROBIAL LOAD

The microbial load of the samples was determined by visibly counting the colony forming unit after 72hrs for fungi

The microbial load/ml was then determined by the formula stated below

$$\text{Count/ml} = \frac{\text{No of colonies on plate}}{\text{Amount plated}} \times \frac{1}{\text{dilution factor}}$$

2.7.1.2.7 DETERMINATION OF PERCENTAGE OCCURRENCE

This was done using the formular stated below

$$\% \text{ occurrence} = \frac{\text{No of a particular isolate on plate}}{\text{Total number isolates on same}} \times 100$$

2.7.1.2.8 SUB-CULTURING OF MICROORGANISMS

Freshly prepared PDA was poured into already sterilized Petri dishes, it was allowed to solidify. From the old plates containing the microorganism, the streaking method will be used to isolate the individual bacteria cultures into a fresh plate for further studies. The fungal cultures was sub-

cultured by carefully taking a fresh growing portion with a sterilized needle into a fresh PDA plate

2.7.1.2.9 MACROSCOPIC AND MICROSCOPIC IDENTIFICATION OF THE ISOLATED FUNGI

Fungi isolated from the groundnut samples were analyzed for morphological using cultural characteristics such as the colony colour. Microscopic features such as shape and texture of conidia were observed under a microscope fitted with a camera (Motic B1 Digital camera) using the cover-slip method in which a little quantity of each culture was transferred onto the base of cover slips buried in PDA

2.7.1.3 CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES

2.7.1.3.1 CULTURAL CHARACTERISTICS

For the bacterial isolates, cultural characteristics will be observed on Nutrient agar plates. The cultural characteristics include. Size, shape, surface, opacity, texture, elevation and pigmentation will be determined by visual observation.

2.7.1.3.2 GRAM REACTION

A wire loop was sterilized in Bunsen burner and allowed to cool then a loopful of growth was collected from the agar plate and applied on a clean grease-free slide then a drop of normal saline was added, emulsified and heat fixed by passing over a flame three times. The smear was flooded with crystal violet for 30-60seconds and then covered with iodine for 30-60seconds and then washed off; it was decolorized with acetone until no colour runs off the slide and rinsed immediately. The slide was covered with safranin for 1 minute and then washed off with clean water. The slide was kept in a rack to air dry after wiping the back with cotton wool. The stained

smear was then examined microscopically under oil immersion at 100 magnification objective lens. Gram-positive bacteria appeared dark purple while gram- negative bacteria appeared pink.

2.7.1.3.3 MOTILITY TEST

Motility test was done by stabbing a slant with the isolated bacteria. If bacteria is not motile, there will only be growth along the stab line.

2.7.1.3.4 CATALASE TEST

Three millilitres (3ml) of hydrogen peroxide solution was poured into a sterile test tube. Then a sterile glass rod was used to collect several colonies of the test and inoculate them into the hydrogen peroxide solution. It was observed for immediate active bubbling for the positive test.

2.7.1.3.5 COAGULASE TEST

A drop of sterile distilled water was placed on each end of a sterile slide. Then a colony of the test organism was emulsified on each spot to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or dotting of the organisms within 10seconds. Plasma was not added to the second suspension which serves as the control.

2.7.1.3.6 OXIDASE TEST.

A piece of filter paper was placed in a clean Petri dish and 2-3 drops of fresh or nascent oxidase reagent was added. A colony of test organism was collected using a glass rod and smeared on the filter paper and observed. Blue-purple color within few a seconds showed a positive test.

2.7.1.3.7 UREASE TEST

The test organism was heavily inoculated onto Christensens urea broth in a bijou bottle using a sterile wire loop and incubated at 35°C- 37°C for 18- 24hours and examined, thereafter a pink color in the medium showed positive test.

2.7.1.3.8 CITRATE TEST

Simon's citrate agar medium was prepared in a slant bijou bottle, then using a sterile wire loop was used to inoculate the test organism onto the slant medium and incubated at 35°C for 48hours after which it was examined for color formation. A bright blue color in the medium gave a positive citrate test. *K. pneumonia* and *E. coil* were employed as positive and negative controls respectively.

2.7.1.3.9 INDOLE TEST

A sterile wire loop was used to inoculate a colony of test organism into 2ml of peptone water containing tryptophan. The tube was stoppered and incubated at 37°C for 24hours. Kovac's reagent was added to the medium. Observation of red coloration on the surface layer within 10minutes showed a positive result.

2.7.1.3.10 CARBOHYDRATE FERMENTATION TEST

The four sugar solutions were prepared and poured into test tubes well stopped with Durham tube for gas collection. The sugar was autoclaved after which a ioopfui of test organisms was introduced into the sugar solution (Buchana, and Caibbons. 1994). A change in color from pink to yellow shows fermentation and collection of gas bubbles in the Durham tube shows gas production which is a positive test. A control was set up without the organism inoculated.

2.7.1.3.11 METHYL RED TEST

Glucose phosphate peptone water was used for inoculation of test organisms and incubated for 48 hours at 37°C after which few drops of methyl red solution was added to the culture and read immediately. Formation of red color immediately showed a positive test.

2.7.2 PHYSIOCHEMICAL ANALYSIS

2.7.2.1 SOIL REACTION (PH)

2.7.2.1.1 PRINCIPLE:

The pH value of an aqueous solution is the negative logarithm of hydrogen concentration. Ion activity is measured by using a pH meter. Soil pH is determined in 1:2.5 soil/water extract.

2.7.2.1.2 REAGENT:

Standard pH solutions with values 2, 4, 7, 9.

2.7.2.1.3 APPARATUS;

- 1- pH meter
- 2- Beaker 50 ml.
- 3- Glass rods.
- 4- Thermometer

2.7.2.1.4 PROCEDURE:

2.7.2.1.4.1 EXTRACTION:

- 1- Add 25 ml distilled water to 10g air-dried sample in a beaker 50 ml. Read the suspension temperature by thermometer.
- 2- Stir at regular intervals for 20-30 minutes.
- 3- Wash the pH meter electrode with distilled water.
- 4- Open the contact switch, wait 5 minutes, adjust temperature knob to room temperature.

2.7.2.1.4.2 CALIBRATION

Rinse the electrode and adjust the pH dial with a standard pH solution followed by another acid or alkaline one.

2.7.2.1.5 MEASUREMENT

- 1- Rinse the electrode with distilled water, then with the soil suspension after stirring.
- 2- Read the pH value of the soil Suspension.

2.7.2.2 ELECTRICAL CONDUCTIVITY (EC)

Electrical conductivity Is commonly used for measuring the electrical resistance in the solution which indicates the total concentration of ionized constituents in solutions. It is closely related to the sum of the cations and anions in the suspension. Accordingly it can be used for indicating the salinity in soil extracts. Electric conductivity can be expressed as millimhos/cm in 1:2.5 soil/water extract.

2.7.2.2.1 APPARATUS:

- 1-Conductivity meter, immersion type with platinized platinum electrodes (direct indicating bridge)
- 2- Beakers 100 ml.
- 3- Thermometer.
- 4-Glass rods.
- 5- Funnels & filter paper.

2.7.2.2.2 REAGENTS

- 1- Potassium chloride solution 0.01 N: Dissolve 0.7456 g of dry potassium chloride in distilled water and make to 1 L at 25°C. This is a standard reference solution, which at 25°C has an E.C. of 1411.8×10^6 (0.0014118) mhos/cm or 1.4118 m mhos/cm.

2- Calcium sulfate dihydrate saturated solution. At 25°C it has an E.C. of 2.2 mmhos/cm.

2.7.2.2.3 PROCEDURES

2.7.2.2.3.1 EXTRACTION:

- 1- Put 10 g air-dry soil in 100 ml beaker, add 25 ml distilled water.
- 2- Stir for 10 minutes, repeat stirring 4 times on 30 minutes intervals.
- 3- Measure the suspension temperature by thermometer

2.7.2.2.3.2 OPERATION:

Before measuring, rise and fill the cell with reagent 1,

- 1- Set the temperature compensation dial,
- 2- Open the contact switch, wait for 5 minutes.
- 3- Balance the bridge with the main dial,

2.7.2.2.3.3 CALIBRATION:

Measure the accuracy of the conductivity meter by using reagent 2, it should give an E.C. 2.2 mmhos/cm.

2.7.2.2.3.4 MEASUREMENT:

- 1- Rinse the electrode with the suspension solution.
- 2- Read the conductivity of the soil suspension.

2.7.2.2.3.5 REMARKS:

1- mhos/cm= 1000 mmhos/cm.

mmhos/cm = 1000 micromhos/cm.

- 2- The electrode must be full of the reagent (till the mark before reading).

3- At switching the contact on, if the bridge will not balance, the conductivity of the extract may be below or over that of the scale limits. So either set on reading with micromhos instead of mmhos or dilute the extract.

4- The cell constant is calculated from the relation:

$$K = EC_{25} \times R_{25}$$

Where K=cell constant, EC_{25} = electrical conductivity at 25°C and R_{25} , reading of cell resistance at 25°C.

Note: It is preferable to determine pH and EC in the same soil suspension 1:2.5.

2.7.2.3 SOIL ORGANIC MATTER:

2.7.2.3.1 WALKLEY-BLACK METHOD

2.7.2.3.1.1 EQUIPMENT:

- 1- 500-ml Erlenmeyer flasks.
- 2- 10 ml pipette.
- 3- 10 and 20 ml dispensers.
- 4- 50 ml burette.
- 5- Analytical balance.
- 6- Magnetic stirrer.
- 7- Incandescent lamp.

2.7.2.3.1.2 REAGENTS:

- 1- H_3PO_4 85%
- 2- H_2SO_4 concentrated (96%)
- 3- NaF, solid

4- Standard 1.00 N $K_2Cr_2O_7$: Dissolve 49.04 g of dried (105°C) $K_2Cr_2O_7$ in water and dilute to 1 litre.

5- 0.5 N Fe solution⁺⁺ : Dissolve 196.1g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 800ml of water containing 20ml of H_2SO_4 and dilute to 1 litre. The Fe^{++} in this solution oxidizes slowly on exposure to air so it must be standardized against the dichromate daily.

6- Ferriin indicator: Dissolve 3.71g of O-phenanthroline and 1.74g of $FeSO_4 \cdot 7H_2O$ in 250ml of water.

2.7.2.3.1.3 PROCEDURE:

1- Weigh out 0.10 of 2.00 dried soil(<60 mesh)and transfer to a 500ml Erlenmeyer flask. The sample should contain 10 to 25 mg of organic C (17 to 43 mg organic matter). For a 1 g sample, this would be 1.2 to 4.3% organic matter. Use up to 2g of sample for light coloured soils and 0.1g for organic soils.

2- Add 10 ml of 1 N $K_2Cr_2O_7$ by means of a pipette.

3- Add 200 ml of concentrated H_2SO_4 by means of dispenser and swirl gently to mix. Avoid excessive swirling that would result in organic particles adhering to the sides of the flask out of the solution.

4- Allow to stand 30 minutes. The flasks should be placed on an asbestos sheet during this time to avoid rapid loss of heat.

5- Dilute the suspension with about 200 ml of water to provide a clearer suspension for viewing the endpoint.

6- Add 10 ml of 85% H₃PO₄ Using a suitable dispenser, and 0.2 g of NaF, using the "calibrated spatula" technique. The H₃PO₄ and NaF are added to complex Fe³⁺, which would interfere with the titration endpoint.

7- Add 10 drops of ferroin indicator. The indicator should be added just prior to titration to avoid deactivation of adsorption onto clay surfaces.

8- Titrate with 0.5 N Fe⁺⁺ to a burgundy endpoint. The color of the solution at the beginning is yellow-orange to dark green, depending on the amount of the unreacted Cr₂O₇⁻ remaining, which shifts to a turbid gray before the endpoint and then changes sharply to a wine red at the endpoint. Use of a magnetic stirrer with an incandescent light makes the endpoint easier to see in the turbid system.

(Fluorescent lighting gives a different endpoint color). If less than 5 ml of Fe⁺⁺ solution was required to backtitrate the excess Cr₂O₇⁻, there was insufficient Cr₂O₇⁻ present, and the analysis should be repeated either by using a smaller sample size or doubling the amount of K₂Cr₂O₇ and H₂SO₄. Alternatively use a Pt electrode to determine the endpoint after step 5 above. This will eliminate uncertainty in determining the endpoint by color change.

9- Run a reagent blank following the above procedure without soil. The reagent blank is used to standardize the Fe⁺⁺ solution daily.

10- Calculate % C and % organic matter:

a. % easily oxidizable organic C

$$\text{Where } C = \frac{(B - S) \times N \text{ of Fe}^{++} \times 12 \times 100}{\text{g of soil} \quad 4000}$$

Where B = ml of Fe⁺⁺ solution used to titrate blank,

$S = \text{ml of Fe}^{++} \text{ solution used to titrate sample, and } 12/4000 = \text{milliequivalent of weight of C in g}$

To convert easily oxidizable organic C to total C, divide by 0.77 (or multiply by 1.30) or other experimentally determined correction factor.

b. % organic matter = %C / 0.58 = %C × 1.72

2.7.2.4 POTASSIUM, SODIUM AND MAGNESIUM;

2.7.2.4.1 EXTRACTION FROM SOIL: by ammonium acetate method.

2.7.2.4.1.1 PRINCIPLE:

Ammonium acetate is known to extract the soil exchangeable potassium among other cations. This is rapid procedure for extraction of potassium separately from other exchangeable metallic cations. Potassium absorbed on the soil particles is exchanged with ammonium of the acetate solution, maximally at the latter's strength. Sodium and magnesium could be also extracted by the same reagent.

2.7.2.4.1.2 APPARATUS:

Shaker, plastic bottle with caps, cylinder, beaker.

2.7.2.4.1.3 REAGENTS: Ammonium acetate 1 N :

To 700 - 800 ml distilled water, add 57 ml. conc. acetic acid 68 ml of conc ammonium hydroxide. Dilute to a volume of 1 liter and adjust to pH 7.0 by the addition of more ammonium hydroxide or acetic acid. The same solution can be also made by dissolving 77 g ammonium acetate in 1 liter dist. Water.

2.7.2.4.1.4 PROCEDURES:

(1) Add 100 ml of ammonium acetate solution to 5g air dried soil (passed through 1-2 mm sieve) in a plastic bottle. Place the bottles in the shaker.

(2) Shake for one hour.

(3) Filtrate through filter paper whatman 41 or S & S 512, M & N 280. Receive in a plastic container. Discard the first third of the filtrate

2.7.2.4.2 DETERMINATION:

2.7.2.4.2.1 POTASSIUM AND SODIUM

2.7.2.4.2.1.1 PRINCIPLES:

Potassium and sodium ions can be determined quantitatively when they are atomized from solution led to burner and excited to spectral emission in a flame. Since the intensity of the light emitted by each element depends primarily on the concentration of its atoms in the flame at any given instant, a measurement of the light intensity produced by a given element makes possible the quantitative determination of that element.

2.7.2.4.2.1.2 APPARATUS: Flame photometer.

...Potassium chloride 1000 ppm:

Dissolve 1.9117g dried KCl in distilled water and make to 1 L volume.

...Sodium chloride 1000 ppm:

Dissolve 2.5422 g dried NaCl in distilled water and make to 1 L volume.

2.7.2.4.2.1.3 STANDARD CURVE SOLUTIONS

Prepare the following dilution 10, 20, 30, ..., 100 ppm from the standard 1000 ppm solution in solution of 1 N ammonium acetate pH 7.0.

2.7.2.4.2.1.4 PROCEDURE:

- (1) Pipet an aliquot of the solution to be analyzed into a 50 ml volumetric flask, complete with 1 N ammonium-acetate solution (pH 7)
- (2) Determine the potassium concentration by use of the flame photometer and the appropriate calibration curve.

2.7.2.4.2.1.5 TERMINATING OPERATIONS

- (1) Introduce distilled water for approximately 3 minutes to clean the intake and the atomizer / burner assembly.
- (2) Close the valve of supplying gas.
- (3) Push down button of gas selection and wait until the flame is extinguished.
- (4) Switch off main switch
- (5) Place cover over the housing after allowing sufficient time for cooling of

2.7.2.4.3 CALCULATION:

- (1) Concentration of K or Na in sample extract can be calculated by slope calculation. For every concentration of the standard solution (10-100 ppm) the concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope.

- (2) Content of K and Na in soil sample:

Content of K or Na as mg/100g soil (at 105°C)

2.7.2.4.2.2 MAGNESIUM:

Magnesium can be determined by atomic absorption.

2.7.2.4.2.2.1 REAGENTS

Standard stock solution 1000 ppm. A dilute standard solution is prepared for making up a series of standards for the calibration curve, from 0.1 to 8 ppm.

2.7.2.5 AVAILABLE SOIL MICRONUTRIENTS

Content of available nutrients is relevant to soil supplying power, as the available form of the nutrient can be easily taken up by plants for indicating the nutritional status of soil, available micronutrients are extracted by diluted solutions of chelating compounds, such as EDTA, EDDHA or DTPA.

2.7.2.5.1 EXTRATION BY DTPA (METHOD):

2.7.2.5.1.1 PRINCIPLE:

The DTPA method has been adopted most frequently on alkaline, calcareous areas for most crops. It is successful for evaluating soil nutritional status and separation deficient from non-deficient soils. It also exhibits correlation with plant response to micronutrient fertilizers. Moreover, this method can extract all the 4 elements (Fe, Mn, Zn, and Cu) in one extraction. Therefore, it is easier, simpler and more economical to follow in routine analysis.

2.7.2.5.1.2 REAGENTS:

DTPA solution (extracting solution):

- (a) 0.1 M triethanolamine (TEA) weigh 14.9 g
- (b) 0.005M diethylenetriamine penta acetic acid (DTPA); weigh 1.967g
- (c) 0.01 M calcium chloride $\text{CaCl}_2, 2\text{H}_2\text{O}$, weighs 1.47g

Dissolve a + b+ c in about 800 ml distilled water, adjust pH to 7.3 with diluted HCl 1:1 (it needs 10 ml). Complete to 1 liter with distilled H₂O

2.7.2.5.1.3 APPARATUS

- (1) Shaker.
- (2) 80 ml cups with caps (plastic).
- (3) Funnels.

(4) Filter paper whatman 42 or S & S 512 or M&N 280.

(5) Automatic pipette 50 ml.

2.7.2.5.1.4 PROCEDURES

(1) Weigh 20g air-dry soil sample passed through 2 ml sieve. Transfer the sample to a cup.

(2) Add 40 ml DTPA solution, fix the cup in the shaker.

(3) Shake for 2 hours (100 stroke/min) at 25°C.

(4) Carry out filtration into cups. Discard the first third of the filtrate.

The rest is ready for determination of available micronutrients.

2.7.2.5.2 DETERMINATION OF MICRONUTRIENTS BY ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS):

2.7.2.5.2.1 PRINCIPLE:

An atom is capable of absorbing the same wavelength of light normally emitted upon excitation. In addition, the transitional intensity of a given wavelength is affected by concentration and thickness of the absorbing medium. According to both principles, the electrically excited element from a hollow cathode lamp causes a known spectral emission of the element. A part of the lamp beam emission is absorbed in the flame by the neutral atom of the same element in the flame atomized sample solution. Absorbance correlates with element concentration in the sample.

2.7.2.5.2.2 REAGENTS

Standard stock solutions 1000 ppm of Fe, Mn, Zn, Cu (in ampule).

2.7.2.5.2.3 APPARATUS:

Atomic absorption spectrophotometer.

2.7.2.5.2.4 PROCEDURES

Preparation of standard-reference solutions:

Fe: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 ppm.

Mn: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

Zn: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

Cu: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

Mg: 0.2, 0.5, 1.0, 2.0, 4.0, 8.0 ppm.

2.7.2.5.2.5 DETERMINATION:

(1) Set the AAS in work according to operation instructions in the manual of AAS apparatus.

Care must be taken that compressed air must be introduced before the fuel gas.

(2) After setting on flame, atomize the sample (which must be clear). The value is recorded on digits and printed on chart.

(3) Make a blank with extracting solution. Set apparatus to zero with the blank value for every element.

2.7.2.5.2.6 CALCULATION;

Using slope calculation: for every concentration of the standard solution (0.2 - 8 ppm) the concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope.

Concentration in ppm = reading \times dilution \times factor \times 100 / d.w.at 105°C.

2.7.2.6 SOIL TEXTURE AND PERCENT COMPOSITION (SILT, SAND AND CLAY)

2.7.2.6.1 PROCEDURE

(1) Sample of the soil was mixed in water and allowed to settle for hours in a McCartney bottle.

(2) Once it settled measurements of each layer was measured and the overall length was taken.

(3) Percentage of each layer (silt, clay and sand) was measured with the formula below;

Length of layer \times 100

Total length

(4) Soil texture was determined by the USDA soil textural triangle below;

2.8 STATISTICAL ANALYSIS

Multivariate statistical analyses were applied to enumerate the possible relation between the tannia and taro accessions based on the collected quantitative and qualitative morphological characters. The morphometric analyses, based on measurements of both qualitative and quantitative characters. Based on a preliminary morphological analysis we divided the collected specimens into two groups – taro and tannia to capture the full range of variations between and within the species.

For each quantitative character, the Shapiro-Wilks normality test was first used to determine their distribution, then depending on their normality analysis of variance or Kruskal-Wallis test was used to analyse their differences, and thereafter box plots were made in R studio version 3.3.2 (R Core Team, 2019). To include the qualitative characters in the multivariate analysis, a matrix was developed based on individual character distribution. Several multivariate approaches were used to compare all the evaluated characters including principal components analysis (PCA), Bray Curtis-based non-metric dimensional scaling (NMDS), unweighted pair group method with arithmetic mean (UPGMA) cluster analysis, and Pearson (linear) correlation in PAST [PAleontological STATistic] package version 3.24 (Hammer *et al.*, 2019). Before the analysis, all the characters were normalized using the software's correlation matrix as previously adopted and described by Wahlsteen and Tyler (2019). Pearson's correlation was used to show the relationship between the traits studied. The PCA was used to analyse matrices of several characters and species to get a general overview of the variation in the two groups. NMDS is a

numerical technique with the capability to produce a dissimilarity-based index data matrix that places data points in a dimensional coordinate system so that relative distances between points reflect the relative dissimilarity between samples (Schiffbauer and Dornbos, 2011; Hammer *et al.*, 2019). This technique can highlight the taxonomic significance of characters included in a study (Liu *et al.*, 2013). UPGMA-based cluster analysis was performed with arithmetic mean, Michener and Sokal (1957) and Gower similarity index (Gower, 1971).

CHAPTER THREE

RESULTS

The physicochemical characteristics of the soil used in the study is presented in Table 2. The results suggest that the soil is slightly acidic and rich in biocarbonate ions but cadmium, nickel, vanadium and arsenic are absent.

Table 2. Physiochemical characteristics of the control and spent crude oil contaminated soil used in the study.

Parameters	Concentration	
	Spent crude oil contaminated soil*	Control
pH	6.25	4.34
Conductivity (Us/cm)	900	189
Chlorine (mg/l)	237	36
Bio carbon (mg/l)	332	35.20
Sodium (mg/l)	129	18.5
Potassium (mg/l)	26.2	5.82
Calcium (mg/l)	14.10	5.13
Magnesium (mg/l)	15.56	1.84
Phosphate (mg/l)	3.39	1.00
Ammonium (mg/l)	6.54	2.10
Total hydrocarbon (mg/l)	42.90	13.22
Iron (mg/l)	2.87	1.64
Lead (mg/l)	0.14	0.04
Copper (mg/l)	0.11	0.03
Cadmium (mg/l)	0.03	0
Zinc (mg/l)	0.43	0.18
chromium (mg/l)	0.09	0.05
Manganese (mg/l)	0.18	0.08
Nickel (mg/l)	0	0
Vanadium (mg/l)	0	0
Arsenic (mg/l)	0	0
Sand (%)	71.74	70
Silt (%)	2.17	3.75
Clay (%)	26.1	26.25
Soil texture	Sandy clay loam	Sandy clay loam

*Contaminated soil contains 250 kg of soil with 10 liters of spent crude oil.

The cultural characteristic of bacterial associated with the soil is presented in Table 3. Four dilutions resulted in the highest number of colonies and five serial dilutions had the least number of colonies. The colony shape varied from circular, irregular to filamentous while the colour ranged from colourless to creamy.

Table 3. Cultural characteristic of bacterial associated with the soil used in the study

NOSD	*CFU	Control					NOC	*CFU	Spent crude oil contaminated soil				
		Shape	Colour	Elevation	Margin	NOC			Shape	Colour	Elevation	Margin	NOC
4(1)	38×10 ⁴	Circular	Colourless	Flat	Entire	19	29×10 ⁴	circular	colourless	convex	entire	9	
		Circular	Cream	Convex	Entire	14		circular	cream	flat	entire	6	
		Irregular	Cream	Convex	Undulate	1		irregular	colourless	flat	undulate	14	
		Irregular	Colourless	Flat	Undulate	2							
		Filamentous	Colourless	Flat	Filiform	2							
4(2)	43×10 ⁴	Irregular	Colourless	Flat	Lobate	2	39×10 ⁴	filamentous	cream	crateriform	filiform	4	
		Filamentous	Colourless	Flat	Filiform	2		circular	cream	convex	entire	17	
		Circular	Cream	Convex	Entire	8		irregular	cream	flat	undulate	4	
		Circular	Colourless	Flat	Entire	25		circular	colourless	flat	entire	14	
		Irregular	Cream	Convex	Undulate	6							
5(1)	27×10 ⁵	Filamentous	Colourless	Flat	Filiform	1	22×10 ⁵	filamentous	cream	umbonate	filiform	6	
		Irregular	Cream	Convex	Undulate	6		circular	cream	convex	entire	3	
		Circular	Cream	Convex	Entire	8		irregular	colourless	crateriform	undulate	4	
		Circular	Colourless	Flat	Entire	12		circular	colourless	flat	entire	9	
5(2)	25×10 ⁵	Circular	Cream	Convex	Entire	13	22×10 ⁵	circular	cream	flat	entire	6	
		Irregular	Cream	Convex	Undulate	5		circular	colourless	convex	entire	12	
		Irregular	Colourless	Flat	Undulate	6		irregular	cream	flat	undulate	4	
		Filamentous	Colourless	Flat	Filiform	1							

Keys:

NOSD – Sample and umber of serial dilutions, NOS – Number of colonies, *Colony forming units

The cultural characteristic of fungi associated with the soil is presented in Table 4. The morphological characteristics include white mycelocolony shape varied from circular, irregular to filamentous while the colour range from colourless to creamy.

Table 4. Cultural characteristic of fungal associated with the soil used in the study

NOSD	*CFU	MC	Control			*CFU	MC	Spent crude oil contaminated soil		
			FI	PO (%)	NOC			FI	PO (%)	NOC
4(1)	42×10 ⁴	White mycelia growth	Unknown	19.05	8	22×10 ⁴	Yellow spotted growth	Yeast cells	77.2	17
		Dull green mycelia growth	<i>Aspergillus</i> spp.	2.38	1		Red spotted growth	Yeast cells	22.7	5
		Red-spotted growth	Yeast cells	2.38	1					
		Dark mycelia growth	<i>Penicillium</i> spp.	4.76	2					
		Yellow spotted growth	Yeast cells	71.43	30					
4(2)	35×10 ⁴	White mycelia growth	Unknown	17.14	6	28×10 ⁴	Dull brown mycelia growth	<i>Aspergillus</i> spp.	3.57	1
		Dull green mycelia growth	<i>Aspergillus</i> spp.	5.71	2		Brown mycelia growth	Unknown	3.57	1
		Dull brown mycelia growth	<i>Aspergillus</i> spp.	2.86	1		Red spotted growth	Yeast cells	75	21
		Dull brown mycelia growth	<i>Aspergillus</i> spp.	5.71	2		Yellow spotted growth	Yeast cells	17.8	5
		Yellow spotted growth	Yeast cells	68.58	24					
5(1)	57×10 ⁵	White mycelia growth	Unknown	7.02	4	54×10 ⁵	Red spotted growth	Yeast cells	7.40	4
		Red-spotted growth	Yeast cells	1.75	1		Yellow spotted growth	Yeast cells	18.5	10
		Dull brown mycelia growth	<i>Aspergillus</i> spp.	3.51	2		White spotted growth	Yeast cells	74.0	40
		Yellow spotted growth	Yeast cells	87.72	50					
5(2)	33×10 ⁵	White mycelia growth	Unknown	6.06	2	49×10 ⁵	Dull green mycelia growth	<i>Aspergillus</i> spp.	2.04	1

Dull brown mycelia growth	<i>Aspergillus</i> spp	3.03	1	Yellow spotted growth	Yeast cells	4.08	2
Brown mycelia growth	<i>Phoma</i> spp.	9.09	3	White spotted growth	Yeast cells	91.8	45
Yellow spotted growth	Yeast cells	81.82	27	Red spotted growth	Yeast cells	2.04	1

Key

MC - Morphological characteristics on PDA, NOSD – Sample and number of serial dilutions, NOS – Number of colonies, PO - Percentage occurrence, *Colony forming units, FI – Predicted fungi species isolated

The results of the proximate and secondary phytochemical analysis of taro species harvested from the contaminated soil revealed the presence of carbohydrates, protein, fat, fibre and ash in varying amounts (Table 5). The crude fat had the least amount of nutrient of 1.21, while the moisture was 74.53%.

The result of the phytochemical constituent of the taro samples revealed the presence of saponin, tannins, alkaloids, flavonoids and polyphenol.

Table 5: Proximate value of the taro harvested from contaminated soil

Parameters	Amount (mg)
Moisture	74.53
Crude Carbohydrates	45.34
Crude Protein	5.23
Crude Fat	1.21
Crude Fibre	34.33
Crude Ash	13.89
Saponin	2.11
Tannins	3.22
Alkaloids	5.23
Flavonoid	2.34
Polyphenols	2.01

Table 6: Fungi Associated with taro harvested from contaminated soil

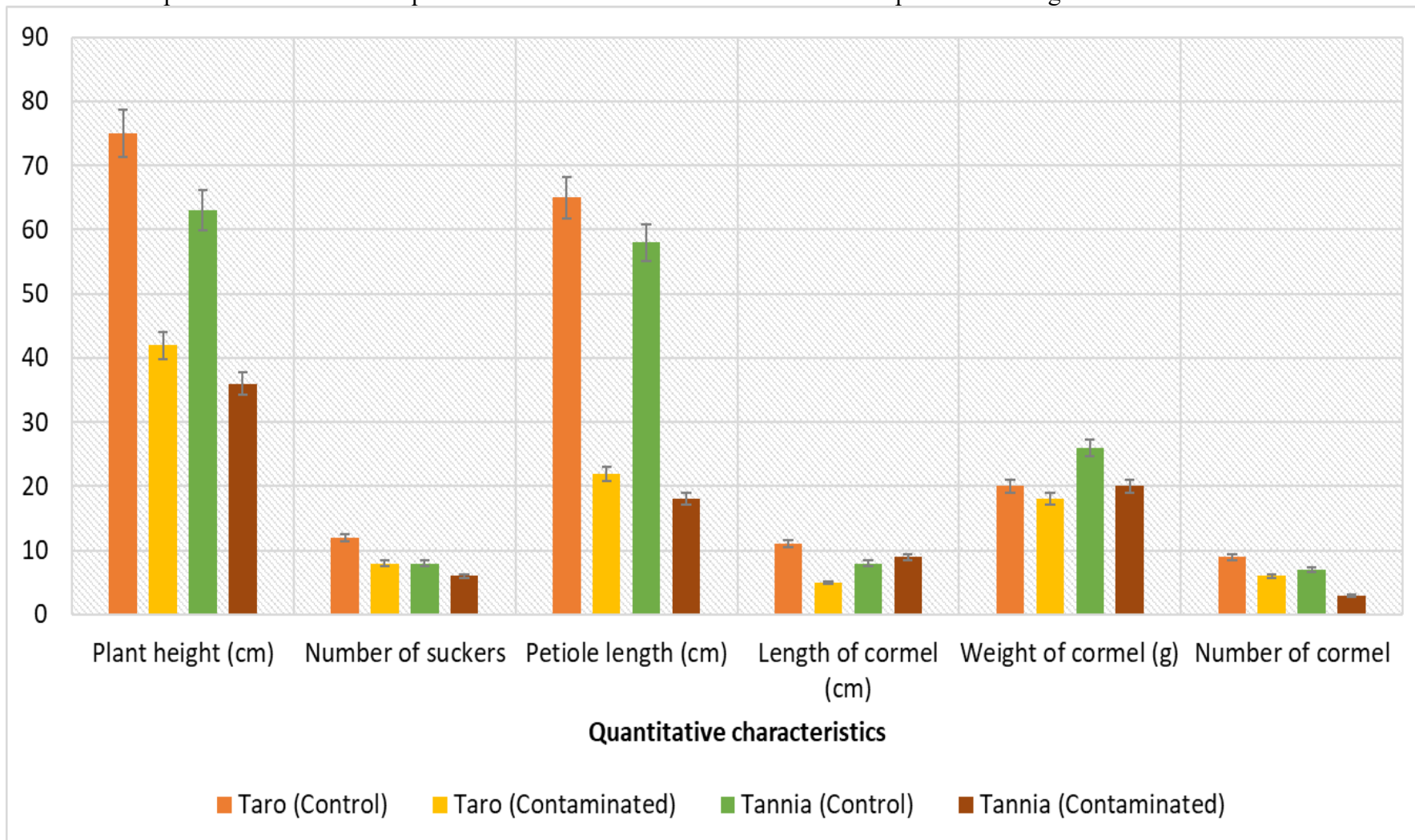
CFU	Morphological description of fungi on PDA	Microscopic description of fungi		Fungi isolated	% occurrence
		Texture of conidia	Shape of conidia		
64×10 ⁵	Black mycelia growth	Rough	Globose	<i>Aspergillus</i> sp.	3.125%
	Grey mycelia growth	Rough	Globose	<i>Penicillium</i> sp.	89.1%
	Lemon mycelia growth	Rough	Sub-globose	<i>Aspergillus</i> sp.	7.81%
127×10 ⁵	Black mycelia growth	Rough	Globose	<i>Aspergillus</i> sp.	5.51%
	Lemon mycelia growth	Rough	Sub-globose	<i>Aspergillus</i> sp.	11.81%
	Grey mycelia growth	Rough	Globose	<i>Penicillium</i> sp.	82.68%
86×10 ⁵	Black mycelia growth	Rough	Globose	<i>Aspergillus</i> sp.	3.94%
	Lemon mycelia growth	Rough	Sub-globose	<i>Aspergillus</i> sp.	1.57%
	Grey mycelia growth	Rough	Globose	<i>Penicillium</i> sp.	62.2%.
75×10 ⁵	Black mycelia growth	Rough	Globose	<i>Aspergillus</i> sp.	8.86%
	Lemon mycelia growth	Rough	Sub-globose	<i>Aspergillus</i> sp.	10.24%
	Grey mycelia growth	Rough	Globose	<i>Penicillium</i> sp.	54.33%.
95×10 ⁵	Black mycelia growth	Rough	Globose	<i>Aspergillus</i> sp.	24.21%
	Grey mycelia growth	Rough	Globose	<i>Penicillium</i> sp.	75.79%

Table 7. Analysis of variance in the quantitative characters

Character	<i>P</i>-value	X² or F value	DF
Plant height	2.13e-05*	11.10	52,1
Number of suckers	0.00*	10.16	52,1
Petiole length	1.82e-05*	12.63	52,1
Length of cormels	1.95e-05*	15.01	52,1
Weight of cormel	0.00*	6.08	52,1
Number of cormel	0.00*	12.29	52,1

*Significant P-value

The effects of spent crude oil on some quantitative characteristics of tannia and taro is presented in Figure 1.



CHAPTER FOUR

DISCUSSION

An evaluation of the effects of spent crude oil on the growth and development of cocoyam as well as the effects on soil and the crop germplasm have been conducted. Observations revealed that the plant cultivated in the control soil grew well whereas the plant cultivated in the contaminated soil did not grow and develop properly. Similar effects were also observed in the result from the contaminated soil and harvested germplasm from the soil.

Taro and tannia are important staple food crop in Nigeria, and it remains an important crop to many cultural and agricultural traditions worldwide (Ooka and Brennan, 2000). According to the authors, the corm of Taro could be eaten fried, boiled, baked or converted into different processed foods like Taro powder, noodles, and cakes. Onuegbu (1999) and Pandukur *et al.* (2016) had previously reported on the fungi associated with the deterioration of healthy Taro (*C. esculenta*). The fungi listed by these authors includes *A. flavus*, *A. niger*, *P. digitatum*, *Botrydiplodia theobromae*, *Sclerotia rolfsii*, *Fusarium solani*, *Erwinia carotovora*, *Rhizopus stolonifer*, *Verticillium lateritium*, *F. verticillioides* and *Alternaria alternata*. However, in this study only *Aspergillus* and *Penicillium* species were found to be associated with the healthy Taro. The low number of fungi isolated in this study from the inner tissues of Taro (*C. esculenta*) when compared to those isolated from Taro (*C. esculenta*) by Onuegbu (1999) and Pandukur *et al.* (2016) might be attributed to the fact the Taro samples analyzed were healthy inner tissues while those of Onuegbu (1999) and Pandukur *et al.* (2016) were deteriorated samples.

The presence of *Penicillium* and *Aspergillus* species in the food sample according to Apinis, (2003) is because their spores are abundant in the environment and can be introduced through

dust and soil. Their presence in the inner tissues of cocoyam are of serious public health concern as these fungi have all been implicated with the production of mycotoxin. (Makun *et al.*, 2009)

Phytochemical compounds are desirable components in our food because of their antioxidant properties. Phytochemicals such as phenolic compounds detected in cocoyam are essential for the growth and reproduction of plants (Doherty *et al.*, 2010). In this study the number of phenolic compounds detected in cocoyam was 6.21mg/l. Flavonoids on the other hand are not only among the most common antioxidants in nature, but also those with the highest antioxidant potency in vitro (Miller *et al.*, 1996). Although their best-described property is to act as antioxidants (Nijveldt *et al.*, 2001). Flavonoids can display a huge array of biochemical and pharmacological effects that affect the function of the immune system and inflammatory processes (Middleton and Kandaswami, 1992). The amount of flavonoids in the taro samples suggests its possible use as an antioxidant, antiviral, anti-allergic and anti-inflammatory agent. There has been no recommended daily allowance for the consumption of phytochemicals; however, a recent, valuable contribution to the phytochemical debate has been made by Wahlqvist *et al.*, (1998) who have proposed developing a food-based Index of Preferred Phytochemical Intake (IPPI). The detection of other food components such as crude protein, crude fat, crude carbohydrates, ash and fibre also indicates the usefulness of Taro (*C. esculenta*) as a source of food. The moisture content in this study was 74.53 and it is quite similar to the results obtained by Onwueme (1999), where the moisture content ranged from 63-85%. The carbohydrate content gotten from Taro in this study was 45.34% and it is high compared to the results gotten by Onwueme (1999) where the carbohydrate content ranged from 13-29%.

CONCLUSION

The results of this study have shown that the microbiological, proximate, phytochemical composition and the morphological characteristics of the two cocoyam species (*Colocasia esculenta* [L.] Schott. and *Xanthosoma sagitifolium* [L.] Schott.) are affected by spent crude oil. Moreover, cocoyam contains food compounds that are beneficial and also of economic importance to man. However, there is the need to conserve this food substance against fungal infestation so that its full economic value can be harnessed.

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