ISOLATION AND IDENTIFICATION OF MICROORGANISM ASSOCIATED WITH GROUND PALM KERNEL SHELL INOCULATED WITH CHICKEN DROPPING AT RATIO 1:1

BY

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OCTOBER, 2015.

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FACULTY OF SCIENCE

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OCTOBER, 2015

CERTIFICATION

This is to Certified that this project work was carried out by AROGUNDADE TEMITOPE ELIJAH, of the department of microbiology, with matric number MCB/11/0328, faculty of science, Federal University Oye –Ekiti, Ekiti State.

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DEDICATION

This project work is dedicated to the supreme ruler of heaven and the earth, the Father to the fatherless, the Almighty God, for His Grace and Mercy through all this years. And also to the entire Family and Household of Julius and Comfort Arogundade.

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ABSTRACT

Interest in microbial degradation of pollutant has increased in recent years and palm kernel shell (PKS) is one of the material that contribute a great pollution to the southern Nigeria. The aim of this study is to isolate and identify microorganism associated with ground palm kernel shell (GPKS) inoculated with chicken dropping at ratio 1:1. 5g of the sample was picked at different interval of five days for microbial analysis and biochemical tests like catalase, sugar fermentation test were also carried out on the samples. Standard method was used to determine the pH and total titratable acid (TTA) of the sample. Microbial analysis results that GPKS contained a variety of microorganism. The probable organism include *Bacillus spp,pseudomonas spp*, and *Aspergillus spp*. The mean viable counts of microorganism (cfu/g) in GPKS ranged from 3.0 x 10^6 to 5.5 x 10^6 which indicate relatively high counts of bacteria in the samples. The pH readings favours organism on the alkaline range. The various organisms can serve as a source of inoculum and the consortia of these organism can help in the biodegradation process of palm kernel shell which in turn can eventually help in solving the problem of agro – pollution in the environment.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BIODEGRADATION

Biodegradation is the chemical dissolution of materials by bacteria, fungi, or other biological means (Richard, 2002). Although often conflated, biodegradable is distinct in meaning from compostable. While biodegradable simply means to be consumed by microorganism, "compostable" makes the specific demand that the object break down under composting conditions. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. Organic material can be degraded aerobically with oxygen, or anaerobically, without oxygen. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process. Biodegradable matter is generally organic material that serves as a nutrient for microorganisms. Microorganisms are so numerous and diverse that, a huge range of compounds are biodegraded, including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances. Decomposition of biodegradable substances may include both biological and abiotic steps (Agamuthu, 2004).

In practice, almost all chemical compounds and materials are subject to biodegradation, the key is the relative rates of such processes (minutes, days, years, centuries, etc.) A number of factors determine the degradation rate of organic compounds. Salient factors include light, water and oxygen (Sims and Cupples, 1999). Temperature is also important because chemical reactions proceed more quickly at higher temperatures. The degradation rate of many organic compounds is limited by their bioavailability. Compounds must be released into solution before organisms can degrade them (Sims, 1991). Biodegradability of natural and synthetic materials. Biodegradability can be measured in a number of ways. Respirometry tests can be used for aerobic microbes. First one places a solid waste sample in a container with microorganisms and soil, and then aerate the mixture. Over the course of several days, microorganisms digest the sample bit by bit and produce carbon dioxide (CO_2), the resulting amount of CO_2 serves as an indicator of degradation. Biodegradability can also be measured by anaerobic microbes and the amount of methane or alloy that they are able to produce. In formal scientific literature, the process is termed bio-remediation (Yoshito, 2000).

1.2 ROLE OF MICROORGANISMS IN BIODEGRADATION OF POLLUTANTS

Biodegradation is described associated with environmental bioremediation. Therefore, biodegradation is nature's way of recycling wastes, or breaking down

organic matter into nutrients that can be used and reused by other organisms. In the microbiological sense, "biodegradation" means that the decaying of all organic materials is carried out by a huge assortment of life forms comprising mainly bacteria, yeast and fungi, and possibly other organisms. Bioremediation and biotransformation methods endeavour to harness the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), Polyaromatic hydrocarbons (PAHs), radionuclides and metals (Lesley, 2012).

1.3 DIFFERENT TYPES OF BIODEGRADATION BY MICROORGANISMS

1.3.1 BACTERIAL DEGRADATION

There are various reports on the degradation of environmental pollutants by different bacteria. Several bacteria are even known to feed exclusively on hydrocarbons (Yakimov, 2007). Bacterial strains that are able to degrade aromatic hydrocarbons have been repeatedly isolated mainly from soil. These are usually gram negative bacteria, most of them belong to the genus Pseudomonas. The biodegradative pathways have also been reported in bacteria from the genera Mycobacterium, Corynebacterium, Aero Monas, Rhodococcusand Bacillus (Mrozik, 2003).

Although many bacteria are able to metabolize organic pollutants, a single bacterium does not possess the enzymatic capability to degrade all or even most of the organic compounds in a polluted soil. Mixed microbial communities have the most powerful biodegradative potential because the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas (Fritsche and Hofrichter, 2005).

1.3.2 MICROFUNGI AND MYCORRHIZA DEGRADATION

Micro fungi are described as a group of organisms that constitute an extremely important and interesting group of eukaryotic, aerobic microbes ranging from the unicellular yeasts to the extensively mycelia molds (Rossman, 1995). Fungi are an important part of degrading micro biota because, like bacteria, they metabolize dissolved organic matter; they are principal organisms responsible for the decomposition of carbon in the biosphere. But, fungi, unlike bacteria, can grow in low moisture areas and in low pH solutions, which aids them in the breakdown of organic matter (Spellman, 2008). Equipped with extracellular multienzyme complexes, fungi are most efficient, especially in breaking down the natural polymeric compounds. By means of their hypha systems they are also able to colonize and penetrate substrates rapidly and to transport and redistribute nutrients within their mycelium.

1.3.3 YEAST DEGRADATION

Several yeasts may utilize aromatic compounds as growth substrates, but more important is their ability to convert aromatic substances cometabolically. Some species such as the soil yeast Trichosporoncutaneumpossess specific energy-dependent uptake systems for aromatic substrates (e.g., for phenol) (Mörtberg and Neujahr, 1985). Furthermore, biodegradation of aliphatic hydrocarbons occurring in crude oil and petroleum products has been investigated well, especially for yeasts.

1.4 PALM KERNEL SHELL (PKS)

Palm kernel shells (PKS) are derived from the oil palm tree (Elaeisguineensis), an economically valuable tree, and native to western Africa and widespread throughout the tropics (Ndoke, 2006). The PKS are obtained after extraction of the palm oil the nuts are broken and the kernels are removed with the shells mostly left as waste. The PKS are hard stony endocarps that surround the kernel and the shells come in different shapes and sizes (Alangaram, 2008).

These shells are mainly of two types the "Dura" and "Tenera". The Tenera is a hybrid which has specially been developed to yield high oil content and it has a thin shell thickness compared to Dura type (Dagwa and Ibhadode, 2008). There are several efforts being made towards the utilization of the PKS (Okafor, 1988).

1.5 CHICKEN WASTE

The poultry industry is one of the largest and fastest growing agro-based industries in the world. The industry is currently facing a number of environmental problems. One of this problem is the accumulation of large amount of wastes, especially manure and litters. This is resulting in pollution problem and unless environmentally and economically sustainable management technologies are evolved. The amount of poultry litters produced in a broiler unit depends on the litter (i.e. bedding materials) management, and feed intake and its digestibility. Different range of materials including wood shaving, cereal straw, husk and paper clipping are used as bedding material (Power and Dick, 2000).

1.6 HUMUS SOIL

Humus is a non-living and finely divided organic matter in soil, derived from microbial decomposition of plant and animal substances. Humus, which ranges in colour from brown to black, consists of about 60 percent carbon, 6 percent nitrogen, and smaller amounts of phosphorus and sulfur. As humus decomposes, its components are changed into forms usable by plants (Rothwock*et al.*, 2008)

Humus is classified into mor, mull, or moder formations according to the degree of its incorporation into the mineral soil, the types of organisms involved in its decomposition, and the vegetation from which it is derived. (Kelley, 1998).

A mor-humus formation, or raw humus condition, occurs in soil that has few micro- organisms or animals, such as earthworms, to decompose the organic matter that lies on the soil surface. Below this surface-litter layer is a distinct, strongly compacted humus layer; a layer of mineral soil underlies the humus. Fungi and small arthropods are the most common organisms (Okly, 1987).

CHAPTER TWO

2.0 LITERATURE REVIEW

Palm kernel shells (PKS) are derived from the oil palm tree (Elaeisguineensis), an economically valuable tree, and native to western Africa and widespread throughout the tropics (Ndoke, 2006). Palm kernel shells are not common materials in the construction industry. This is either because they are not available in very large quantities as sand or gravel, or because their use for such has not been encouraged. For some time now, the Nigerian government has been clamouring for the use of local materials in the construction industry to limit costs of construction. There has therefore been a greater call for the sourcing and development of alternative, non-conventional local construction materials. In Nigeria, the oil palm tree generally grows in the rain forest region close to the coastal areas and adjacent to some inland waterways. Omange (2001) wrote that palm kernel shells are used mostly as a source of fuel for domestic cooking in most areas where they occur. He stated further that the shells are often dumped as waste products of the oil palm industry.

Ndoke (2006) stated that the two predominant varieties of palm fruits namely Tenera and Dura; produce about 1.5 million tons of palm kernel shells per annum in Nigeria. Palm kernel shells have been used as aggregates in light and dense

concretes for structural and non-structural purposes. Ndoke (2006) went further to show that the 28 day compressive strength of concrete with palm kernel shells as aggregate range between 0.3 and 20.5N/mm^2 depending on the proportion in the mix

2.1 MANAGEMENT OF POULTRY LITTER

The poultry industry is one of the largest and fastest growing agro-based industries in the world. There is an increasing demand for poultry meat mainly due to its acceptance by most societies and its relatively low cholesterol content. The poultry industry is currently facing a number of environmental problems. One of the major problems is the accumulation of large amount of wastes, especially manure and litter, generated by intensive production is voided by a layer as Large-scale accumulation of these wastes may pose disposal and pollution problems unless environmentally and economically sustainable management technologies are evolved (Power and Dick,2000; Kelleher *et al.*, 2002; Sharpley*et al.*, 2007).

Most of the manure and litter produced by the poultry industry is currently applied to agricultural land. When managed correctly, land application is a viable way to recycle the nutrients such as nitrogen (N), phosphorus (P) and potassium (K) in manure. However, pollution and nuisance problems can occur when manure is applied under environmental conditions that do not favour agronomic utilisation of

the manure-borne nutrients (Sharpley*et al.*, 1998; Casey *et al.*, 2006; Kaiser *et al.*, 2009)

2.2 POULTRY LITTER PRODUCTION

The quantity of poultry litter produced in a broiler unit depends on the litter (i.e. bedding material) management, and feed intake and its digestibility. A range of materials including wood shavings, cereal straw, husk and paper clippings are used as bedding materials (Swain and Sundaram, 2000). Three common practices are adopted for litter management in broiler units (Bernhart, 2010). These include single use litter, partial re-use and multi-use litter.

2.3 COMPOSITION OF CHICKEN DROPPING.

Chicken dropping contains all 13 of the essential plant nutrients that are used by plants. These include nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), copper (Cu), zinc (Zn), chlorine (Cl), boron (B), iron (Fe), and molybdenum (Mo). Plant nutrients originate from the feed, supplements, medications, and water consumed by the animals. Using poultry manure as a fertilizer for crops or trees may provide a portion, or all, of the plant requirements (Obeng, 1997). The amount of nutrients provided depends on the nutrient content of the manure (lb of nutrient/ton of manure) and the amount of manure applied (ton of manure/acre). The amount of manure applied per acre (called the application rate) is typically based on the nitrogen needs of the plants.

However, phosphorous requirement can also be used to determine the application rate (Diez, 2010)

2.4 MICROBIAL LOAD

Poultry manure contains a large and diverse population of viruses, bacteria, fungi andProtozoa. Microbial concentrations in poultry litter can exceed 1010 cells/g (Acosta-Martinez and Harmel*et al.*, 2006; Cook *et al.*, 2008; Rothrock*et al.*, 2008a), and gram positive bacteria (i.e. Actinomycetes, Clostridia/Eubacteria, Bacilli/Lactobacilli)account for nearly 90% of the microbial diversity (Lu *et al.*, 2003; Enticknap,2006; Lovanh*et al.*, 2007). While microbes perform a variety of different enzymatic and metabolic processes within the litter environment, two microbial groups of special interest to the poultry industry are nitrogen mineralizing microbes and pathogens (Moore, 2006).

2.5 OBECTIVES OF THE STUDY

The main objective of this study was to determine the biodegradation of palm kernel shell using chicken litters. However, the specific objectives are:

- To determine the microbial load of the various samples
- To isolate and identify the various organisms present
- To have the knowledge on how the consortia microorganisms in chicken dropping can help in the biodegradation of the palm kernel shell

CHAPTER THREE

3.0 MATERIALS AND METHOD.

3.1 MATERIALS

Petri dishes, Conical flask, alluminium foil, microscope, needle and syringe, autoclave, incubator, beaker, spatula, Ziploc bag, ice block nylon, test tube, test tube rack, cotton wool, measuring cylinder, weighing balance, inoculating loop, Bunsen burner, bijou bottle, nutrient agar, Sabroid dextrose agar, yeast extract agar, 3% hydrogen peroxide.

3.2 METHODS

3.2.1 SAMPLE COLLECTION AND PREPARATION

The palm kernel shell was collected from a local palm oil industry in Oye local government area, Ekiti state, Nigeria. After crushing at site and properly hand – picked to separate the cake from the shell.

1kg of the palm kernel shell (PKS) was weighed and washed with 1.5 litre of sterile distilled water containing small amount of detergent in order to remove dirt's and dust.

The weighed palm kernel shell was further oven- dried at $70^0_C$for one week. The dried palm kernel shell was grounded to obtain a powder form of the sample. The powdered sample was then kept in air-tight container for further analysis.

Chicken droppings were collected from fuoye poultry.

Humus soil was collected from three (3) different locations in Oye local government area.

3.2.2 PREPARATION OF MEDIA

* **Nutrient agar preparation**: Nutrient agar (2.8g) was weighed using weighing balance and dispensed into a clean conical flask and mixed with 100ml of distilled water. Proper dissolution was done to ensure that the agar medium dissolves properly with the distilled water. The conical flask was firmly corked with cotton wool and foil paper to prevent it from pouring away during sterilization and to also prevent contamination after sterilization. The corked conical flask was placed in the autoclave along with other glass wares and autoclaved at $121^0_C$for 15 minutes. After sterilization, the media were allowed to cooled to about $40\text{-}45^0_C$before aseptically poured into the petri dishes and allow to solidify at room temperature.

* **Sabroid dextrose agar preparation**: Sabroid dextrose agar (6.5g) was weighed using the weighing balance and dispensed into a clean conical flask and mixed with 100ml of distilled water. Proper dissolution was done to ensure that the agar

medium dissolves properly with the distilled water. The conical flask was firmly corked with cotton wool and foil paper to prevent it from pouring away during sterilization and to also prevent contamination after sterilization. The corked conical flask was placed in the autoclave along with other glass wares and autoclaved at $121^0_C$for 15 minutes. After sterilization, the media were allowed to cooled to about 40-45^0_Cbefore aseptically poured into the petri dishes and allow to solidify at room temperature.

* **Yeast extract agar preparation**: yeast extract agar (2.3g) was weighed using weighing balance and dispensed into a clean conical flask and mixed with 100ml of distilled water. Proper dissolution was done to ensure that the agar medium dissolves properly with the distilled water. The conical flask was firmly corked with cotton wool and foil paper to prevent it from pouring away during sterilization and to also prevent contamination after sterilization. The corked conical flask was placed in the autoclave along with other glass wares and autoclaved at $121^0_C$for 15 minutes. After sterilization, the media were allowed to cooled to about 40-45^0_Cbefore aseptically poured into the petri dishes and allow to solidify at room temperature.

3.2.3 SERIAL DILUTION

Serial dilution was carried out on the uncrushed palm kernel shell for identification of microorganisms that was present in the sample.

10g of the uncrushed palm kernel was weighed into a Ziploc bag using a weighing balance with the flame on after the nylon has been zero on the weighing balance. 0.1% peptone water was then dispensed into the nylon containing the 10g uncrushed palm kernel shell still on the weighing balance until it was 100g all together with the flame still on, that makes our stock. The stock was then shaken very well using vortex machine in other to dislodge all the organism in the palm kernel shell into the peptone water for five to ten minutes.

Using unused syringe without needle, 1ml was taken from the stock and dispensed into test tube number one containing 9ml of 0.1%peptone water in the presence of flame. The test tube was then shaken very well using vortex machine. Using sterile syringe, 1ml was taken from test tube number one and dispensed into test tube number two and vortex. The same procedure was repeated for test tube number three using sterile syringe with needle, and so on until it reaches test tube number ten.Serial dilution procedure was also carried out on the chicken droppings for microbial analysis.

3.2.4 POUR PLATE.

The work bench was swabbed very well with ethanol before putting the sterilized petri dishes. The petri dishes was labelled properly. For example, ATUB5, meaning AT-ArogundadeTemitope, u- uncrushed palm kernel shell, B- bacteria, 5-dilution 5. For bacteria, 3plates were poured using dilution 5 and 6. 2 plates for 5 and one plate for 6. For mold, 3 plates were also poured using dilution 2 and 3, 2plates for 2 and one plate for 3. For yeast, 3 plates were also poured using dilution 4 and 5, 2 plates for 5 and one plate for 4.

Using 2ml syringe, 0.2ml was taken from the test tube according to the dilution to be used for each plate. The plates were left on the work bench for some time after pouring for it to solidify. The various plates were tape with masking tape to prevent contamination and incubated. Mold and yeast were incubated at 27^0c, while bacteria were incubated at 37^0c for 18 to 24 hours.

3.2.5 SUB CULTURE

After 24hrs of incubation, the plates were observed for growth and the count on the plates were taken using colony counter. The plates were then sub cultured in other to isolate a pure culture from the plate.

3.2.6 IDENTIFICATION AND MORPHOLOGICAL CHARACTERIZATION OF ISOLATEDORGANISMS.

*** GRAM STAINING**

A thin smear of cell suspension was made on a clean slide and then allowed to air-dry, then heat fixed by passing the slide over the blue flame. The smear was flooded with crystal violet for 1 minute and washed with water and then flooded with some drops of iodine for 1 minute, the iodine decreases the solubility of the purple dye forming dye- iodine complexes, which was then washed with water. The purple dye- iodine complexes was then decolourised with 95% ethanol for 30 seconds and rinsed with water. The smear was then counter stained with safranin for 60 seconds and the excess stain was washed with water. The smear slide was then allowed to air dry, and a drop of oil immersion was dropped on the smear and viewed under the microscope using oil immersion objective (x100).

3.2.7 BIOCHEMICAL CHARACTERIZATION OF ISOLATED ORGANISM.

Biochemical test were done to the bacterial isolates to identify the specific physiologic characteristics. Biochemical reaction that are specific for every medium and reagent were the basis for identifying the unknown bacterial isolates from the samples. The biochemical test include:

***CATALASE TEST**

A colony of the organism was picked and placed in a drop of 3%hydrogen peroxide on a clean slide. Effervescence caused by the liberation of oxygen as gas bubbles indicated the production of catalase by the bacterium while an absence of gas bubbles indicated a negative result.

***GRAMS REACTION**

A colony of the organism was picked and placed in a drop of 3% potassium hydroxide on a clean slide. Using the inoculating loop the organism was mixed very well with the solution and lifted up, the stinging of the organism to the loop indicate a positive reaction and the absence of stinging of the organism to the loop indicate a negative reaction.

***SUGAR FERMENTATION TEST.**

Sugar fermentation coupled with acid production, which can be formed during the reaction is detected by the use of Durham's tube in inverted position into a given tube. Sugars used were sucrose, lactose, fructose and mannitol. A nutrient broth was prepared by weighing exactly 2.8g of nutrient agar into a different conical flask and labelled accordingly and made up to 100ml with distilled water. After it has settled the broth was decanted. Exactly 1g of each sugar was weighed into these different conical flask, after it has dissolved very well, phenol red was then

added as an indicator. A 10ml of each of the sugar solution was dispensed into different test tube with Durham's tube inserted into each tube in an inverted position. The tubes were labelled appropriately and covered tightly with cotton wool and alluminium foil paper and sterilized in an autoclave for 20mins at 121^0c. These test tubes were allowed to cool down before inoculated with the isolate of the test organism and incubated at 37^0c for 24hrs. Acid production was observed by a change in colour from red to yellow. This indicates acid production by the inoculated organisms by utilizing the sugars. Appearance of bubbles in the Durham's tube indicates gas production. If otherwise, gas is not produced.

3.2.8 PRESERVATION OF ISOLATED ORGANISM.

Preparation of slant was done in other to keep the isolated organism.

Nutrient agar (11.2g) was weighed using weighing balance and dispensed into a clean conical flask and mixed with 200ml of distilled water. Proper dissolution was done to ensure that the agar medium dissolves properly with the distilled water. After proper dissolution of the agar, it was then dispensed into bijoe bottle and sterilized in the autoclave. After sterilization, the bottle were slanted at angle 60^0 and was allowed to solidify and makes our slant. The slant were then incubated with the isolated organism.

3.2.9 BIODEGRADATION OF GROUND PALM KERNEL SHELL INOCULATED WITH CHICKEN DROPPING AT RATIO 1:1

Biodegradation of Ground palm kernel shell (GPKS) plus chicken droppings at (Ratio 1:1).

100g of grounded palm kernel shell was weighed using weighing balance into a container after the container has been surface sterilized using ethanol. 20ml of sterile distilled water was then added to it in other for the sample to be moisturized. 100g of chicken dropping was also added to it and mixed together properly using spatula inside the container.

Reading was taken from day zero, day five (5), by taking 5g from the sample for analysis.

The same procedure was repeated for day ten (10), day fifteen (15), day twenty (20) and day thirty (30) respectively.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

TOTAL VIABLE MICROBIAL COUNT OF SAMPLES

4.1.1 Total viable count from uncrushed palm kernel shell.

Table 1 shows the total viable microbial count isolated from uncrushed palm kernel shell. The count shows that uncrushed palm kernel shell contained a large number of bacteria which ranges from (0.7×10^6 cfu/g to 2.5×10^6cfu/g) indicating heavy proliferation ratio of bacteria than those ofYeast and mould in the sample with count ranging from (1.0×10^4) to (1.3×10^4).

4.1.2 Total viable count from chicken dropping

Table 2 shows the total viable microbial count isolated from chicken dropping. The various count shows that chicken dropping contained a large number of bacteria with the count ranging from (3.6×10^6cfu/g to 5.4×10^6cfu/g) indicating that bacteria is the predominant organism than those of yeast and mould in the sample with count ranging from (1.1×10^5) to (2.5×10^3)

4.1.3 Total viable count from humus soil.

Table 3 shows the total viable microbial count isolated from humus soil. The various count shows that humus soil contained varieties of microorganism with the count of bacteria ranging from 2.5×10^6 to 3.5×10^6cfu/g indicating that bacteria is more predominant than yeast (1.9×10^5) and mould (2.5×10^3) which have lesser count to bacteria.

TABLE 1: TOTAL VIABLE MICROBIAL COUNT ISOLATED FROM UNCRUSHED PALM KERNEL SHELL (UPKS).

ISOLATE CODES	18hrs (cfu/g)	24hrs (cfu/g)
BACTERIAL COUNT		
UPKSB5	0.7 x 10	2.5 x 10
UPKSB5	0.6×10^{6}	1.4×10^{6}
UPKSB6	-	0.2×10^{7}
YEAST COUNT AT 72hrs		
UPKSY4	1.0×10^{5}	
UPKSY5	1.1×10^{6}	
UPKSY5	1.3 x 10	
MOULD COUNT AT 72hrs		
UPKSM2	0.9×10^{3}	
UPKSM2	0.8 x 10	
UPKSM3	1.3×10^{4}	

KEY: UPKSB – Uncrushed palm kernel shell bacteria.

UPKSY – Uncrushed palm kernel shell yeast.

UPKSM – Uncrushed palm kernel shell mould.

TABLE 2: TOTAL VIABLE MICROBIAL COUNT ISOLATED FROM CHICKEN DROPPING (CD).

ISOLATE CODES	18hrs (cfu/g)	24hrs (cfu/g)
BACTERIAL COUNT		
CDB5	3.6 x 10	5.4 x 10
CDB5	0.8×10^6	2.7×10^6
CDB6	0.3×10^7	3.0×10^7
YEAST COUNT AT 72HRS		
CDY4	1.1×10^5	
CDY5	0.9×10^6	
CDY5	0.7 x 10	
MOULD COUNT AT 72HRS		
CDM2	3.0×10^3	
CDM2	2.5 x 10	
CDM3	1.5×10^4	

KEY: CDB – Chicken dropping bacteria

CDY – Chicken dropping yeast

CDM – Chicken dropping mould

TABLE 3: TOTAL VIABLE MICROBIAL COUNT ISOLATED FROM HUMUS SOIL (HS).

ISOLATE CODES	18hrs (cfu/g)	24hrs (cfu/g)
BACTERIAL COUNT		
HSB5	2.5 x 10	3.5 x 10
HSB5	2.0×10^6	2.7×10^6
HSB6	1.5×10^7	2.0×10^7
YEAST COUNT AT 72HRS		
HSY4	3.0×10^5	
HSY5	2.0×10^6	
HSY5	1.9 x 10	
MOULD COUNT AT 72HRS		
HSM2	2.5×10^3	
HSM2	2.0 x 10	
HSM3	1.5×10^4	

KEY: HSB – Humus soil bacteria

HSY – Humus soil yeast

HSM – Humus soil mould

4.2 TOTAL VIABLE MICROBIAL COUNT FROM DEGRADING GROUND PALM KERNEL SHELL INOCULATED CHICKEN DROPPING AT RATIO 1:1

4.2.1 Total viable bacterial count from degrading ground palm kernel shell

Table 4 shows the total viable bacterial count isolated from degrading ground palm kernel shell at various interval of five (5) days. Fromdayzero, day five (5) and to day thirty (30) respectively. The various counts of bacterial at these interval especially from day 0 with count ranging from 3.0 x $10^6$to 4.0 x 10^6cfu/g, day five (5) from 3.5 x 10^6 to 4.5 x 10^6cfu/g to day fifteen (15) with count from 4.2 x 10^6 to 5.5 x 10^6cfu/g show that, there is an increase in the count of bacteria isolates. From day twenty (20) to day thirty (30) with count ranging from 2.2 x 10^6 to 2.7 x 10^6cfu/g show that, there is a decrease in the number of bacterial isolates.

4.2.2 Total viable yeast count of isolates from degrading ground palm kernel shell inoculated with chicken dropping at ratio 1:1

Table 5 shows the total viable yeast count isolated from degrading ground palm kernel shell at various interval of five days, from day 0, day 5 to day 30 respectively. The various count of yeast isolates at these interval from day 0 to day 15 indicated that, there is an increase in the count of yeast isolates, with count

ranging from 2.5 x 10^5 to 4.5 x 10^5while there is a small decrease in the count of yeast isolates from day 20 to day 30 with count ranging from 2.0 x $10^5$to 2.5 x 10^5.

4.2.3 Total viable mould count of isolates from degrading ground palm kernel shell inoculated with chicken dropping at ratio 1:1

Table 6 shows the total viable mould count isolated from degrading ground palm kernel shell at various interval of five days, from day 0, day 5, and to day 30 respectively. The various count of mould isolates at these interval from day 0 to day 15 point to an increase in in the count of mould isolates with count ranging from 1.5 x $10^4$to 5.5 x 10^3 while there is a decrease in the count from day 20 to day 30 with count from 2.0 x 10^3 to 2.7 x 10^3.

TABLE 4: TOTAL VIABLE BACTERIAL COUNT OF ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INNOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 1:1

ISOLATE CODES	18hrs (cfu/g)	24hrs (cfu/g)
DAY 0		
DGPKSB1	3.0×10^6	4.0×10^6
DGPKSB2	2.5×10^6	3.0×10^6
DGPKSB3	1.5×10	2.5×10
DAY 5.		
DGPKSB1	3.0×10^6	4.5×10^6
DGPKSB2	2.5×10	3.5×10
DGPKSB3	1.9×10^7	2.4×10^7
DAY 10.		
DGPKSB1	3.5×10	4.6×10
DGPKSB2	3.0×10^6	4.0×10^6
DGPKSB3	2.5×10^7	3.6×10^7
DAY 15		
DGPKSB1	5.0×10^6	5.5×10^6
DGPKSB2	4.2×10^6	4.7×10^6
DGPKSB3	4.0×10	4.1×10
DAY 20		
DGPKSB1	2.5×10^6	3.0×10^6
DGPKSB2	2.2×10	2.7×10
DGPKSB3	1.5×10^7	2.0×10^7
DAY 30		
DGPKSB1	1.3×10	2.0×10
DGPKSB2	1.2×10^6	1.5×10^6
DGPKSB3	0.9×10^7	1.0×10^7

KEY: DGPKS – Degrading ground palm kernel shell

DGPKSB – Degrading ground palm kernel shell

TABLE 5: TOTAL VIABLE YEAST COUNT OF ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INNOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 1:1.

ISOLATE CODES	COUNT
DAY 0	
DGPKSY1	2.5×10^5
DGPKSY2	2.2×10^6
DGPKSY3	3.5×10^6
DAY 5	
DGPKSY1	5.0×10^5
DGPKSY2	4.5×10^6
DGPKSY3	4.0×10^6
DAY 10	
DGPKSY1	5.5×10^5
DGPKSY2	4.6×10^6
DGPKSY3	3.9×10^6
DAY 15	
DGPKSY1	4.5×10^5
DGPKSY2	3.5×10^6
DGPKSY3	3.0×10^6
DAY 20	
DGPKSY1	2.0×10^5
DGPKSY2	1.9×10^6
DGPKSY3	1.5×10^6
DAY 30	
DGPKSY1	2.0×10^5
DGPKSY2	1.5×10^6
DGPKSY3	1.2×10

KEY: DGPKS- Degrading ground palm kernel shell

DGPKSY – Degrading ground palm kernel shell

TABLE 6: TOTAL VIABLE MOULD COUNT OF ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INNOCULATED WITH CHICKEN DROPPING AT RATIO 1:1.

ISOLATE CODES	COUNT
DAY 0	
DGPKSM1	3.0×10^3
DGPKSM2	2.2×10^3
DGPKSM3	1.5×10
DAY 5	
DGPKSM1	4.9×10^3
DGPKSM2	4.0×10^3
DGPKSM3	3.7×10^4
DAY 10	
DGPKSM1	4.5×10^3
DGPKSM2	4.2×10^3
DGPKSM3	3.9×10^4
DAY 15	
DGPKSM1	5.5×10^3
DGPKSM2	5.0×10^3
DGPKSM3	4.0×10^4
DAY 20	
DGPKSM1	2.5×10^3
DGPKSM2	2.0×10
DGPKSM3	1.5×10^4
DAY 30	
DKPKSM1	2.2×10
DGPKSM2	2.0×10^3
DGPKSM3	1.8×10^4

KEY: DGPKS – Degrading ground palm kernel shell

DGPKSM- Degrading ground palm kernel shell mould

4.3 MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF BACTERIA ISOLATES FROM SAMPLES.

4.3.1 Morphological and microscopic characteristics of bacterial isolates from uncrushed palm kernel shell (UPKS).

Table 7 shows the morphological and microscopic characteristics of bacteria isolates colonies from uncrushed palm kernel shell (UPKS) with various characteristics favouring bacteria that are Cocci in their shapes and have smooth surface.

4.3.2 Morphological and microscopic characteristics of bacterial isolates from chicken dropping

Table 8 shows the morphological characteristics of bacteria isolates colonies from chicken dropping with some colonies having Cocci shapes and yellow in colour, while some are rod in their shapes with colour and smooth surface.

4.3.3 Morphological and microscopic characteristics of bacterial isolates colonies from humus soil

Table 9 shows the morphological characteristics of bacteria isolates colonies from humus soil with the various characteristics indicating that bacteria isolates that are Cocci in shape and have smooth surface are more predominant.

TABLE 7: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF BACTERIA ISOLATES COLONIES FROM UNCRUSHED PALM KERNEL SHELL (UPKS).

ISOLATE CODES	COLOUR	SHAPE	SURFACE	ELEVATION
UPKSB1	Creamy	Cocci	Smooth	Raised
UPKSB2	Creamy	Cocci	Rough	Raised
UPKSB3	Creamy	Rod	Smooth	Flat

KEY: UPKS – Uncrushed palm kernel shell

UPKSB – Uncrushed palm kernel shell bacteria

TABLE 8: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF BACTERIA ISOLATES COLONIES FROM CHICKEN DROPPING (CD).

ISOLATE CODES	COLOUR	SHAPE	SURFACE	ELEVATION
CDB1	Creamy	Cocobacillus	Smooth	Raised
CDB2	Yellow	Cocci	Smooth	Raised
CDB3	Creamy	Rod	Rough	Raised

KEY: CD – Chicken dropping

CDB – Chicken dropping bacteria

TABLE 9: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF BACTERIA ISOLATES COLONIES FROM HUMUS SOIL (HS).

ISOLATE CODES	COLOUR	SHAPE	SURFACE	ELEVATION
HSB1	Creamy	Cocci	Rough	Raised
HSB2	Creamy	Rod	Smooth	Raised
HSB3	Creamy	Cocci	Smooth	Flat

KEY: HS – Humus soil

HSB – Humus soil bacteria

4.4 MORPHOLOGICAL AND MICROSCOPIC CHARACTERIZATION OF BACTERIAL ISOLATES COLONIES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INOCULATED WITH CHICKEN DROPPING AT RATIO 1:1

Table 10 shows the morphological and microscopic characteristics isolates colonies from degrading ground palm kernel shell at various interval of five (5) days, from day 0, day 5, and to day 30 respectively. The various characteristics of bacterial isolates at these interval indicated that bacteria that are Cocci in shapes, smooth in their surface and have creamy colour are more predominant than bacterial isolates with rod shapes and rough surface.

TABLE 10: MORPHOLOGICAL AND MICROSCOPIC CHARACTERIZATION OF BACTERIA ISOLATES COLONIES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INNOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 1:1

ISOLATE CODES	COLOUR	SHAPE	SURFACE	ELEVATION
(DAY 0)				
DGPKSB1	Creamy	Cocci	Smooth	Flat
DGPKSB2	Creamy	Rod	Rough	Raised
DGPKSB3	Creamy	Cocci	Smooth	Flat
(DAY 5)				
DGPKSB1	Creamy	Cocci	Smooth	Raised
DGPKSB2	Creamy	Cocci	Rough	Flat
DGPKSB3	Creamy	Rod	Smooth	Raised
(DAY 10)				
DGPKSB1	Creamy	Cocci	Smooth	Raised
DGPKSB2	Creamy	Cocci	Smooth	Flat
DGPKSB3	Creamy	Cocci	Rough	Raised
(DAY 15)				
DGPKSB1	Creamy	Cocci	Smooth	Flat
DGPKSB2	Creamy	Cocci	Smooth	Raised
DGBKSB3	Creamy	Cocci	Smooth	Raised
(DAY 20)				
DGPKSB1	Creamy	Cocci	Smooth	Flat
DGPKSB2	Creamy	Cocci	Smooth	Raised
DGPKSB3	Creamy	Cocci	Smooth	Raised
(DAY 30)				
DGPKSB1	Creamy	Cocci	Smooth	Raised
DGPKSB2	Yellow	Cocci	Rough	Raised
DGPKSB3	Creamy	Cocci	Smooth	Flat

KEY: DGPKS – Degrading ground palm kernel shell

DGPKSB – Degrading ground palm kernel shell bacteria

4.5 BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES FROM SAMPLES.

4.5.1 Biochemical characterization of bacterial isolates colonies from uncrushed palm kernel shell.

Table 11 shows the biochemical characterization of bacterial isolates from uncrushed palm kernel shell. The biochemical results favours bacterial that catalase positive, gram's reaction positive and most of these isolates produce acid during sugar fermentation test than the isolates that are negative in this regard.

4.5.2 Biochemical characterization of bacterial isolates colonies from chicken dropping

Table 12 shows the biochemical characteristics of bacterial isolates from chicken dropping. The various biochemical test result indicated that bacterial isolates that are catalase positive, positive in gram's reaction and produce acid and gas during sugar fermentation test are more predominant.

4.5.3 Biochemical characterization of bacterial isolates colonies from humus soil.

Table 13 shows the biochemical characterization of bacterial isolates from humus soil. The result indicated that catalase positive bacteria, acid producing bacteria in sugar fermentation are more predominant.

TABLE 11: BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES COLONIES FROM UNCRUSHED PALM KERNEL SHELL (UPKS).

ISOLATES

TESTS	UPKSB1	UPKSB2	UPKSB3
GRAM STAINTING	+	-	+
CATALASE	+	+	+
GRAM'S REACTION (3% KOH)	+	-	+
SUGAR TEST			
FRUCTOSE	A/G	A/G	A/G
LACTOSE	A/-	A/G	A/G
MANNITOL	A/G	A/G	A/-
SUCROSE	A/G	A/-	A/-
PROBABLE ORGANISM	*Pseudomonas aeruginosa*	*Bacillus spp*	*Bacillus spp*

KEY: UPKS – Uncrushed palm kernel shell

UPKSB – Uncrushed palm kernel shell bacteria

A/G – Acid production and Gas production

A/- = Acid production only and no Gas production

-/- = No acid and gas production

- = Negative

+ = Positive

TABLE 12: BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES COLONIES FROM CHICKEN DROPPING (CD).

ISOLATES

TESTS	CDB1	CDB2	CDB3
GRAM STAINTING	+	-	+
CATALASE	+	+	-
GRAM'S REACTION (3%KOH)	+	-	+
SUGAR TEST			
FRUCTOSE	A/G	A/G	A/G
LACTOSE	A/-	A/-	A/-
MANNITOL	A/G	A/G	A/G
SUCROSE	A/-	A/-	A/G
PROBABLE ORGANISM	*Micrococcus spp*	*Bacillus spp*	*Nitrobacterspp*

KEY: CD – Chicken dropping

CDB – Chicken dropping bacteria

A/G – Acid production and Gas production

A/- = Acid production only and no Gas production

-/- = No acid and gas production

- = Negative

+ = Positive

TABLE 13: BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES COLONIES FROM HUMUS SOIL (HS).

ISOLATES

TESTS	HSB1	HSB2	HSB3
GRAM STAINTING	+	-	+
CATALASE	+	+	+
GRAM'S REACTION (3%KOH)	+	-	+
SUGAR TEST			
FRUCTOSE	A/G	-/-	A/-
LACTOSE	A/G	A/G	A/G
MANNITOL	A/-	A/G	A/G
SUCROSE	A/G	A/G	A/G
PROBABLE ORGANISM	*Pseudomonas aeruginosa*	*Bacillus spp*	*Staph aureus*

KEY: HS – Humus soil

HSB – Humus soil bacteria

A/G – Acid production and Gas production

A/- = Acid production only and no Gas production

-/- = No acid and gas production

- = Negative

+ = Positive

4.6 BIOCHEMICAL CHARACTERIZATION OF BACTERIAL ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL INOCULATED WITH CHICKEN DROPPING AT RATIO 1:1.

Table 14 shows the biochemical characterization of bacterial isolates from degrading ground palm kernel at different interval of five days, from day 0, day 5, and to day 30 respectively. The various biochemical test results of bacterial isolates at these interval indicated that bacterial isolates that are catalase positive, gram's reaction positive and acid and gas producing isolates during sugar fermentation test are more predominant than bacterial isolates that are negative in this regard.

TABLE 14: BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES COLONIES FROM DEGRADING GROUND PALM KERNEL (DGPKS) INOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 1:1.

ISOLATE CODES	GRAM STAINING	CATALASE	GRAM'S REACTION (3%KOH)	SUGAR TEST F	L	M	S	PROBABLE ORGANISM
(DAY 0)								
DGPKSB1	+	+	+	A/G	A/G	A/G	A/G	*Bacillus spp*
DGPKSB2	-	+	-	A/-	A/-	A/G	A/-	*Micrococcus spp*
DGPKSB3	+	-	+	A/G	-/-	A/G	A/G	*Staphylococcus spp.*
(DAY 5)								
DGPKSB1	+	+	+	A/G	A/G	A/G	A/G	*Pseudomonas spp*
DGPKSB2	+	+	+	A/G	-/-	A/G	A/G	*Nitrobacterspp*
DGPKSB3	+	+	+	A/G	A/-	A/-	A/G	*Pneumococcus spp*
(DAY 10)								
DGPKSB1	-	+	+	A/G	A/G	A/G	A/G	*Bacillus spp*
DGPKSB2	+	+	-	A/-	A/G	A/-	A/G	*Pseudomonas aeruginosa*
DGPKSB3	+	-	+	A/G	A/G	A/G	A/G	*Staphylococcus spp*
(DAY 15)								
DGPKSB1	+	+	+	A/G	-/-	A/G	A/G	*Micrococcus spp*
DGPKSB2	+	+	+	A/-	A/G	A/G	A/-	*Pneumococcus spp*
DGPKSB3	+	+	-	A/-	A/-	A/G	-/-	*Bacillus spp*
(DAY 20)								
DGPKSB1	-	+	-	A/G	A/G	A/G	A/G	*Micrococcus spp*
DGPKSB2	+	+	+	A/G	A/G	A/-	A/G	*Bacillus sp*
DGPKSB3	+	+	+	A/-	A/G	A/G	A/-	*Staphylococcus spp*
(DAY 30)								
DGPKSB1	+	+	+	A/G	A/G	A/G	A/G	*Micrococcus spp*
DGPKSB2	+	+	+	A/-	A/G	A/G	A/G	*Pseudomonas spp*
DGPKSB3	+	+	+	A/G	A/G	-/-	A/G	*Enterococcus spp*

KEY: DGPKS – Degrading palm kernel shell, F – Fructose S – Sucrose, L – Lactose,
DGPKSB – Degrading palm kernel shell bacteria M - Mannitol
A/G – Acid production and Gas production - = Negative+ = Positive
A/- = Acid production only and no Gas production -/- = No acid and gas production.

4.7 MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGAL ISOLATES FROM SAMPLES.

4.7.1 Morphological and microscopic characteristics of fungal isolates from uncrushed palm kernel shell.

Table 15 shows the cultural morphology and microscopic observation of fungal isolates from uncrushed palm kernel shell.

4.7.2 Morphological and microscopic characteristics of fungal isolates from chicken dropping.

Table 16 shows the cultural and microscopic observation of fungal isolates from chicken dropping based on their conidia, sporangium, mycelium, spores, and hyphae.

4.7.3 Morphological and microscopic characteristics of fungal isolates from humus soil.

Table 17 shows the various characteristics of fungal isolates from humus soil.

TABLE15: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGAL ISOLATES FROM UNCRUSHED PALM KERNEL SHELL

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
YEAST			
UPKSY1	Whitish colony growth	The apex radiating from the entire surface, conidia are 1-celled and globose	*Candida stellate*
UPKSY2	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
UPKSY3	Smooth white coloured colony	Mycelium are not extensive. Conidia are 1-celled ovoid to fusoid.	*Candida oleophila*
MOULD			
UPKSM1	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
UPKSM2	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
UPKSM3	White mycelia	Sparse mycelia, non- septate hyphae conidiophore	*Rhizopusstolonifera*

KEY: UPKSY – Uncrushed palm kernel shell for yeast

UPKSM- Uncrushed palm kernel shell for mould

TABLE16: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGAL ISOLATE FROM CHICKEN DROPPING.

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
YEAST			
CDY1	Rough creamy colony growth	Growing a spherical to sub- spherical budding yeast- like cell or blastoconidia	*Candida tropicalis*
CDY2	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
CDY3	Whitish colony growth	The apex radiating from the entire surface, conidia are 1-celled and globose	*Candida stellate*
MOULD			
CDM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
CDM2	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
CDM3	Yellowish growth colony	A single, simple, dark and narrow conidiophores	*Aspergillus herbarious*

KEY: CDY – Chicken dropping yeast

CDM- Chicken dropping mould

TABLE17: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGAL ISOLATE FROM HUMUS SOIL

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
YEAST			
HSY1	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
HSY2	Rough creamy colony growth	Growing a spherical to sub- spherical budding yeast- like cell or blastoconidia	*Candida tropicalis*
HSY3	Smooth white coloured colony	Mycelium are not extensive. Conidia are 1-celled ovoid to fusoid.	*Candida oleophila*
MOULD			
HSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
HSM2	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
HSM3	White mycelia	Sparse mycelia, non- septate hyphae conidiophore	*Rhizopusstolonifera*

KEY: HSY – Humus soil yeast

HSM- Humus soil mould

4.8 MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGAL ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INOCULATED WITH CHICKEN DROPPING AT RATIO 1:1.

Table 18 shows the morphological and microscopic characteristics of fungal isolates from degrading ground palm kernel shell at various interval of five days, from day 0, day 5, and to day 30. These characteristics are based on their conidia, sporangium, mycelium, spores and hyphae.

TABLE 18: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGAL ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 1:1.

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
		(DAY 0)	
DGPKSM1	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
DGPKSM2	White mycelia	Sparse mycelia, non-septate hyphae conidia	*Rhizopus stolonifera*
DGPKSY	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
		(DAY 5)	
DGPKSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
DGPKSM2	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
DGPKSY	Smooth white coloured colony	Mycelium are not extensive. Conidia are 1-celled ovoid to fusoid.	*Candida oleophila*
		(DAY 10)	
DGPKSM1	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
DGPKSM2	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
DGPKSY	Rough creamy colony growth	Growing a spherical to sub-spherical budding yeast- like cell or blastoconidia	*Candida tropicalis*
		(DAY 15)	
DGPKSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
DGPKSM2	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
DGPKSY	Whitish colony growth	The apex radiating from the entire surface, conidia are 1-celled and globose	*Candida stellate*
		(DAY 20)	
DGPKSM1	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
DGPKSM2	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
DGPKSY	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
		(DAY 30)	
DGPKSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
DGPKSM2	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
DGPKSY	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*

KEY: DGPKSY – Degrading ground palm kernel shell yeastDGPKSM –Degrading ground palm kernel shell mould

4.9 pH READING FROM DEGRADING GROUND PALM KERNEL SHELL INOCULATED WITH CHICKEN DROPPING AT RATIO 1:1.

Table 19 shows the pH reading from degrading palm kernel shell at various interval of five days, from day 0, day 5, and to day 30 respectively. The various pH readings at these interval showed an increase in the reading, which favours the organism on the alkaline range than organism on the acidic range.

TABLE 19: pH READING FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 1:1 USING pH METER.

ISOLATE CODES	pH READING
(DAY 0)	
DGPKS	6.02
(DAY 5)	
DGPKS	7.77
(DAY 10)	
DGPKS	8.50
(DAY 15)	
DGPKS	9.40
(DAY20)	
DGPKS	11.20
(DAY30)	
DGPKS1	12.50
DGPKS2	11.70
DGPKS3	13.20

KEY: DGPKS – Degrading ground palm kernel shell

4.9.1.0 TOTAL TITRATABLE ACID (TTA).

Table 20 shows the total titratable acid (TTA) reading from degrading ground palm kernel shell at various interval of five days, from day 0, day 5, and to day 30 respectively. The various TTA reading at these interval showed an increase in the reading.

TABLE 20: TOTAL TITRATABLE ACID (TTA) READING FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INNOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 1:1

ISOLATE CODES	BURETTE READING (AT WHICH THE COLOUR CHANGES) g/L
(DAY 0)	
DGPKS	9.4 3.52
(DAY 5)	
DGPKS	11.5 4.31
(DAY 10)	
DGPKS	16.5 6.18
(DAY 15)	
DGPKS	14.0 5.25
(DAY 20)	
DGPKS	18.5 6.93
(DAY 30)	
DGPKS1	14.0 5.25
DGPKS2	16.0 6.00
DGPKS3	17.0 6.37

$$TA = \frac{\text{Number of Millilitre of Naoh}}{\text{Number of Millilitre of Sample}} \times 0.1 \times 7.5$$

Key: Number of Millilitre of Sample = 20ml

Concentration of NaOH = 0.1m

Number of Millilitre of NaOH = 9.4

① $\frac{9.4}{20} \times 0.1 \times 7.5$

$= 3.52 \text{ g/L}$

② $\frac{11.50}{20} \times 0.1 \times 7.5 = 4.31 \text{ g/L}$

3) $\frac{16.50}{20} \times 0.1 \times 7.5 = 6.18 \text{ g/L}$

4) $\frac{14.00}{20} \times 0.1 \times 7.5 = 5.25 \text{ g/L}$

4.2 DISCUSSION

After the microbial analysis performed on the various samples, the organism present in uncrushed palm kernel shell, chicken droppings, humus soil were isolated. Higher count of (2.5×10^6) cfu/g was found in plate one with dilution factor 5 and the lowest count of (0.2×10^7) was found in plate three with dilution factor 6 when using uncrushed palm kernel shell as the sample. Higher count of (5.4×10^6) was also found in plate one with dilution factor of 5 and lowest count of (3.0×10^7) in plate three with dilution factor of 6 when chicken dropping was used as the sample. Higher count of (3.5×10^6) was also found in plate one with dilution factor 5 and lowest count of (2.0×10^7) was found in plate three with dilution factor 6 using humus soil as the sample. Count was also recorded when grounded palm kernel shell plus chicken dropping was used as the sample, higher count of (4.0×10^6) was found in plate one with dilution factor 5 and lowest count of (2.5×10^7) was found in plate three with dilution factor 6. Sample was also picked at day 5 of mixing grounded palm kernel shell plus chicken dropping together and analyzed formicrobial load and count was also recorded, higher count of (4.5×10^6) was found in plate one with dilution factor 5 and lowest count of (2.4×10^7) was found in plate three with dilution factor of 6. Different count were also observed for yeast and mold with different dilution factor. The microbial analysis results indicate that the various samples contained a variety of microorganisms

which include bacillus sp, pseudomonas aeruginosa and Aspergillus spp. The results are in consonance with (Verla, 2014), which confirm the presence of various microorganisms in the sample.

The p^H reading also favours organisms on the alkaline range.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Through the evaluation and analysis of microbial load of the various samples which include chicken dropping and humus soil, and the various biochemical tests carried out on the samples which include catalase test, sugar fermentation test etc., the results show that the samples contained heavy load of variety of microorganisms which can serve as a source of innoculum and consortia of these microorganisms can help in the biodegradation of palm kernel shell which in turn can eventually help in solving the problem of agro pollution in the environment.

5.2 RECOMMENDATION

From the observation and the result recorded in this study, the following recommendation are proffered as thus:

*There is enough inoculum that can help in the biodegradation of palm kernel shell.

*The mixture of the sample can serve as manure.

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