**CHAPTER 1**

**INTRODUCTION**

A fungus is a eukaryote that digests food externally and absorbs nutrients directly through its cell walls. Most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae. Fungi are heterotrophs and, like animals, obtain their carbon and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal) and are called biotrophs; others obtain their nutrients from dead plants or animals and are called saprotrophs. Some fungi infect a living host, but kill host cells in order to obtain their nutrients; these are called necrotrophs. Fungi were once considered to be primitive members of the plant kingdom, just slightly more advanced than bacteria. Fungi are an ancient group not as old as bacteria, which fossil evidence suggests may be 3. 5 billion years old but the earliest fungal fossils are from the Ordovician, 460 to 455 million years old (Redecker *et al.* 2000). Based on fossil evidence, the earliest vascular land plants didn't appear until approximately 425 million years ago, and some scientists believe that fungi may have played an essential role in the colonization of land by these early plants (Redeker *et al.* 2000). Mushrooms exquisitely preserved in amber from the Late Cretaceous (94 million years ago) tell us that there were mushroom forming fungi remarkably similar to those that exist today when dinosaurs were roaming the planet (Hibbett *et al*. 2003). Fungi are an important group of plant pathogens most plant diseases are caused by fungi but fewer than 10% of all known fungi can colonize living plants (Knogge, 1996). Plant pathogenic fungi represent a relatively small subset of those fungi that are associated with plants. Most fungi are decomposers, utilizing the remains of plants and other organisms as their food source. Other types of associations include the role of fungi as decomposers, as beneficial symbionts, and as cryptic plant colonizers called endophytes. Most fungi are associated with plants as saprotrophs and decomposers.

Soil is normally considered as the ﬁne earth which covers land surfaces as a result of the in situ weathering of rock materials or the accumulation of mineral matter transported by water, wind, or ice. The distinctive feature of soil is that to this weathered mineral material is added organic material. This organic material may be both living and dead. The dead organic matter will include little altered and freshly added dead plant roots and leaf and other plant litter, dead fauna, and organic material in various stages of decomposition from little modiﬁed relatively fresh materials to the complex decomposed material called humus. It is this mixture of mineral and organic material which gives the soils their distinctive characteristics.

The soil is a highly complex system with many components playing diverse functions and could be due mainly to the activity of soil organisms (Chiang and Soudi, 1994). Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth (Nagamani *et al*., 2006). Microorganisms are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformation and mineralization activities in soils. Type of cultivation and crop management practices are found to have greater influence on the activity of soil microflora (Mc. Gill *et al.,* 1980). Continuous use of chemical fertilizers over a long period may cause imbalance in soil microflora, as result affecting indirectly biological properties of soil leading to soil degradation (Manickam and Venkataraman, 1972). Fungi are fundamental for soil ecosystem functioning (Warcup, 1951).

Soil is the upper layer of most of the earth’s surface and varies in depth from inches to over twenty feet. It is a product of weathered rock, but quite distinct in its characteristic. Soils are excellent cultural media for the growth of many types of organisms (Angelov, (2008). This includes bacteria, fungi, algae, protozoa and viruses. A spoonful of soil contains billions of microorganisms. In general the majority of microbial population is found in the upper six to twelve inches of soil and the number decreases with depth (Cattle, *et al.*, 2002). The number and kinds of organisms found in soil depend upon the nature of soil, depth, season of the year, state of the cultivation, reaction, organic matter, temperature, moisture, aeration, etc.

**AIM**

This research is aimed at isolation and identification of fungi in compost and garden soils.

 **OBJECTIVES**

* To isolate fungi from the soil using routine culture media.
* To identify the fungi community from the soil sample collected from Godfrey Okoye University farms and wastes surrounding.

 **CHAPTER 2**

 **LITERATURE REVIEW**

**ORIGIN OF FUNGI**

The origin of fungi was estimated at between 660 million and up to 2.15 billion years ago and the divergence of the fungal animal from the plant lineage at between 780 million and up to 2.5 billion years ago (Taylor and Berbee 2006). The divergence of the two major lineages of higher fungi, Ascomycota and Basidiomycota, was dated at between 390 million years and up to 1.5 billion years ago (Berbee and Taylor 1993, 2007; Taylor and Berbee 2006). Fungi make up a major clade of eukaryotes that includes yeasts, molds, lichens, and mushrooms. The ancestor of fungi was probably a flagellated, aquatic unicellular organism, but the group is now dominated by terrestrial, filamentous forms that lack flagella. Fungi obtain nutrition as saprotrophs (decomposers) or biotrophs (mutualists and parasites). Fungi play a critical role in the carbon cycle and have huge economic impacts as decayers, pathogens, and industrial microorganisms (David, 2017). According to David Habbett (2017), for much of the history of mycology, the only characters available for estimating evolutionary relationships of fungi were macro morphology (for groups that produce macroscopic structures, such as mushrooms) and anatomical features (e.g., shapes and staining reactions of spores). Starting in the 1960s, electron microscopy made it possible to observe subcellular characters, such as features associated with flagella or the mitotic apparatus, which were used to address the higher level relationships and the limits of fungi. Until the late 20th century, fungal systematics routinely developed phylogenetic hypotheses and classifications through subjective analyses of non-molecular characters, but they rarely applied cladistics methods. After the Polymerase Chain Reaction (PCR) was developed in the late 1980s, however, mycologists turned toward analyses of DNA characters, particularly ribosomal RNA (rRNA) gene sequences. Many of the older anatomy-based hypotheses were upheld by analyses of molecular data, but there were also surprises, particularly involving morphologically cryptic taxa (e.g., yeasts) or highly derived forms that lack informative anatomical characters (e.g., gasteromycetes, such as stinkhorns and puffballs). Around the beginning of the 21st century, multilocus phylogenetic analyses using PCR amplification of protein-coding as well as rRNA genes became standard, which combined dense taxon sampling with moderately large numbers of molecular characters. Currently, fungal systematics is being further transformed by analyses of whole genomes and environmental DNA sequences (metagenomics).Before the introduction of [molecular methods](https://en.wikipedia.org/wiki/Molecular_phylogenetics) for phylogenetic analysis, [taxonomists](https://en.wikipedia.org/wiki/Taxonomy_%28biology%29) considered fungi to be members of the [plant kingdom](https://en.wikipedia.org/wiki/Plant) because of similarities in lifestyle: both fungi and plants are mainly [immobile](https://en.wikipedia.org/wiki/Sessility_%28zoology%29), and have similarities in general morphology and growth habitat (Bruns 2006). Like plants, fungi often grow in soil and, in the case of [mushrooms](https://en.wikipedia.org/wiki/Mushroom), form conspicuous [fruit bodies](https://en.wikipedia.org/wiki/Fruit_bodies), which sometimes resemble plants such as [mosses](https://en.wikipedia.org/wiki/Mosses). The fungi are now considered a separate kingdom, distinct from both plants and animals, from which they appear to have [diverged](https://en.wikipedia.org/wiki/Genetic_divergence) around one billion years ago (Bruns 2006; Baldauf, et *al* 1993). They reproduce by both sexual and asexual means, and like [basal](https://en.wikipedia.org/wiki/Primitive_%28phylogenetics%29) plant groups (such as [ferns](https://en.wikipedia.org/wiki/Fern) and [mosses](https://en.wikipedia.org/wiki/Moss)) produce [spores](https://en.wikipedia.org/wiki/Spore). Similar to mosses and algae, fungi typically have [haploid](https://en.wikipedia.org/wiki/Haploid) nuclei (Shoji, *et al.,* 2006).The cells of most fungi grow as tubular, elongated, and thread-like (filamentous) structures called [hyphae](https://en.wikipedia.org/wiki/Hypha), which may contain multiple nuclei and extend by growing at their tips. Each tip contains a set of aggregated [vesicles](https://en.wikipedia.org/wiki/Vesicle_%28biology%29) cellular structures consisting of [proteins](https://en.wikipedia.org/wiki/Protein), [lipids](https://en.wikipedia.org/wiki/Lipid), and other organic molecules called the [Spitzenkörper](https://en.wikipedia.org/wiki/Spitzenk%C3%B6rper) (Andia *et al*, 2006; Zabriskie *et al,* 2000).Soil is a [mixture](https://en.wikipedia.org/wiki/Mixture) of [organic matter](https://en.wikipedia.org/wiki/Organic_matter), [minerals](https://en.wikipedia.org/wiki/Minerals), gases, liquids, and organisms that together support life (Chesworth, 2008). The Earth's body of soil is the [pedosphere](https://en.wikipedia.org/wiki/Pedosphere), which has four important functions: it is a medium for plant growth; it is a means of [water storage](https://en.wikipedia.org/wiki/Water_storage), supply and purification; it is a modifier of [Earth's atmosphere](https://en.wikipedia.org/wiki/Atmosphere_of_Earth); it is a habitat for organisms; all of which, in turn, modify the soil.

Soil interfaces with the [lithosphere](https://en.wikipedia.org/wiki/Lithosphere), the [hydrosphere](https://en.wikipedia.org/wiki/Hydrosphere), the [atmosphere](https://en.wikipedia.org/wiki/Atmosphere), and the [biosphere](https://en.wikipedia.org/wiki/Biosphere). (Chesworth, 2008). The term *pedolith*, used commonly to refer to the soil, translates to *ground stone*. Soil consists of a solid phase of minerals and organic matter (the soil matrix), as well as a [porous](https://en.wikipedia.org/wiki/Porosity) phase that holds gases (the soil atmosphere) and water (Richard *et al.,* 2007). Accordingly, soils are often treated as a three [state](https://en.wikipedia.org/wiki/State_of_matter) system of solids, liquids, and gases (David, *et al.,* 2006).

Soil is a product of the influence of [climate](https://en.wikipedia.org/wiki/Climate), [relief](https://en.wikipedia.org/wiki/Terrain) (elevation, orientation, and slope of terrain), organisms, and its [parent materials](https://en.wikipedia.org/wiki/Parent_material) (original minerals) interacting over time (Alfred, *et al.,* 1975). It continually undergoes development by way of numerous physical, chemical and biological processes, which include [weathering](https://en.wikipedia.org/wiki/Weathering) with associated [erosion](https://en.wikipedia.org/wiki/Erosion). Given its complexity and strong internal [connectedness](https://en.wikipedia.org/wiki/Connectedness), it is considered an [ecosystem](https://en.wikipedia.org/wiki/Ecosystem) by [soil ecologists](https://en.wikipedia.org/wiki/Soil_ecology) (Ponge, 2015). Soil is a major component of the [Earth](https://en.wikipedia.org/wiki/Earth)'s [ecosystem](https://en.wikipedia.org/wiki/Ecosystem). The world's ecosystems are impacted in far-reaching ways by the processes carried out in the soil, from [ozone depletion](https://en.wikipedia.org/wiki/Ozone_depletion) and [global warming](https://en.wikipedia.org/wiki/Global_warming), to [rainforest destruction](https://en.wikipedia.org/wiki/Rainforest_destruction) and [water pollution](https://en.wikipedia.org/wiki/Water_pollution). With respect to Earth's [carbon cycle](https://en.wikipedia.org/wiki/Carbon_cycle), soil is an important [carbon reservoir](https://en.wikipedia.org/wiki/Carbon_sink), and it is potentially one of the most reactive to human disturbance Hernandez, *et al.,* (2002) and climate change Ivan *et al.,* (2006). As the planet warms, it has been predicted that soils will add carbon dioxide to the atmosphere due to increased [biological](https://en.wikipedia.org/wiki/Soil_biology) activity at higher temperatures, a [positive feedback](https://en.wikipedia.org/wiki/Positive_feedback) (amplification).(Powlson, 2005). This prediction has, however, been questioned on consideration of more recent knowledge on soil carbon turnover. (Thomas, *et al.,* 2016).

Soil acts as an engineering medium, a habitat for [soil organisms](https://en.wikipedia.org/wiki/Soil_organisms), a recycling system for [nutrients](https://en.wikipedia.org/wiki/Nutrients) and [organic wastes](https://en.wikipedia.org/wiki/Organic_waste), a regulator of [water quality](https://en.wikipedia.org/wiki/Water_quality), a modifier of [atmospheric composition](https://en.wikipedia.org/wiki/Atmospheric_chemistry), and a medium for [plant growth](https://en.wikipedia.org/wiki/Plant_growth), making it a critically important provider of [ecosystem services](https://en.wikipedia.org/wiki/Ecosystem_services) (Mackay, *et al.,* (2010). Since soil has a tremendous range of available niches and habitats, it contains most of the Earth's genetic diversity. A gram of soil can contain billions of organisms, belonging to thousands of species, mostly microbial and in the main still unexplored (Daniel, *et al.,* (1998). Soil has a [mean](https://en.wikipedia.org/wiki/Mean) [prokaryotic](https://en.wikipedia.org/wiki/Prokaryote) density of roughly 108 organisms per gram, Nunan *et al.,* (2014) whereas the ocean has no more than 107 prokaryotic organisms per milliliter (gram) of seawater. [Organic carbon](https://en.wikipedia.org/wiki/Soil_organic_matter) held in soil is eventually returned to the atmosphere through the process of [respiration](https://en.wikipedia.org/wiki/Cellular_respiration) carried out by [heterotrophic](https://en.wikipedia.org/wiki/Heterotrophic) organisms, but a substantial part is retained in the soil in the form of [soil organic matter](https://en.wikipedia.org/wiki/Soil_organic_matter); [tillage](https://en.wikipedia.org/wiki/Tillage) usually increases the rate of soil respiration, leading to the depletion of soil organic matter (Andrews *et al.,* (2000). Since plant roots need oxygen, ventilation is an important characteristic of soil. This ventilation can be accomplished via networks of interconnected [soil pores](https://en.wikipedia.org/wiki/Pore_space_in_soil), which also absorb and hold rainwater making it readily available for uptake by plants. Since plants require a nearly continuous supply of water, but most regions receive sporadic rainfall, the [water-holding capacity](https://en.wikipedia.org/wiki/Soil_water_%28retention%29) of soils is vital for plant survival. (Robert, *et al.,* 1962).

Soils can effectively remove impurities, kill disease agents, Alexander, *et al.,* (2000) and degrade contaminants, this latter property being called natural attenuation. Typically, soils maintain a net absorption of [oxygen](https://en.wikipedia.org/wiki/Oxygen) and [methane](https://en.wikipedia.org/wiki/Methane), and undergo a net release of [carbon dioxide](https://en.wikipedia.org/wiki/Carbon_dioxide) and [nitrous oxide](https://en.wikipedia.org/wiki/Nitrous_oxide). Soils offer plants physical support, air, water, temperature moderation, nutrients, and protection from toxins. Soils provide readily available nutrients to plants and animals by converting dead organic matter into various nutrient forms. (Benites, *et al.,* (2005). Compost is [organic matter](https://en.wikipedia.org/wiki/Organic_matter) that has been [decomposed](https://en.wikipedia.org/wiki/Biodegradation) in a process called composting. This process [recycles](https://en.wikipedia.org/wiki/Recycling) various organic materials otherwise regarded as waste products and produces a [soil conditioner](https://en.wikipedia.org/wiki/Soil_conditioner) (the compost). Gilbert, *et al.,* (1997).Compost is rich in nutrients. It is used for example in [gardens](https://en.wikipedia.org/wiki/Garden), [landscaping](https://en.wikipedia.org/wiki/Landscaping), [horticulture](https://en.wikipedia.org/wiki/Horticulture), [urban agriculture](https://en.wikipedia.org/wiki/Urban_agriculture) and [organic farming](https://en.wikipedia.org/wiki/Organic_farming). The compost itself is beneficial for the land in many ways, including as a soil conditioner, a [fertilizer](https://en.wikipedia.org/wiki/Fertilizer), addition of vital [humus](https://en.wikipedia.org/wiki/Humus) or [humic acids](https://en.wikipedia.org/wiki/Humic_acids), and as a natural [pesticide](https://en.wikipedia.org/wiki/Pesticide) for soil. In [ecosystems](https://en.wikipedia.org/wiki/Ecosystems), compost is useful for erosion control, land and stream reclamation, wetland construction, and as landfill cover. [Earthworms](https://en.wikipedia.org/wiki/Earthworm) and [fungi](https://en.wikipedia.org/wiki/Fungus) further break up the material. Bacteria requiring oxygen to function ([aerobic bacteria](https://en.wikipedia.org/wiki/Aerobic_bacteria)) and fungi manage the chemical process by converting the inputs into heat, [carbon dioxide](https://en.wikipedia.org/wiki/Carbon_dioxide), and [ammonium](https://en.wikipedia.org/wiki/Ammonium) (Gilbert, *et al.,* (1997).With the proper mixture of water, oxygen, carbon, and nitrogen, micro-organisms are able to break down organic matter to produce compost. The composting process is dependent on micro-organisms to break down organic matter into compost. There are many types of microorganisms found in active compost of which the most common are: [Bacteria](https://en.wikipedia.org/wiki/Bacteria); The most numerous of all the [microorganisms](https://en.wikipedia.org/wiki/Microorganism) found in compost. Depending on the phase of composting, [mesophilic](https://en.wikipedia.org/wiki/Mesophile) or [thermophilic](https://en.wikipedia.org/wiki/Thermophile) bacteria may predominate. [Actinobacteria](https://en.wikipedia.org/wiki/Actinobacteria); Necessary for breaking down paper products such as newspaper, [bark](https://en.wikipedia.org/wiki/Bark_%28botany%29), etc. [Fungi](https://en.wikipedia.org/wiki/Fungi)- [molds](https://en.wikipedia.org/wiki/Molds) and [yeast](https://en.wikipedia.org/wiki/Yeast) help break down materials that bacteria cannot, especially [lignin](https://en.wikipedia.org/wiki/Lignin) in woody material. [Protozoa](https://en.wikipedia.org/wiki/Protozoa)- Help consume bacteria, fungi and micro organic particulates. [Rotifers](https://en.wikipedia.org/wiki/Rotifers); Rotifers help control populations of bacteria and small protozoans. In addition, [earthworms](https://en.wikipedia.org/wiki/Earthworm) not only ingest partly composted material, but also continually re-create aeration and drainage tunnels as they move through the compost.

A farm is an area of land that is devoted primarily to [agricultural](https://en.wikipedia.org/wiki/Agriculture) processes with the primary objective of producing [food](https://en.wikipedia.org/wiki/Food) and other [crops](https://en.wikipedia.org/wiki/Crop); it is the basic facility in food production. Gardening is the practice of growing and cultivating plants as part of [horticulture](https://en.wikipedia.org/wiki/Horticulture). In gardens, [ornamental plants](https://en.wikipedia.org/wiki/Ornamental_plant) are often grown for their [flowers](https://en.wikipedia.org/wiki/Flower), [foliage](https://en.wikipedia.org/wiki/Leaf), or overall appearance; useful plants, such as [root vegetables](https://en.wikipedia.org/wiki/List_of_root_vegetables), [leaf vegetables](https://en.wikipedia.org/wiki/Leaf_vegetable), [fruits](https://en.wikipedia.org/wiki/Fruit), and [herbs](https://en.wikipedia.org/wiki/Herb), are grown for consumption, for use as [dyes](https://en.wikipedia.org/wiki/Dye), or for [medicinal](https://en.wikipedia.org/wiki/Medicine) or [cosmetic](https://en.wikipedia.org/wiki/Cosmetics) use. Gardening is considered by many people to be a relaxing activity.

Gardening ranges in scale from [fruit](https://en.wikipedia.org/wiki/Fruit) [orchards](https://en.wikipedia.org/wiki/Orchard), to long [boulevard](https://en.wikipedia.org/wiki/Boulevard) plantings with one or more different types of [shrubs](https://en.wikipedia.org/wiki/Shrub), [trees](https://en.wikipedia.org/wiki/Tree), and [herbaceous plants](https://en.wikipedia.org/wiki/Herbaceous_plant), to residential [yards](https://en.wikipedia.org/wiki/Yard_%28land%29) including lawns and foundation plantings, to plants in large or small containers grown inside or outside. Gardening may be very specialized, with only one type of plant grown, or involve a large number of different plants in mixed plantings. It involves an active participation in the growing of plants, and tends to be labor-intensive, which differentiates it from [farming](https://en.wikipedia.org/wiki/Agriculture) or [forestry](https://en.wikipedia.org/wiki/Forestry). Garden soil contains humus compound. Humus is a large group of natural organic compounds, found in the soil, formed from the chemical and biological decomposition of plant and animal residues and from the synthetic activity of microorganisms while compost soil is a decayed remains of organic matter that has rotted into a natural fertilizer.

Fungi mediate nearly every aspect of organic matter production, decomposition, and sequestration, with concomitant roles in the mineralization and cycling of nitrogen and phosphorus. Filamentous fungi support plant production through mycorrhizal associations that enhance the acquisition of water and nutrients. Above ground, ubiquitous fungal endophytes can confer resistance to thermal and drought stress and reduce herb ivory (Porras-Alfaro and Bayman, 2011). Fungi also support carbon and nitrogen fixation by algae and cyanobacteria through lichen associations and, in arid and Polar Regions, the formation of biotic crusts that also mediate soil atmosphere exchanges, water infiltration, and stabilize surface soils against erosion (Pointing and Belnap, 2012). As agents of organic matter decomposition, fungi mediate the creation and protection of soil organic matter (SOM), as well as its mineralization. Surface litter becomes SOM when the residue of humic material and microbial products reaches a threshold composition of 70% acid insoluble material (Berg and McClaugherty, 2003), at which point the cost of further degradation is not energetically favorable without additional inputs of more labile material (Moorhead *et al.,* 2013). However, surface litter decomposition may not be the principal pathway of SOM formation in many ecosystems. Fungi are linked to numerous other organisms in complex soil food webs. The spectrum of organisms that feed on or parasitize fungi in soil is extremely diverse, spanning viruses, bacteria, protists, insects, small mammals, and other fauna. Similarly, the spectrum of living organisms that are subject to attack by fungi is equally diverse. Fungi that are placed into one guild designation often engage in other sorts of interactions, and the extent of these phenomena is poorly known. As with fungal linkages as mycorrhizae and decomposers, other food web linkages range from nonspecific to highly specific. Some of the most widespread trophic linkages of soil fungi appear to be related to nitrogen demand imparted by the high carbon: nitrogen ratios of plant materials that comprise the main energy source. A number of wood decay fungi obtain supplemental nitrogen by feeding on nematodes (Thorn and Barron, 1984; Barron, 2003). Some of these fungi are also able to prey on bacteria (Barron, 1988). Some of the best-known nematode trapping fungi actively produce enzymes to degrade cellulose or lignocellulose, leading Barron (2003) to suggest that these fungi are primarily decomposers that have particularly dramatic adaptations for nitrogen supplementation. Mycorrhizal fungi also engage in various interactions that may seem unexpected given their guild designation. For example, some EMF fungi can supplement nitrogen by attacking living animals. Such as collembolans (small wingless insects known as springtails; Klironomos and Hart, 2001).

Organisms in the soil are categorized as micro or macro organisms and all have a critical role to play in making soil living, dynamic, and productive. (Knudsen, (2006). Microorganisms: Those which cannot be seen with the naked eye; Bacteria: In a teaspoon (5-7 grams of soil) there are typically 100 billion bacteria representing one aspect of a well-developed and functional microscopic ecosystem beneath the soil surface. (Knudsen, 2006).There are four general categories of bacteria in the soil; decomposers, mutualists, pathogens (these are generally on the mind of most people), and lithotrophs. Lithotrophs are critical to nitrogen recycling and pollutant degradation. (Inghan, 2000) Soil bacteria are critical to water dynamics, nutrient recycling, disease suppression, and the health and productivity of soils. Protozoa: Single cell animals requiring bacteria to eat and water for movement. They will also feed on each other and can reduce bacterial diseases by consuming large number of pathogenic bacteria. The regulation of bacterial populations through grazing releases excess nitrogen in the form of ammonium (NH4+)providing nitrogen for plant growth as protozoa generally occupy soil zones within the rhizosphere adjacent to root structures. Nematodes: Small diameter microscopic worms living in the water surrounding root zones are the final microbiological life form responsible for living soil. They can provide both benefits and problems in the garden. Excessive irrigation favors some parasitic omnivore nematodes which may greatly reduce production. Otherwise, similar to other micro life forms discussed above, nematodes consume fungi, bacteria, protozoa, and each other returning nutrients to the soil and maintaining a balanced ecosystem under the soil. (Neher, 2001). Macro organisms: Those which can be seen with the naked eye: Arthropods; which include mites, millipedes, spiders, scorpions, and beetles are the primary macro organisms responsible for maintaining the health of our living soils. They constitute a population of predator/prey organisms which facilitate nutrient release, recycling, and utilization. They are grouped as shredders, predators, herbivores, and fungal feeders. (Moldenke, 2001). All occupy critical roles with respect to maintaining healthy, living, and productive soils. Larger arthropods live on the soil surface and shred organic debris, and each other, into small parts aiding in decomposition and incorporation into the soil. If a gardener has ever spread organic mulch on the soil, and then marveled at how quickly it seems to vanish, blame the shedders! Examples are millipedes, sow bugs, earwigs, and some beetles. The results of all this frenetic interaction are copious streams of recycled organic material and body parts circulating throughout the soil ecosystem. Earthworms: The final candidate for evaluation is the most visible feature of living soil systems. Most importantly they alter and mix soil structure to better facilitate water movement and holding capacity, bury and shred surface organic material, provide channels for root growth and development, maintain nutrient dynamics, facilitate plant growth, and stimulate microbial activity. (Edwards, 2001).

 **CHAPTER 3**

**3.1 MATERIALS**

Wire loop, test tube, Petri dishes, conical flask, distilled water, ethanol, scalper, foil, weighing balance, hand glove, face mask, masking tape, lacto phenol and cotton blue, microscope, autoclave, forceps, filter paper, U-shaped glass rod, slides, pipette, sterilized bags, glass spreader, cotton wool, measuring cylinder, Bunsen burner, Saboraud dextrose agar ,cover slip, incubator.

**3.2 METHODOLOGY**

**3.2.1 COLLECTION OF SOIL SAMPLES**

 The soil sample were collected from two different fields at Godfrey Okoye University, thinker’s corner. The first sample was collected from the farm i.e. garden soil and the second sample was collected from compost soil. The soil was taken at 15 cm depth and put into small sterilized bags for laboratory analysis.

**3.2.2 ISOLATION OF FUNGI FROM THE SOIL**

 The soil dilution and spread plate method on media such as Saboraud Dextrose agar used in the isolation techniques. Antibiotics e.g. Chloramphenicol, was added into the media to prevent the growth of any bacteria on the plate, so that only fungi can grow on the plate.

**3.2.3 PREPARATION OF SOIL DILUTIONS**

* One gram of the soil sample was weighed and added to 9mls of sterile water. The suspension was well shaken, and labeled as “A”.
* Before the soil settles, 1ml of the suspension was removed with a sterile pipette and transferred into another 9ml sterile water, vortex thoroughly and labeled “B”.
* This dilution steps were repeated three times and each time with 1ml of the previous suspension and into another 9 ml of sterile water. Labeled sequentially as tubes C, and D.
* This result in serial dilution of 10-1 through 10-4 grams of the soil per ml. Petri dishes of each dilutions, containing Saboraud dextrose agar medium.

**3.2.4 MAKING SPREAD PLATES FOR FUNGAL CULTURE**

* Fungal colonies were picked from three prepared Saboraud dextrose agar plates and labelled as A, B, and D. Vortex samples A, B, and D and 0.1ml of each suspension was removed using sterile pipette onto each plate. This increases the dilution value further, by a factor of ten (A=10-1, B=10-2, D=10-4).
* Next, a glass tube was dipped into ethanol and then into the flame for a few seconds to ignite and burn off the ethanol. This will sterilizer the spreader.
* The spreader was held above the first plate until the flame was extinguished. The plate was quickly opened, holding the lid close by. The spreader was dipped into the agar away from the inoculum to cool, and then spread the drop of inoculum around the surface of the agar until traces of free liquid disappear. Replace the plate lid.
* The spreader was re-flamed and the process repeated with the next plate, working quickly so as not to contaminate the agar with an air born organisms.
* The fungal plates were incubated at room temperature for up to 4 to 7 days for sporation to occur. The plate were inverted during the incubating period to prevent drops of moisture from condensation from falling onto the agar surface.

**3.3 IDENTIFICATION OF FUNGI USING SLIDE CULTURE TECHNIQUE**

With a pair of forceps, a sheet of sterile filter paper was placed into the Petri dish. A sterile U-shaped glass rod was placed on the filter paper inside the petri dish. (Rod can be sterilized by flaming, if held by forceps). Enough sterile water was poured (about 2ml) on filter paper to completely moisten it. The forceps was used to place a sterile slide on U-shaped rod. The scalpel was flamed to sterilized and cut 5 mm square block of the medium from the plate of saboraud agar. A block of agar was picked, by inserting the scalpel and carefully transfer this block aseptically to center of the slide. Four sides of the agar square with spores or mycelia fragments of fungus to be examined. Be sure to flame and cool the loop prior to picking up spores. A sterile cover slip was placed on the upper surface of the agar cube. Place the cover on the Petri dish and incubate at room temperature for 4 to 5 days. After 4 to 5 days, examine the slide under low power. If growth has occurred there will be growth of hyphen and production of spores.

**3.4 STAINING OF FUNGI USING LACTO PHENOL BLUE**

Then the forceps are sterilized using the Bunsen burner and allowed to cool. Using the scalpel blade the block of SDA containing the fungal culture is removed from the center of the slide and is disposed. Using a pipette a drop of lacto phenol solution is placed onto a clean glass slide (on the center). One ml of ethanol was dropped using a sterile pipette to avoid air bubble. The cover slip is gently placed on the slide by lowering it down. The slide is now read and examined under the microscope. Fungi may be detected by the presence of characteristic structure such as micro/macroconida, spores and hyphae. Using the low power ×10 obj. to locate the object. Using the high power ×40 obj. to confirm the presence of fungal structure.

 **NOTE:** Identification of the unknown cultures was done using cultural characteristics together with the microscopic picture to compare with the related picture in the laboratory manual.

**CHAPTER 4**

**RESULTS**

Fungal growth occurred on the two soil samples. They have different colors, textures, elevations, shape, margins.(Table 1 ) and (Table 2).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compost soil | Color | Texture | Elevation | Shape | Margin |
| Sample ASample BSample D | Milkya)Milkyb) Milkya)Milkyb)Milky |  Wrinkleda)Mucoidb)Wrinkleda) Mucoidb)Wrinkled | Flata) Flatb) Flata) Raisedb) Flat | Rhizioda) Irregularb) Rhizoida) Irregularb)Irregular | Lobatea)Lobateb) Lobatea)Lobateb)Lobate |

 **TABLE 1: MOPHOLOGY OF THE FUNGI IN COMPOST SOIL**

 **TABLE 2: MOPHOLOGY OF FUNGI IN GARDEN SOIL**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| GardenSoil | Color | Texture | Elevation | Shape | Margin |
| Sample ASample BSample D | a)Milkyb)Milkya)Milkyb)Milkya)Milkyb)Milky | a)Wrinkledb)Mucoida)Mucoidb)Wrinkleda)Mucoidb)Wrinkled | a)Flatb) Convexa)Raisedb)Flata)Raisedb)Flat | a)Rhizoidb) Irregulara)Irregularb)Rhizoida)Irregularb)Irregular | a)Undulateb)Entirea)Lobateb)Lobatea)Lobateb)Lobate |

The slide culture helped in the identification of the fungi. The three isolates obtained were identified as *Aspergillus niger*, *Aspergillius fumigatus*, *Mucor* sp. and all three fungi grow in the two soil samples (Table 3).

The microscopic identification of the three cultures showed that they have *Aspergillius niger* (fig.1), *Aspergillius fumigatus* (fig.2), *Mucor* sp (fig.3).

**TABLE 3: IDENTIFICATION OF MICROBIAL ISOLATES TWO SOIL SAMPLES.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial number** | **Sample name** | **Color** | **Name of fungal identified** |
| **1** | Sample D(a) | Black | *Aspergillus niger*  |
| **2** | Sample D(b) | Green | *Aspergillius* *fumigatus* |
| **3** | Sample D(c) | White | *Mucor* sp. |



**Fig.1**: *Aspergillius* *fumigatus*



**Fig. 2:** *Aspergillus niger*



**Fig. 3:** *Mucor* sp

 **CHAPTER 5**

**DISCUSSION**

 Soil is a complex system containing flora of various microorganisms including bacteria, algae, fungi. Fungi are one of them, it is estimated to have 1.5 million species of fungi in the world at different surroundings with about 5% of these identified formally. In the present study, the isolated fungi were identified on the basis of cultural, microscopic and morphological characteristics. The environmental factors such as the soil pH, moisture, temperature, organic carbon and nitrogen play an important role in the mycoflora distribution (Kumar *et al.,* 2015). These are the main factors affecting the fungal population that was very high in the analyzed soil samples. Three soil samples were isolated and the identified fungi were found to have history of causing diseases in human beings with respect to other research done by many scientists. In this result fungi growth occur more in compost soil because it contains more nutrients than garden soil. The identified soil fungi from the two soil sample are *Aspergillus niger, Aspergillius fumigatus* and *Muco*r sp. In sample D (a) identified as the *Aspergillius niger* is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear canal and tympanic membrane. In sample D (b), identified as *Aspergillius* *fumigatus*is the most frequent cause of invasive fungal infection in immunosuppressed individuals, which include patients receiving immunosuppressive therapy for autoimmune or neoplastic disease, organ transplant recipients, and AIDS patients. *A. fumigatus* can cause chronic pulmonary infections, allergic disease in immunocompetent hosts. In sample D(c), identified as *Mucor* sp. which can cause mucormycosis. Mucormycosis frequently infects the sinuses, brain, or lungs. While infection of the oral cavity or brain are the most common forms of mucormycosis, the fungus can also infect other areas of the body such as the gastrointestinal tract, skin, and other organ systems. Also *Mucor* sp are known to cause disease in man through inhalation of the spores in the air e.g. systematic mucormycosis in diabetic patients. The mycofloral analysis was in agreement some other studies such as Ratnasri *et al*., (2014) and Megha Bhutt *et al.,* (2015).

**CONCLUSION**

In this work we were able to observe that the soils contains different types of fungal species. The two common soil fungi obtained were *Aspergillus* spp and *Mucor* sp. In these work the fungal species which were isolated can be pathogenic in nature for human begins e.g. farmers work in the farm and Enugu State Waste Association of Management in Africa (ESWAMA) who pack dirty are liable to inhale these spores in the air. It can cause respiratory disease and can produce toxins that infects the liver e.g. Aflatoxins. Also some are known to cause disease in man through inhalation of the spores in the air e.g. systematic mucormycosis in diabetic patients.

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