

**ISOLATION OF PATHOGENIC BACTERIA FROM ANTHILL SOIL (EKOSODIN  
COMMUNITY, UNIVERSITY OF BENIN, UGBOWO CAMPUS), BENIN CITY.**

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**UNIVERSITY OF BENIN**

**BENIN CITY**

**NOVEMBER, 2022.**

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**AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF  
ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE  
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BACHELOR OF SCIENCE (B.sc) DEGREE IN ENVIRONMENTAL MANAGEMENT  
AND TOXICOLOGY**

**NOVEMBER, 2022.**

**CERTIFICATION**

This is to certify that this project work was done by **OSAMUYI DESMOND EBOIGBE** with matriculation number **LSC1605243**, in the Department of Environmental Management and Toxicology, Faculty of Life Sciences under my supervision.

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**DR. B. J. ENAGBONMA**  
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**DR. C.F. AMAECHI**  
**(PROJECT COORDINATOR)**

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**PROF. A. A. ENUNEKU**  
**(HEAD OF DEPARTMENT)**

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**DATE**

**DECLARATION**

I OSAMUYI DESMOND EBOIGBE DECLARE THAT ISOLATION OF PATHOGENIC BACTERIAL FROM ANTHILL SOIL IS MY OWN WORK AND THAT ALL THE SOURCES THAT I HAVE USED OR QUOTED HAVE BEEN ACKNOWLEDGED BY MEANS OF COMPLETE REFERENCES AND THAT THIS WORK HAS NOT BEEN SUBMITTED BEFORE FOR ANY OTHER DEGREE AT ANY OTHER UNIVERSITY.

OSAMUYI DESMOND EBOIGBE

.....

DATE

## **DEDICATION**

This report is dedicated to God almighty for his endless grace, provision, supply and provision and also giving me the strength to complete this project work. This study is also dedicated to my family, Ella O. Eboigbe, Presley Eboigbe, Precious Eboigbe, my mum and dad Mrs Grace, Mr. Jacob Eboigbe, and my big brother Mr. Osamudiamen Eboigbe for their unwavering support. I will not forget to appreciate all my friends for their moral support and encouragement during my project work.

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## ABSTRACT

Anthill soils play a crucial role in sustainable agriculture practice as a source of locally available source of fertilizer which is essential in plant growth and development, and as such improve soil fertility. However, the pathogenicity of anthill soils have not been fully looked at. The aim of this study is to know the pathogenicity status of the antihill soil. Soil samples were collected from four different anthills from two locations (Institutional and residential areas in a sterile container) and their corresponding adjacent soils (control). The analysis carried out were bacterial count after aliquots of serially diluted soil were plated, cultural characteristics by culturing isolates, afterwards sub culturing. Colony count was done alongside phenotypic identification to know the (size, shape, elevation and margin). Morphological identification (Gram Staining) was done to differentiate, positive from negative. Biochemical and pathogenicity test were carried out using (protease and lipase) test. Bacteria such as *E. coli*, *Bacillus sp*, *Pseudomonas sp*, *serratia sp*, *Enterobacter cloacae*, *staphylococcus sp* and *Salmonella sp* were found. It is therefore recommended that for effective utilization of anthill soils for agricultural crop production, further studies should be carried to better understand the premise that anthill soil maybe dangerous rather than beneficial in the long run.

## CHAPTER ONE

### 1.0

### INTRODUCTION

Ants (Hymenoptera: Formicidae) are among the most successful dominant organisms on Earth (Senula *et al.*, 2019). Ants dominate almost every terrestrial ecosystem and possess diverse nesting and foraging ecologies and are rich in species, with over 15,000 species in 334 genera (Diamond *et al.*, 2012). Due to substantial increase in agricultural practices over the past years and the social / environmental importance to maintain sustainable agriculture, there has been a narrow focus on the influence of cultural values on soil biological diversities, the vital role soil ecosystems and its diversities play in maintaining ecological functions (Horlings and Marsden, 2011). Ants are successfully found in almost all terrestrial environment of the world, ranging from deserts to subarctic tundra. Approximately one-third of the world's animal biomass of the Amazonian terra firme rain forest is made of ants and termites according to rent report. 8 million ants are seen in each hectare of soil, in relation to one million termites. An endogenic exopterygotous insect is termite, and belongs to the order Isopteran, and it's one of the most abundant successful organisms that are found in the soil ecosystem (Sofa *et al.*, 2020). It's now a well known fact that anthill soils could play an imperative role as an alternative to inorganic fertilizer for farmers who do not have resources to procure chemical fertilizers. There is need to get acquainted with the vital role, which anthill soils play in sustainable agriculture as a locally available resource of organic fertilizers at the disposal of farmer, who also benefit monetarily, and the soil fertility will be improved as well. The distribution of anthills, composition and their impact on soil differ greatly depending on land use and vegetation (Menta, 2012).

(Tittonell *et al.*, 2013) indicates that soil infertility is one of the greatest biophysical problems to agricultural practices in sub-Saharan region of Africa and is accompanied with series of degradation processes which affects one another to produce a downward spiral in productivity and quality of the environment. For example, the resultant effects of tillage, inappropriate and insufficient applications of organic matter and nutrient, unarguably result in a decline in soil organic matter. The essential plant nutrients are affected as a result, breakdown of the physical structure of the soil, reduced water infiltration rate and storage capacity. Beyond this, most subsistence farmers battle with other degradation processes including acidification, erosion and salinization. The reduced level of soil fertility also depend on biological and physical degradation of soils and agronomy. There is a strong relationship between poverty and land degradation, national policies and institutional failures. (Rizk and Slimane, 2018). The degradation of the fertility of the soil is connected to anthropogenic (human) activities and environmental problems, of which malnutrition is the end product. In the opinion of (Chisanga *et al.*, 2019), they communicated and indicated that in southern Zambia most subsistent farmers, use anthill soil as organic fertilizer to improve the productivity of the crop to escape the challenges associated with soil fertility. It is well known that anthill soils can minimize the loss of nutrient and act as a manure that help to retain soil texture and moisture (Gachimbi *et al.*, 2005). The practice of anthill soil utilization include digging, heaping and spreading the soil on the field. A short account evidence in some parts of Zambia and Malawi have indicated that maize crop cultivated and fertilized with ant-hill soil was observed to be high strength and relatively gives a high crop yield. High costs associated with inorganic fertilizer could be one of the many reasons most farmers have adopted the use of anthill soil as an organic fertilizer in their agricultural production which could be far from their reach including the availability of soil

nutrients like nitrogen. (Chisanga *et al.*, 2019), found out that anthill soil is being collect by Africa farmers and apply to fields of crops as the resource could be rich in available soil nutrients such as nitrogen, phosphorous and organic carbon compared to adjacent soil. However, there is no enough information with regard to the quantities supply required per hectare to improve the productivity of the crop. The application of anthill soil in crop production by farmers has been reported by learned individuals in Zambia (Siame, 2005), Uganda (Okwakol and Sekamate, 2007), noted that farmers either apply anthill to specific crops or spread the soil from anthills in the fields. A typical example of agriculture production seening around anthill is the chitemene method of agriculture cited in southwestern Tanzania (Chisanga *et al.*, 2019). Some farmers in Malawi have been reported to plant various kind of crops which include bananas (*Musa spp.*) close to anthills. In Uganda, the case is quite seen differently as farmers plant tomatoes (*Solanum spp.*), onions (*Allium spp.*) pumpkins (*Cucurbita spp.*) and maize beside anthills (Okwakol and Sekamate, 2007). In Zimbabwe, okra (*Abelmoschus esculentus*), late-season planted maize, and pumpkins shows that they require good supply of water and nutrients, cultivated on anthills practically. In Niger, subsistence farmers prefer to cultivate sorghum on anthills soils than adjacent soils. In some region, farmers break anthill soil into pieces and spread them in the field. For instance, in southern Zambia, farmers remove part of the anthill soil and make sure that the essential components, the base and colony are not altered. This soil is then transferred to the field and mixed up with the top soil before it's rainy season. In regions where farmers practiced conservation farming, anthill soil is placed in planting basins or container (Siame, 2005) and in ripped lines. In South Africa, some patches of good well-cared sugarcane, known as "isiduli", are avialible sugarcane cultivated on sandy soils. These result in some anthills normally leveled by ploughing (Cadet *et al.*, 2004). Similarly, in Zimbabwe,

farmers are reported to use anthill soil to improve the fertility of the soil. Ants together with termites and earthworms, belong to the major groups called invertebrates which influence soil processes in land ecosystems (Culliney, 2013). Ants alters the physical and chemical parameters of the soil by bioturbation and accumulation of organic substances. Due to the building of underground galleries, material mixing and mounding, the soil from anthill is characterized by the strong formation of soil horizons, drainage, aeration and increased porosity, modified texture and reduced bulk density. Food storage is one of the major factors that contributes to the increase of soil organic matter content in the anthill nests, accumulation of faeces, aphid cultivation and ant remains contributes also (Dostál *et al.*, 2005). Although nest construction and food storage are the most vivid evidence for soil changes, an alternative theory suggests that the initial soil conditions could be the or reflect the differences in ant nest soil and adjacent soil during nest enactment. (Vasconcelos *et al.*, 2000). This hypothesis shows that fertilized queens initiate the construction of their nests selectively in patches with higher soil fertility. Till now, there is very little knowledge as regard the effect of soil properties on colony founding. Johnson (1998) tested the effect of the kind of soil and moisture on colony establishment of two species of *Pogonomyrmex* and noted a significant effect for both factors. However, in another study done by (Wagner *et al.*, 2004), in an analysis of mounds of various ages, there was no information that colonies of *Pogonomyrmex barbatus* prefer more fertile soils during soil enactment phase. Whatever the cause of soil modification maybe, ant influenced soil environment, affects the activity of abundance of other soil biodiversity, such as soil microbes (fungi, actinomycetes bacteria,) , soil insects and nematodes (Dauber *et al.*,2000). Ultimately, soil biotic and abiotic changes established by ants have effects on the performance and diversity of vascular plants (King, 1977; Carlson and Whitford, 1991; Dean *et al.*, 1997). The effect of ant influenced soil

modification on plant activities and vegetation path is documented properly. Ant nests are well known to improve germination, seed production, seedling survival, and plant growth. Plant species associated with anthills is usually different from species growing in surrounding areas. In spite of the broad knowledge regarding the effect of soil environment of ant nests on elevated ground plant performance, surprisingly little knowledge exists on its effect on plant below the ground parts ( Blomquist *et al.*, 2000). Typically, lack of ant disturbance, distribution and parameters of roots and rhizomes in grasslands affects vertical gradient of soil nutrients, soil water and bulk density; rhizome and root biomass decline with depth (Dostál *et al.*, 2005). The presence of ants, especially those that are building clear mounds, can influence substantially the biomass and structure of roots and rhizomes via changes in the soil surrounding. Roots and rhizomes are plastics morphologically in relation to abiotic components of the soil. They may respond by proliferation, changed nutrient uptake or structural modification to affect the soil physical parameters (pore, compaction, structure) and distribution of nutrients and water. There exists various ant species that enact clear mounds in European grasslands such as *Lasius flavus*, *Lasius niger*, and various species of genus *Tetramorium caespitum*. (Kovář *et al.*, 2001). However, *L. flavus* is the most ecologically successful ant species that influence the character of the grasslands by establishing prominent and numerous soil nests. Colonies of this said ant species may fill up to about 2500 mounds ha<sup>-1</sup>. Soil nests of this species are permanent structures maintained by long lasting soil transport to the surface (Jacubczyk *et al.*, 1972). The effect of *L. flavus* on vegetation paths has been demonstrated repeatedly (Dean *et al.*, 1977; Kovář *et al.*, 2001). The practices of farmer by using anthill soil as fertilizer in agriculture have been reported, and scientific evidence are well much available for most of them (Watson, 1977; Nyamapfene, 1986). For example, some studies have shown that sugar cane produce is five times

greater with the application of “isiduli” in the field somewhere (Cadet *et al.*, 2004). Similarly, grass growth and plant biomass have been reported to be outrageously greater around anthills in relation to the open surrounding found in Eastern Cape of south Africa. Research have shown that increase in grass growth surrounding anthill is attributed to the accumulation of surface runoff at the bottom resulting in increased yield in dry seasons, giving plants greater opportunity to survive worst environmental conditions such as drought (Steinke and Nell, 1989). Researchers have worked on also the mineral composition of anthills and the surrounding soils (Watson, 1977; Steinke and Nell, 1989; Cadet *et al.*, 2004; Masanori and Tooru, 2004; Brossard *et al.*, 2007; Chikuvire *et al.*, 2007). There is however, not enough information on the characteristics of specific and suitable anthill soils being used in agricultural productivity. Most studies have also shown that anthills consist of significantly greater concentrations of exchangeable cations and total nitrogen (N) than the adjacent soils (Watson, 1977; Steinke and Nell, 1989; Jones, 1990; Holt and Lepage, 2000; Chikuvire *et al.*, 2007). In tropical wet–dry climates, downleveled erosion is seen to improve soil fertility more around anthill soils than the leached soils far from it (Malaisse, 1978). In addition, soil from anthills has several good effects on crops which include suppression of weed. For example, *Cubitermes* soil was found to suppress the weed, *Striga* infection on sorghum crops in West African nation of Burkina Faso (Andrianjaka *et al.*, 2007).

## **1.1 Aim and Objective**

The aims of this study was to determine the pathogenicity of isolates gotten from ant hill soil

The specific objectives were to:

- Isolate and enumerate the bacteria present in anthill soil from different locations.

- Identify bacteria isolates using cultural, morphological and biochemical characteristics.
- Evaluate the pathogenic bacteria present in anthill soil.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Formation of Anthills

In the opinion of a number of experts, termites and other soil fauna perform vital functions in the formation of anthills (Chisanga *et al.*, 2007). During the building of anthills, woody debris for the nests and vast amounts of honeydew and insects are gathered to serve as food in the colony. Studies have shown that active anthills contain larger supplies of organic matter and inorganic nutrients including calcium, potassium, magnesium, sodium and phosphorus, than the adjacent soils (Chisanga *et al.*, 2007). These ants are effective in the transformations of the following:

- (i) Physical characteristics of the soil including porosity and infiltration.
- (ii) Faunal biomass and the communities of soil microorganisms.
- (iii) Organic matter decomposition rates.

Ground-dwelling ants' soil activities are visible during anthill development. Ants' construction efforts transform the soil into nutrient-rich pockets that promote seed emergence. Changes in soil physical-chemical qualities have been connected by scientists to anthill formation, while others have assessed the linkages between these activities and the distribution patterns of some plants

(Dean *et al.*, 1997) and vegetation succession (King, 1977). Few researchers have linked soil enrichment to plant growth. As a result, there is a higher need to take suitable activities to describe anthills created in different locations if they are to be employed in soil fertility programmes, because anthill nutrient content may be connected to an area's locale.

## **2.2 Opportunities and Constraints of Utilizing Anthill soils in Crop Production**

Several researchers have reported the benefits of employing anthill soil as a crop supplement. Mavehangama and Mapanda (2012), for example, investigated the nutrient status of organic soil amendments from selected wards of Zimbabwe's Chivi district and discovered that the use of organic amendments such as anthill soil was a common practice with the goal of improving soil productivity in Zimbabwe's communal farmlands. These scientists also discovered that the disparities in the nutrient supply capacity of different types of animal dung and soil amendments, including anthill soil, have not been well explored. According to (Mavehangama and Mapanda, 2012), these variances will alter the optimal quantities of each form of amendment that may be required to attain a desired crop yield. (Nyamangara and Nyagumbo, 2011) investigated the interactive effects of selected nutrient resources and tied-ridging on plant growth performance in a semi-arid, smallholder farming environment in central Zimbabwe and discovered that anthill soil and leaf litter are worthwhile investments for financially constrained farmers because they can improve the chemical and possibly physical properties of the soil.

They also discovered that organic fertilizers, such as anthill soil, buffer soils from acidification better than mineral fertilizers, and proposed that farmers who use it would benefit from the potential hydrogen, (pH) moderation effect, which would ensure the availability of nutrients such

as phosphorous, which is normally locked up in acidic soils. In northern Malawi, instead of planting crops directly on the anthills, some farmers decided to take the soil from the anthill and mix it with goat manure in a 1: 1 ratio before applying it, thereby improving the plant nutrient content properties of the anthill soil and reducing the amount of manure required. This invention, which offers a low-cost alternative to inorganic fertilizers, was assessed to have high potential for scaling up to other resource-poor farmers. Furthermore, considerable yields were reported without scientific investigation. This suggests a possible low-input strategy to mitigate the impact of high inorganic fertilizer prices. Despite persuasive literature on the nutritional content of anthill soils, nothing has been recorded and explored to determine the utilization of anthill soil as a source of nutrients for maize cultivation. In light of this, appropriate rates of anthill soil application must be established as part of the integrated soil fertility management (ISFM) component of sustainable agriculture. According to research conducted by ZARI (2014) in Zambia, anthill soil may provide yields greater than 1000kg/ha when properly put in agricultural fields. However, more research is needed on soil management strategies, treatment rates, and crop response in medium to high rainfall scenarios. However, because to restrictions, some farmers do not level anthills, despite scientists' belief that anthill soil may provide an alternative to inorganic fertilizers (Logan *et al.*, 2002). Scientists have also discussed why farmers do not favour levelling anthills in order to maximize land utilization and allow mechanical tillage operations. Such difficulties have been observed to overlook spiritual and economic significance. The experts' advice also ignores the reality that levelling anthills may not be viable in the long run. It is also said that the challenge associated with the usage of anthill soil in agricultural production has frequently revolved on how to obtain the appropriate quantities necessary to meet the nutritional demands of crops. Transportation and handling charges are typically beyond the

farmer's ability. According to (Wood, 1988), the anthill production rates are too low to be used for yearly seasonal crop production and by commercial farmers. Understanding the restrictions of using Anthill soils in crop production may allow scientists to identify solutions and new means of inducing quicker anthill formation.

### **2.2.1 Characteristics of a Suitable Anthill for Crop Production**

#### **2.2.2 Chemical Properties**

Soil chemical qualities include pH, salinity, organic matter, phosphorus concentrations, cation-exchange capacity, nutrient cycling, and concentrations of possible pollutants such as heavy metals, radioactive chemicals, and others, as well as those essential for plant growth and development. The chemical state of soil determines soil-plant relationships, water quality, buffering capacity, nutrient and water availability to plants and other organisms, contaminant mobility, and other physical characteristics such as crusting (Kheyrodin, 2014). Eneji *et al.* (2015) conducted a comparison of the chemical properties of anthill soil and surrounding soils at the University of Agriculture in Makurdi, Nigeria, and discovered that differences between the chemical properties of the anthill and surrounding soils were caused by ecosystem services provided by termites, including bioturbation and soil formation, nutrient transportation and cycling, litter decomposition, soil animal and microbial diversity, among others. A similar study (Joseph *et al.*, 2002) evaluated termite mounds as islands, evaluating woody plant assemblages relative to termitarium size and soil properties, and discovered that anthills are habitat of high socioeconomic importance. The termitaria, which are richer in minerals like Ca, Mg, K, and Na and also the accumulation of all these bases increase the pH, are also found to be more important habitats. When comparing the characteristics of anthill soil to those of natural grassland soil, other studies by (Kaschuk *et al.* 2006) revealed that soil samples taken from the top, middle, and

bottom of the anthills as well as from nearby areas had higher concentrations of K, P, Mg, O, and C and lower pH values. In Nigerian anthill soil samples, Fe<sub>2</sub>O<sub>3</sub>, SiO<sub>3</sub>, K<sub>2</sub>O, CaO, Al<sub>2</sub>O<sub>3</sub>, and Mg were measured by Ekakitie and Osakwe (2014). They discovered varying oxide concentrations that were caused by parent materials in the soil, nearby vegetation, fertilizer usage, and bush fire. The oxides have a crucial role in generating the solid form and water resistance. Additionally, according to the literature, anthill soils are rich in calcium, phosphorus, and organic matter, all of which are essential for optimal crop development. Additionally, plants absorb nutrients from anthill soil very quickly. Local farmers who cannot afford to buy pricey inorganic fertilizers are finding this soil to be an excellent alternative. The density of the anthill soil is extremely low, but it is still possible to collect it, crush it, and combine it with top soil for subsistence farming (Dhembare, 2013). It was also discovered that the pH and the contents of organic carbon, nitrogen, phosphorus, calcium, and magnesium were significantly higher in anthill soils than in nearby areas, with an inverse trend for Al content. Along the slopes, there were noticeable changes in the exchangeable Al and pH between the soil and anthill. However, it has been noted that there are few research on the chemical features of anthill soils, and most of these studies have concentrated on macronutrients, with little information being provided on the nutritional levels of micronutrients like Fe, Mn, Zn, and Cu, among others. As such, the chemical characterization of anthills becomes very essential.

### **2.2.3 Physical Properties**

The arrangement of solid particles and pores influences the physical characteristics of the soil. Examples could include the thickness of the topsoil, the bulk density, porosity, aggregate stability, texture, crusting, and compaction. Basically, they are signs of restrictions on root development, seedling emergence, infiltration, or water transport along the soil profile

(Kheyrodin, 2014). Due to their habit of burrowing and their capacity to alter physical characteristics, such as infiltration, water retention capability, etc., of their anthills, ants play a significant role in determining the physical soil properties of anthills during construction, according to Cammeraat *et al.* (2002) and Dashtban *et al.* (2009). In agricultural soils where heavy equipment and pesticide usage are believed to impair soil porosity and organic matter, the effect of ants on water infiltration and erosion is essential (Cerdeira and Jurgensen, 2008). According to the findings of aggregate fractioning, Schaefer (2001) noted that a larger portion of anthill walls are made up of large aggregates that are cemented by termite body fluids (fraction N 2.00mm), which are quickly broken down into smaller particles, increasing the aggregate fractions to less than 0.500mm. The majority of the stable micro-aggregates in latosols are made up of them. The integration of organic matter, which is only present at the surface and has diminishing values with depth (B horizon), where tiny micro-aggregates may be found, results in the formation of larger organo-mineral aggregates in the upper slope and hill top. This shows that newly formed anthill materials are constituted of welded aggregates, which result in bigger cemented clods (N 1.00 mm). Following the abandonment of anthills, these are further destroyed by weathering and erosion processes. Without heavily relying on statistical evidence, micro morphological observations substantially confirm this concept, and when examined at the microscopic level, thin portions of anthill wall and nearby soils clearly demonstrate tiny aggregates that are only partly held together. Since mica is essentially nonexistent on the surface of Latosols, it is recognized as being randomly disseminated inside the clay plasma of the bigger aggregates, indicating a deep turnover of soil material in the anthills.

#### **2.2.4 Biological Community in Anthill Soils Bacteria, Fauna Biomass and other Microbiota Organisms**

More and more, parasites are understood to be vital components of ecosystems and societies. Understanding the ecological interactions between parasites and their hosts is necessary to comprehend the drivers of parasite abundance and community composition, as well as their cascade effects (Hatcher *et al.*, 2012). We lack a comprehensive review of ant communities as hosts to a wide variety of parasites, despite the fact that the parasites of many other social species (such as humans, lions, and wolves) have been thoroughly investigated. Whether the ants, one of the ecologically effective groups of creatures on Earth, represent lacunae of parasite biodiversity is a crucial subject. The variety of parasites infecting ants is likely to be great, according to extensive taxonomy records and natural history notes (Russell *et al.*, 2012), but there hasn't been a thorough synthetic analysis. As a result, the goal of this work is to describe the known records of parasitic organisms infecting ants for both their taxonomic and functional biodiversity, as well as to evaluate the biology and life histories of these creatures. Over the past few decades, there have been more reports of bacteria linked to ants because better technologies have made it possible to identify bacterium species that were formerly either unculturable or recognizable in the lab. The presence of bacterial infections and their companions within ant colonies has been the subject of several surveys (Russell *et al.*, 2012), frequently in an effort to find possible biocontrol agents. In their study of the bacterial complex associated with various species of ants, the soil they live in, and their anthills, Kotova *et al.* (2013) discovered that *Bacillus* dominated the majority of anthills—more than 80%—while the *Formica* anthill was characterised by the *Flavobacterium*—*Bacteroides*—*Cytophaga* group. Actinomycetes were also discovered to be common in *Formica* sp. and *Lasius* sp. anthills. However, *Streptomyces* bacteria (68.5%) were the predominant dominants of the bacterial community, whereas numerous *Bacteroides* (28%) were detected in the anthills of *Tetramorium*. Numerous staphylococci (20%) were also found in the *L.*

*flavus* anthills. Except for *F. cunicularia*, all the analysed ants' bacterial complexes contained actinomycetes from the genus *Streptomyces*. The many bacteria, actinomycetes, and fungi in anthills, according to Pokarzhevskij (1981), cause many minute soil invertebrates to emerge, including springtails. Ants play an important role in maintaining a stable microclimate in these anthills, which has a significant impact on the unique structure of a microarthropod community (Horstman and Schmid, 1986). Anthill formation and expansion affect the diversity and quantity of springtails. Large, ancient domes with generally consistent humidity tend to have springtail populations that are substantially higher than those of the nearby soil and litter.

### **2.3 Soil enzymes in Anthill soils**

In the soil system, soil enzymes perform crucial biochemical tasks for the breakdown of organic materials. They play a key role as catalysts in a number of crucial chemical processes required for the survival of soil microbes. They also help stabilize soil structure, the breakdown of organic waste, the production of organic matter, and the cycling of nutrients. In the soil, enzymes are constantly being synthesized, stored, inactivated, and/or degraded, contributing significantly to agriculture and primarily to nutrients. In order to ensure enzyme immobilization and stability, activities of enzymes in soils pass through intricate biochemical processes that are coordinated with one another and with ecological processes. Any soil type, in this regard, has a variety of enzymes that affect soil metabolic processes, which depend on physical, chemical, microbiological, and biochemical features. Due to the varied amounts of organic matter, types of living organisms, and rates at which biological processes take place in each kind of soil, the enzyme levels in soil systems vary. The catalytic contribution of enzymes and various substrates, which act as microorganisms' energy sources, results in the biochemical processes in practice.

### **2.3.1 Nutrient dynamics in Anthill soils**

The impacts of earthworms on soil formation were the subject of some of Darwin's early studies, although pedologists and geomorphologists have paid considerably less attention to the chemical, physical, and mineralogical characteristics of soil than to the soil fauna. The habitat in which they reside is altered by many soil organisms, though, via physical and biotic conditioning, both in absolute terms and relative to the availability of nutrients. According to Dokuchaev, who made this observation in the early days of pedology, "soil animals were not only soil residents, but played a fundamental part in most soil responses." About 3000 species of termites (Isoptera), which are social insects, are now recognized, with an estimated 75% of those species being categorized as soil-feeding termites. Termites that feed on dirt do not have any cellular organic matter that has been combined with clay minerals. Five compartments make up their stomach, and each one exhibits a varied pH range (up to 12.5) as well as a variable level of oxygen and hydrogen (Brune *et al.*, 1995; Brune and Kühl, 1996; Donovan *et al.*, 2001). These characteristics are unquestionably significant and may be correctly referred to as nutrients that contribute to anthill soil chemical and physical modifications. Additionally, compared to nearby soils, anthills are found to have greater pH and microbial populations (Black and Okwakol, 1997). Erosion redistributes the collected material, which has an impact on the fertility and microstructure of the soil (Jungerius *et al.*, 1999; Schaefer, 2001). Additionally, termites build galleries that promote soil permeability and water penetration, and these galleries are then filled with topsoil components. The creation of deep, homogenous latosols is aided by rainfall. It is unknown whether termites pick particles or the soil physically fractionates in their bellies, but the composition of clay in anthills is typically 20% greater than that of the surrounding soils, according to Jouquet *et al* (2002). It is also accurate to say that clay minerals change when they

are treated in the mouth or as they go through the digestive system. In this context, Schaefer (2001) noted that high pH levels caused kaolinite to become less crystalline after travelling through termite stomachs. Although termite involvement in aggregate stabilization, organo-mineral microaggregation, increased porosity, organic matter turnover, nutrient concentration, particle size sorting, anthill soil transportation, and erosion effects are discussed in the literature, there is still a paucity of knowledge regarding pedogenesis, landscape evolution, and nutrient dynamics in anthill-based agricultural systems.

### **2.3.2 Water Productivity and Soil moisture retention in Anthill soils**

According to Ali and Talukder (2008), water productivity (WP), which is represented as crop production per unit volume of water, is used to characterize the link between the crops produced and the amount of water utilized in crop production. Crop production can be represented as total dry matter yield, seed (or grain) yield (kg), or, when discussing several crops, yield can be converted to monetary units (e.g USD or any other legal tender in a given situation). The amount of water may be defined in several ways. One of the main elements influencing water productivity in agricultural production is soil moisture retention. Evaporation of water from the soil surface promotes plant development during germination and seedling establishment, as well as other growing stages. The water storage and release qualities of soil are determined by its texture and organic matter concentration. When the soil dries quickly, the osmosis process is disrupted, reducing yield and water output. The nutritional state of forthcoming crops, particularly nitrogen, can have a considerable impact on the rate of growth of leaf area, resulting in soil evaporation losses. Organic matter in the soil environment is subjected to chemical reactions involving microbial activity and nutrients. There is limited information available on the water productivity and soil retention of anthill soils. Nevertheless, other research shows that

anthill soils typically include a lot of clay, which improves their ability to store water. When anthill dirt is applied to common soil types with limited water retention capacity, the soil's moisture content increases and crop development is increased. This suggests that anthill soils may produce a lot of water. To demonstrate the efficacy and efficiency under agricultural production settings, however, more study is required.

### **2.3.3 The potential of Anthill Soils in Integrated Soil Fertility Management (ISFM)**

Integrated soil fertility management (ISFM) is described by Place *et al.* (2003) as a collection of best agricultural techniques utilized in conjunction with best cultural practices, such as the use of suitable germplasm, fertilizer, and organic resources (BAPs). Small-holder farmers in Sub-Saharan Africa (SSA) and development organizations appear to be warming up to this element. By raising farmers' understanding of the range of possible alternatives and how they could complement or replace one another, ISFM implies expanding the range of options open to them. According to Vanlauwe *et al.* (2015), ISFM can be a channel for increasing crop yield while optimizing the agronomic efficiency (AE) of applied inputs, hence assisting in sustainable intensification. The ISFM among smallholder farms takes into account the degree of variability in soil fertility conditions and the soil difficulties that go beyond those solved by fertilizer and organic inputs, such as anthill soils. The many biophysical conditions that are typical of smallholder farming systems have an impact on crop productivity and the AE. In this context, it is essential for improving AE to apply inputs, including management approaches, strategically. Furthermore, the capacity and output goals of the farmer directly influence management decisions. In SSA, restoring soil fertility is viewed as being crucial to helping with efforts to combat poverty. Because most of Africa's poor live in rural areas, where there is a reported decrease in per capita arable land from 0.53 to 0.35 hectares throughout the period, soil fertility

is essential. It is necessary to intensify agriculture quickly and sustainably. Investment in soil fertility is necessary for intensification, greater agricultural production, and improved rural lives. Numerous limitations are present in African soils, including physical soil loss due to erosive forces, nutritional shortages, low levels of organic matter, aluminium and iron toxicity, acidity, crusting, and moisture stress. Some of these limitations are present in tropical soils naturally, but degradation processes connected to land management make them worse. Two-thirds of agricultural land is thought to be deteriorated, with wind and water erosion accounting for 85% of this degradation. Soil nutrient deficit is made worse by smallholder farmers' infrequent use of fertilizer inputs. The average amount of fertilizer used in Africa in the late 1990s was 9 kg per hectare, and it appears that little has changed since then (Henao and Baanante, 2001). In Western and Eastern Africa, it is estimated that each year, approximately 60-100 kg of N, P, and K per acre are lost to erosion, leaching, and agricultural harvests (e.g. Stoorvogel and Smaling, 1990; De Jager *et al.*, 1998). The secret may lie in encouraging the use of locally accessible organic resources like anthill soils for boosting soil fertility as a substitute for cash-strapped farmers who are unable to purchase inorganic fertilizer. Less is known, though, about the rates at which Anthill soils should be applied, alone or in conjunction with other soil amendments, for the best crop production. This necessitates study into the best ways to combine organic and inorganic fertilizers with Anthill soil in order to create an effective Integrated Soil Fertility Management programme for use by small-scale farmers in areas with these resources.

#### **2.3.4 Effect of Anthill Soils on Plant Growth**

The ISFM approach recognizes the necessity for both organic inputs (such as anthill soils and bovine manure) and mineral inputs for crop development as they interact and complement one another (Buresh *et al.*, 1997; Vanlauwe *et al.*, 2002) and speed up plant growth. Smallholder

farmers most frequently use cow dung, compost, crop residue integration, fallowing (natural and improved), intercropping of legumes, and biomass transfer as organic soil nutrients. Although we are focusing on soil nutrient management measures, additional management activities that contribute to soil fertility include soil conservation and tillage techniques, weed control, and cropping options. The conventional wisdom was that organic resources are providers of important soil nutrients such as nitrogen (N). According to Palm *et al.* (2001), several academics have conducted study on estimating the availability of N from organic resources as impacted by resource quality and the physical environment. Research has more recently focused on the benefits of organic matter that go beyond fertilizer replacement, such as the provision of additional macro- and micronutrients, a decrease in phosphorus sorption capacity, an increase in carbon/organic matter, a reduction in soil-borne pests and diseases through crop rotations, and an improvement in soil moisture status (Vanlauwe *et al.*, 2002). The ways that the organic processes contribute to soil fertility varies significantly in certain important ways. Nitrogen-fixing plants are added to the soil through agricultural operations without diminishing the soil's nutrients. Different organic sources will have different nutritional contents and methods for making the organic compounds available to the crop, as well as different advantages for soil fertility (e.g. weed reduction). Organics' efficacy is also determined by agronomic methods. Other organics, such as anthill soils, which are present at the farm level, might potentially have a significant role in increasing crop output due to their increased Nitrogen content, which is essential in plant development. It is recognized, however, that organic and mineral inputs cannot be completely substituted for one another and are both necessary for sustained crop production due, in part, to

1. Reasons for utilizing fertilizers or organic resources alone may not be adequate or appropriate for addressing certain crop development restrictions.

2. In the short term, the possibility for greater benefits provided by beneficial interactions between organic and inorganic inputs.
3. The many functions that each of these inputs play in the long run. When they are utilized in tandem, they assist to cut crop production expenses.

One significant area of complementarity is the enhancement of organic matter status and the services it supports by organic resources, such as anthill soil, while limiting nutrients may be targeted by mineral inputs. An effort has been made to quantify the amount accrued as well as the mechanisms in charge of producing them. According to Vanlauwe *et al.* (2002), there are obvious connections between the usage of urea and organic treatments such agricultural leftovers. Although the list of significant interactions between organic and mineral inputs shown above is not complete, it is frequently shown that these inputs only have additive impact. However, the additive effects are frequently preferable in terms of total yields and net financial return due to falling marginal gains from a single type of input. There are never any negative interactions seen. In a nutshell, we point out that there is a tonne of research demonstrating how organic matter alone may significantly increase crop yields in agriculture. The good short- and long-term effects of ISFM technologies combining organic and mineral nutrition sources are supported by scant but considerable evidence. We need more economic studies of these systems and proof from farmer-managed practices. It is vital to highlight that cereal crops have been the focus of the majority of agronomic research on ISFM. However, a large portion of the organic and inorganic fertilizer use by smallholder farmers is concentrated on higher-value crops, for which the benefits of organics like anthill soil and ISFM are still not well understood. Smallholder farmers' on-farm Integrated Soil Fertility Management (ISFM) techniques According to several researchers, many smallholder farmers in Africa employ a variety of ISFM

techniques, including intercropping legumes (cowpeas, soybeans, beans, groundnuts, etc.) and using cattle manure, which are tried-and-true methods. In Nairobi's semiarid and semi humid regions, farmers used manure in amounts ranging from 86% to 91%, respectively. However, just 40% of farmers employed compost, and those that did so were few, especially in the drier regions. According to reports, 70% of farmers in the extremely humid western highlands employed manure, 41% used compost, and 20% used biomass to increase tree fallow. In Zimbabwe, rotations incorporating legumes and green manure systems were widespread in 48 and 23 percent of extension sites, respectively (Gambara *et al.*, 2002). Alley farming methods have been found to be more prevalent in regions of Nigeria (Adesina and Chinau, 2002), as have *Mucuna* fallows in Benin and Cameroon (Manyong and Houndekon, 2000). Despite differing adoption rates of organic and mineral nutrients by area, the usage of organic methods such as natural fallowing and animal manure has always been greater than the use of artificial fertilizers. In Rwanda, just 2% of plots received mineral fertilizer, which was concerning. Less data is available on the quantity of organic nutrients used, however it is well known that smallholder farmers frequently struggle with rising opportunity costs, leading to a sparse production and application of organic nutrients.

#### **2.4 Economic Benefits of Anthill soils**

In their study of the indigenous use of anthill soils and their sustainability in a village that grows rice in the central plain of Laos, Miyagawa *et al.* (2011) found that if the resource is abundant, it can be used as a fertilizer for rice growing to increase rice yield without having to purchase chemical fertilizer. The academics also noted that no farmer offered or sold dirt from their own property that was used to build anthills. In terms of the local communities' farming practises, it was primarily intended for self-sufficiency. Furthermore, it was found that anthills were utilized

for the construction of charcoal kilns and as a bed for the growth of flora, in addition to being used as a soil amendment and fertilizer. There is presently little information available on the economic advantages of using anthill soil for crop production, despite the fact that this depends on the anthill's construction.

## **CHAPTER THREE**

### **3.0**

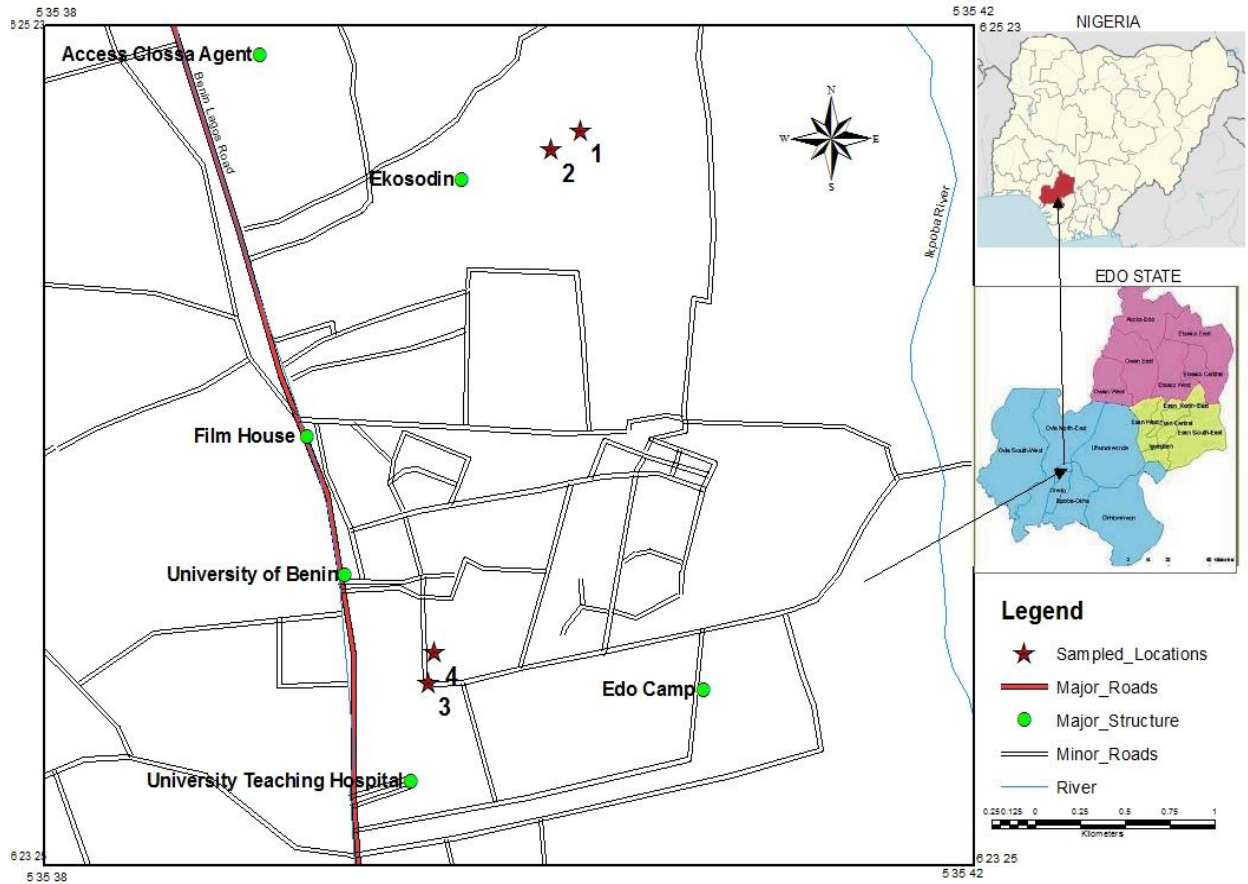
### **Materials and Methods**

#### **3.1 Study Area**

This present study was undertaken in Benin City, the capital of Edo State which is situated in the southern part of Nigeria. The geographical location of the city is at latitudes 6° 11' and 6° 29'N, and longitudes 5° 33' and 5° 47'E. It is located in the humid tropical rainforest belt of Nigeria.

#### **3.2 Collection of samples**

Anthill soil Samples of 50g were collected from 0-15 cm depth (that is digging to 0-15cm to the ground where the activities of the ants had effect) using 5cm in diameter split tube of soil auger from the anthill (10 cm apart) in duplicates from Residential 1A and 1B (Control) and Institutional 2A and 2B (Control). The format of sample collected were (i) from the anthill point (ii) from adjacent position of the anthill in University of Benin for institutional and in Ekosodin for residential both in Benin City, Edo State Nigeria. The samples were preserved in ice bottle at 6 degree celcius and transported aseptically the following day to Microbiology Laboratory, university of Benin, Benin city for microbiological analysis.



**FIG.1: MAP OF STUDY AREA SHOWING THE SAMPLED IOICATIONS**

6 23 25

**Figure 3.1: Map of the study area indicating the sampling locations**



**Plate 3.1: Residential anthill soils**



**Plate 3.2: Institutional anthill soil**

### **3.3 Enumeration and Isolation of Total Heterotrophic Bacterial Count**

Ten-fold serial dilution of the samples were prepared aseptically in sterile physiological saline. An aliquot of 0.1 ml was inoculated using the pour plating technique. Appropriate media were used for fungal and bacterial enumeration. Tryptone soy agar (supplemented with fluconazole) for bacteria and potato dextrose agar (supplemented with chloramphenicol) for fungi. Plates were cultured at  $28\pm 2^{\circ}\text{C}$  for 24 hours. The number of colony forming unit per milliliter (cfu/ml) was calculated according to standard (Willey *et al.*, 2008).

### **3.4 Phenotypic Identification of Bacteria from Samples**

Following successful pour plate technique, isolation and culture was made from a single colony and characterized using cultural, morphological and biochemical methods using the Bergey's manual. Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, citrate utilization, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Holt *et al.*, 1994).

#### **3.4.1 Morphology Identification**

##### **3.4.1.1 Gram Staining**

Methods described in Cheeseborough (2005) were employed in gram staining. This test was done to confirm the cell type of the bacterial isolates. Gram staining techniques was used for differentiation between Gram- positive and Gram- negative bacteria. Organisms that retain the primary stain are called Gram positive (+) while those that do not retain the primary stain when decolourized are called Gram negative (-). The non- retention of the stain is due to the cell composition. Gram stain reagents used include; crystal violet (primary stain), iodine (as mordant), alcohol (decolourizer) and safranin (counter stain). Procedure: A smear of the

organism to be Gram stained was made on a sterile slide using an inoculating wire loop. The slide was air dried and heat fixed by passing it gently over the flame. The smear was stained with 1% violet for minute and washed with distilled water. Gram iodine was added as a mordant for one minute, after which alcohol was added for 30 seconds. The slide was then rinsed with distilled water. The slide was finally flooded with counter stain, safranin for one minute and then washed off with distilled water and then air dried. The preparation was observed under the microscope with an oil immersion and their morphology [shape], arrangement and gram result was obtained.

### **3.4.2 Biochemical Identification**

#### **3.4.2.1 Oxidase Test**

The oxidase test was carried out according to Cheeseborough (2005). This is mainly used to differentiate between pseudomonas from other gram- negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochrome oxidase enzyme. Staphylococcus aureus and Escherichia coli which are gram positive and gram negative respectively were employed as control. A piece of filter paper using sterilizes wire loop 2- 3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl- 3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds.

#### **3.4.2.2 Urease Test.**

Urease was done as described by Cheeseborough (2005). This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like Proteus mirabilis from other

non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 degree centigrade for 24 hours. A change in colour from yellow to red- pink confirmed the presence of urease.

### **3.4.2.3 Indole Production Test**

The indole production test was carried out according to Cheeseborough (2005). This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The best is usually used in differentiating gram- negative bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degree centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacteria isolates was inoculated into the peptone broth. The inoculated media was incubated 37 degree centigrade for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150ml of amylalcohol, 10g dimethylamino benzaldehyde and 150ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

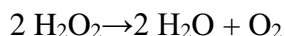
### **3.4.2.4 Citrate Utilization Test**

The citrate utilisation test was carried out as described in Cheeseborough (2005). This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is simon`s citrate agar. In the preparation, 22 grams of commercially available simon`s citrate agar was dissolved in litre of distilled water and sterilized by autoclaving at 121 degree centigrade for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stablign the medium on the tubes using sterile

straight inoculation wire containing culture. The tubes were incubated at 37 degree centigrade for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration.

#### **3.4.2.5 Catalase Test**

The catalase test was carried out according to Cheeseborough (2005). This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.



#### **3.4.2.6 Sugar Fermentation and Production of Gases using Triple Sugar Iron Agar (TSI)**

The tests for sugar fermentation and the production of gases were carried out according to Cheeseborough (2005). TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hr. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chart was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

#### **3.4.2.7 Pathogenicity Test (Protease Test)**

The protease test was carried out following methods described by Cheeseborough (2005). The extracellular protease activity of the isolates will be assayed on TSA plates supplemented with 1% casein (v/v). Colonies grown on tryptone soy broth (TSB) agar were suspended in 3mL of Mueller Hinton broth. The density of this suspension was adjusted to 0.5 McFarland standard, which is the equivalent of  $1.5 \times 10^8$  cells/mL. 1 ml sample of this suspension was inoculated on TSA plates supplemented with 1% casein and incubated at 37°C for 24 to 48h. Zone of clearance due to casein hydrolysis was considered a positive result while no clearance was considered a negative result.

#### **3.4.2.8 Lipase Test**

Lipase test was done as described by Cheeseborough (2005). The lipase activity of the isolates was assayed on tryptone soy agar (TSA) plates supplemented with 1% Tween 80 (v/v). Colonies grown on tryptone soy broth (TSB) agar were suspended in 3mL of Mueller Hinton broth. The density of this suspension was adjusted to 0.5 McFarland standards, which is the equivalent of  $1.5 \times 10^8$  cells/mL. 1ml sample of this suspension was inoculated on tryptic soya agar (TSA) and incubated at 37°C for 24 to 48h.

### **3.5 Statistical Analysis**

The data were analysed using the SPSS package version 21.0. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined.

## CHAPTER FOUR

### 4.0

### RESULT

This study shows the analysis of anthill soil for its various biological activities. however, some bacteria were found to inhabit the anthill soil in residential sample (Ekosodin community), institutional (Ugbowo campus, University of Benin). Some of these bacteria include *E. coli*, *Bacillus sp*, *Pseudomonas sp*, *serratia sp*, *Enterobacter cloacae*, *staphylococcus sp* and *Salmonella sp*. Sampling was carried out in residential area of anthill soil and in adjacent likewise in institutional area. The result obtained shows a slight increase in bacteria load from the anthill soil than in adjacent, for instance from the result of the residential and institutional samples, it was observed that the bacteria CFU/ml count was recorded higher in anthill soil compared to that of adjacent soil with ( $\log_{10}$  cfu/g)  $7.57\pm 0.05$  and  $6.53\pm 0.18$  for residential and  $7.25\pm 0.01$  to  $6.45\pm 0.08$  for institutional samples. From the result it was also observed that the bacteria CFU/ml count for main residential soil was higher than that of the institutional and The residential adjacent was higher than institutional adjacent. The virulence factor of these isolates showed that samples isolated from this study using various test appropriate, Dnase, Haemolysin and Gelatinase were pathogenic and had the ability to cause disease.

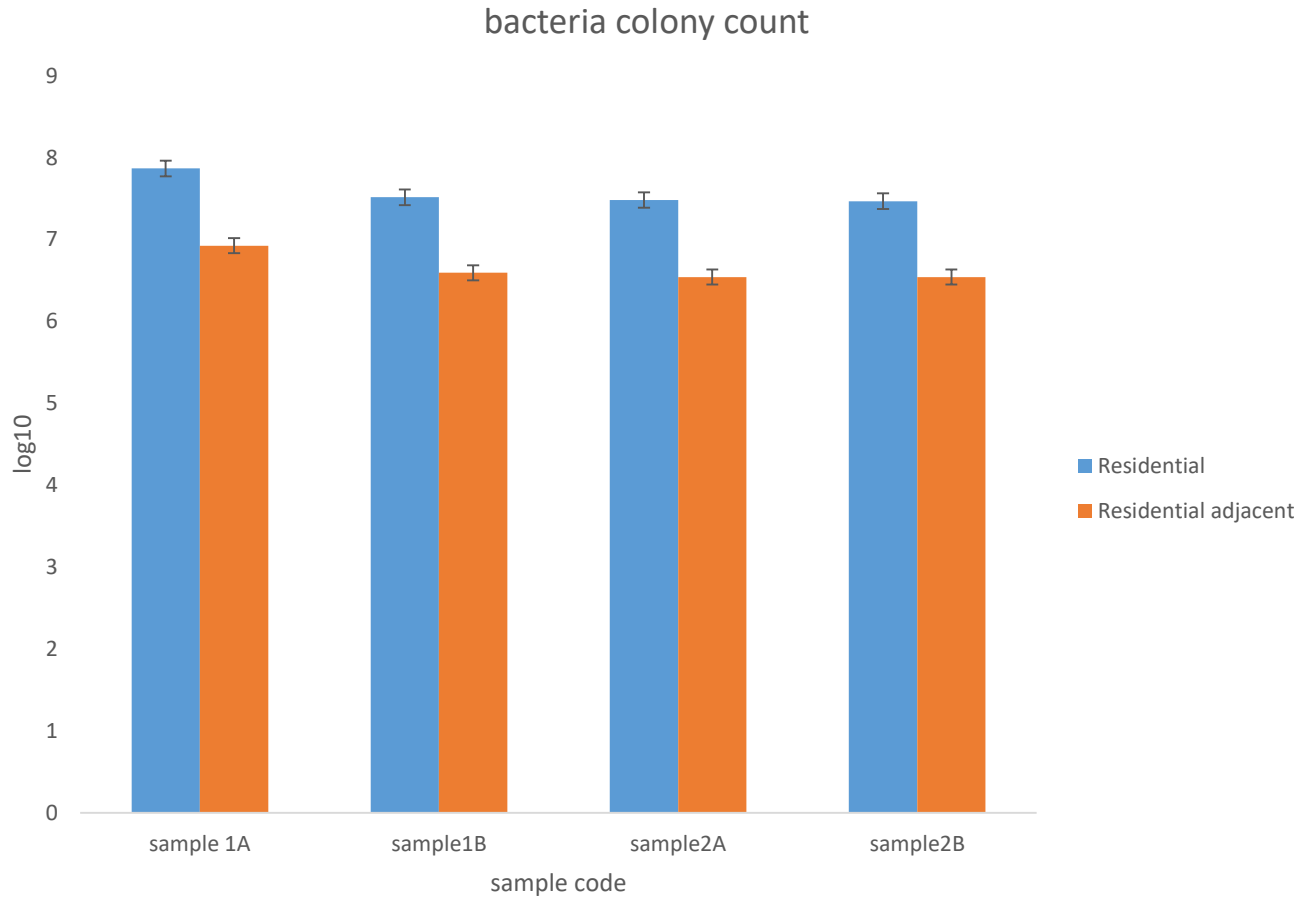


Figure 1: Residential and adjacent heterotrophic bacteria colony count ( $\log_{10}$  cfu/g)

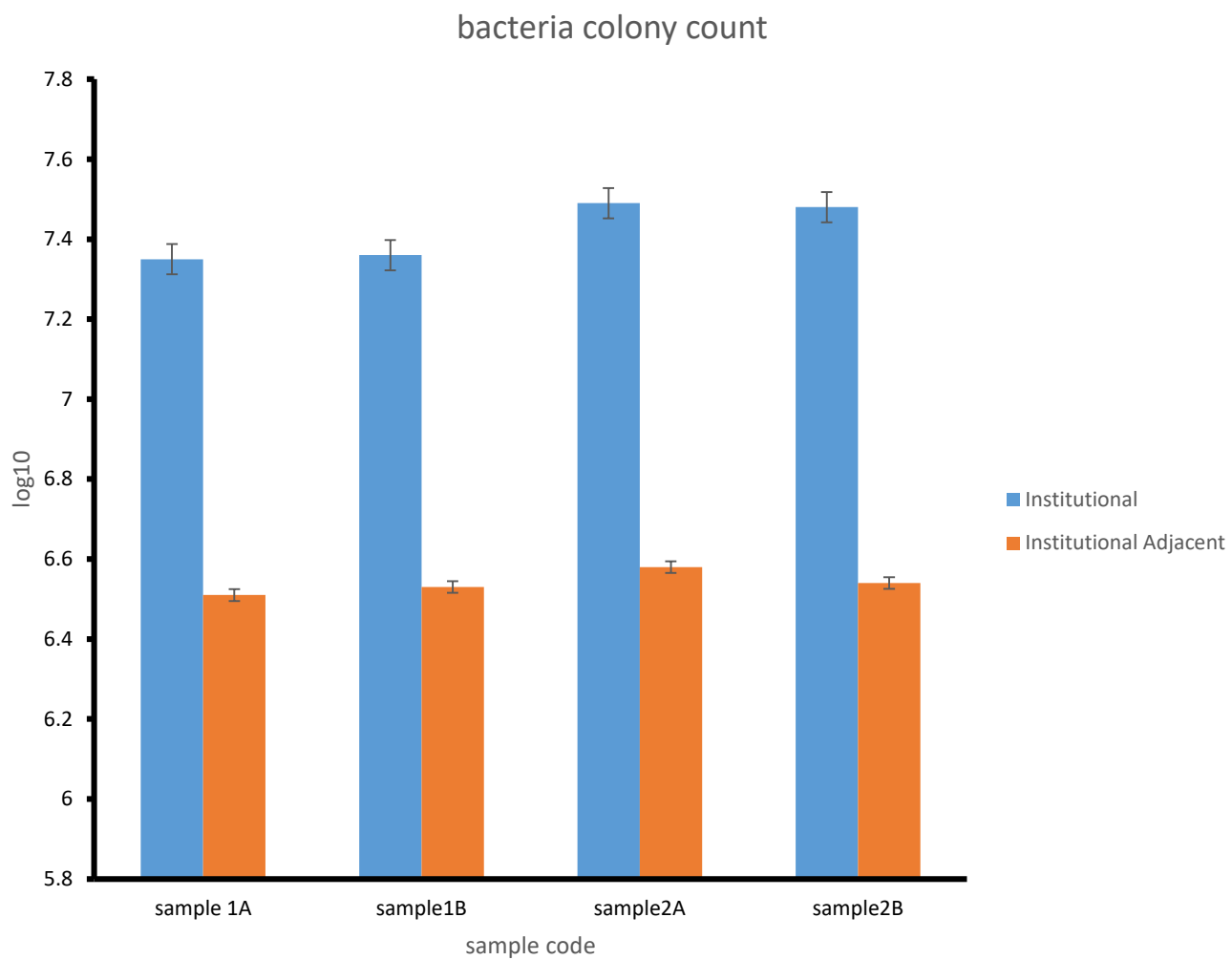


Figure 2: Institutional and adjacent heterotrophic bacteria colony count ( $\log_{10}$  cfu/g)

Table 1: Cultural and morphological characteristics of bacteria isolates

Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Elevation	Convex	Convex	Flat	Convex	Convex	Convex	Convex
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Size	Small	Small	Small	Small	Small	Small	Small
Morphological characteristics							
KOH	+	+	+	-	-	-	+
Gram stain	-	-	-	+	+	+	-
Cell morphology	Rod	Rod	Rod	Cocci	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Clusters	Single	Chains	Clusters
Biochemical characteristics							
Catalase	+	+	+	+	+	+	+
Coagulase	-	-	-	+	-	-	-
Indole	+	-	-	-	-	-	-
Oxidase	-	-	-	-	-	+	-
Citrate	-	-	+	+	+	+	+
Urease	-	-	+	+	-	-	-
H <sub>2</sub> S	-	+	-	-	-	-	-
Glucose	+	+	+	+	+	-	+
Lactose	+	-	-	+	-	-	-
Sucrose	-	-	+	+	+	-	+
Gr. Diff.	Green metallic Sheen (EMB)	Black (SSA)		Yellow (MSA)	Straw (BCA)	Lemon (PCA)	Cream
Identity	<i>E. coli</i>	<i>Salmonella</i> sp	<i>Serratia</i> sp	<i>S. aureus</i>	<i>Bacillus</i> sp.	<i>Pseudomonas</i> sp	<i>Enterobacter cloacae</i>

Keys:

Gr. Diff. Agar = Growth on different agar

MSA = Mannitol salt agar

BCA = Bacillus cereus agar

SSA = Salmonella shigella agar

PCA = Pseudomonas cetrimide agar

EMB = Eosin methylene blue agar

Table 2. Phenotypic virulence determinants of bacterial isolates obtained from anthill soil

Bacterial Isolates	DNase	Hemolysin	Gelatinase
<i>Escherichia coli</i>	+	B	-
<i>Pseudomonas sp</i>	+	B	+
<i>Staphylococcus aureus</i>	+	B	+
<i>entrobacter cloacae</i>	+	$\gamma$	-
<i>Salmonella sp</i>	+	$\gamma$	-
<i>Serratia sp</i>	+	B	+
<i>Bacillus sp</i>	+	B	-

Key: + = positive (present) - = negative (absent)

Table 3: Distribution of bacteria isolates from soil obtained from different anthills

ISOLATE	Institutional		Residential	
NAME	Institutional	adjacent	Residential	adjacent
<i>Enterobacter sp</i>	+	-	+	-
<i>Bacillus sp</i>	+	+	+	+
<i>Pseudomonas sp</i>	+	-	+	+
<i>E.coli</i>	+	-	+	-
<i>Salmonella sp</i>	+	-	+	+
<i>Serratia sp</i>	-	+	-	+
<i>Staphylococcus sp</i>	-	-	+	-

Key: + is positive

- Is negative

## CHAPTER FIVE

### 5.0

### DISCUSSION

This study was aimed at isolating and characterizing pathogenic bacterial isolates from anthill soil. Owing to the fact that anthill soils have been used as organic fertilizers in various parts of the world, especially in developing countries, there is a pressing need to check if these soils employed as fertilizers, to yield plant growth, are pathogenic. Some isolates found in the anthill soil includes *E. coli*, *Bacillus sp*, *Pseudomonas sp*, *serratia sp*, *Enterobacter cloacae*, *staphylococcus sp* and *Salmonella sp*. These isolates were similar to that found by (Maron *et al.*,2011; Mommer *et al.* 2018 and Schnitzer *et al.* 2011).

Virulence test (Dnase, Haemolysin and gelatinase) from the results all the isolates were positive to DNase. *Enterobacter cloacae* and *Salmonella sp.* showed gamma Haemolysin. *Pseudomonas sp*, *staphylococcus sp*, *serratia sp* were positive to gelatinase.

From the result obtained from both residential and institutional samples, it was observed that the bacteria CFU/ml count was higher in anthill soil compared to that of adjacent soil with ( $\log_{10}$  cfu/g)  $7.57 \pm 0.05^a$  and  $6.53 \pm 0.18^b$  for residential and  $7.25 \pm 0.01$  and  $6.45 \pm 0.08$  for institutional respectively. The institutional samples showed less bacterial counts than the residential samples ( $\log_{10}$  cfu/g)  $7.25 \pm 0.01^a$  and  $7.57 \pm 0.05^b$ , this may be due to the biochemical activities, direct and indirect influence of other microbes habitating the soil and the complex chemical reactions, owing to the availability of organic deposits that are more prevalent around the soil in residential areas. These values correspond with Mommer *et al.* (2018). There was a 0.05 significance difference according Duncan anova.

*Bacillus sp.* had the highest distribution across all the sampled locations. *E. coli* and *Salmonella sp.* had 75% distribution across the sampled locations and *staphylococcus sp.* had the lowest distribution as it was found in only one of the sampled locations

It is well known established fact that microbes are omnipresent and series of physical environmental factors influence the presence, proliferation and count of bacteria in the microbial community. The higher population of bacteria recorded in anthill soil than in the adjacent soils could be traced to the environmental conditions and ant activities which may directly inhibit some bacteria and perhaps prevent the invading of some bacteria. The variation in the count at different samples is supported by the publication of Marshal *et al.*, (2008).

Most of the bacteria isolates gotten from this study like *staphylococcus* and *Escherichia coli* are pathogenic to both animal and plant. Consumable plants grown in this anthill soil can easily be infected by these pathogens which later become an opportunistic pathogen to human. Disease outbreaks linked to human pathogen, contamination of fresh plant produce are a growing concern worldwide (Berg *et al.*, 2005; van Baarlen *et al.*, 2007; Tyler & Triplett, 2008; Whipps *et al.* 2008; Holden *et al.*, 2009; Teplitski *et al.*, 2009; Critzer & Doyle, (2010). Nevertheless, many of the human pathogenic bacteria can be highly competitive for nutrients and produce various antimicrobial metabolites allowing them to colonize and proliferate on plant surfaces in the presence of the indigenous microbial communities, such as in the case of anthill microbes.

Interestingly, the mechanisms involved in rhizosphere colonization and antimicrobial activity of pathogenic bacteria appear to be similar to the mechanisms involved in virulence and colonization of human tissues (Berg *et al.*, 2005; van Baarlen *et al.*, 2007; Holden *et al.*, 2009). The presence of *E.coli* and *salmonella sp* became worrisome as a series of studies have clearly shown that human pathogenic bacteria can enter the food production chain not only after harvest

and during handling of fresh food products, but also during the pre-harvest stages of crop production. Pre-harvest entry can occur via contaminated manure and irrigation water, animals, or seeds. Their ability to survive in this kind of soil (Anthill soil) and to colonize the plant exemplifies that human pathogenic bacteria are not solely adapted to propagate and survive in the animal gastrointestinal tracts. Instead, there appear to be a continuum of available microsites on different hosts that allows for cross-kingdom jumps by human pathogenic bacteria (van Baarlen *et al.*, 2007; Holden *et al.*, 2009; Kaestli *et al.*, 2012). In this context, Tyler & Triplett (2008) suggested that human pathogens may use plants 'as an alternative host to survive in the environment and as a vehicle to re-colonize animal hosts once ingested'.

*Bacillus* are predominant soil bacteria widely used in industrial applications, particularly antibiotics production having medically, agriculturally and veterinary importance Gonzalez-Lamothe *et al.*, 2009; Osbourn *et al.*, 2011). Their abilities of endospore formation and antibiotics production have to be regarded as an advantage for the colonization of environment. The good thing is that some of these pathogenic bacteria are capable of producing antibiotics which in other tone can be beneficial pharmaceutically.

Protection from microbial pathogens is essential in social insects, like ants, that reside in moist, dense living conditions, where individuals are highly related (Stow and Beattie 2008; Yek and Mueller 2011). A common adaptation found only in ants is the presence of metapleural glands, which are paired organs on the sides of ants that secrete a waxy, acidic anti-microbial substance (Beattie *et al.* 1985; Bot *et al.* 2002; Yek and Mueller 2011). The antiseptic secretions of metapleural glands appear to suppress the spread of fungal and bacterial disease in ant colonies (Poulsen *et al.*, 2002). Ants also secrete anti-microbial compounds from mandibular glands. Plants survive and grow better in ant-occupied soil, a pattern attributed to heightened moisture

and nutrients, as well as protection from aboveground predators and fire (Giladi 2006; Hanzawa *et al.*, 1988; Prior *et al.*, 2014; Wagner *et al.* 2004; Warren and Giladi 2014).

## CONCLUSION

Anthill soil samples from Residential (Ekosodin Community) and Institutional (University of Benin, Ugbowo campus) have shown to possess pathogenic bacteria such as *E. Coli*, *Bacillus sp.*, *Pseudomonas Sp.*, *Enterobacter Serratia sp.* Etc. The constant search for pathogenic bacteria is a point of focus, as the spread of multidrug resistant pathogen causing life threatening diseases is at the top gear. Hence, effort should be intensified to search for new compounds particularly from microbes to combat and curb the threat of increasing population of antibiotic-resistant bacteria. The presence of *Escherichia coli*, *salmonella*, *serratia* and *Enterobacter* as pathogens is a major concern. This study therefore concluded that anthill soil sediment houses series of bacteria species. Some of these bacteria strains are pathogenic with virulence factors. However, it is important to note that most of these bacteria could be multiple drug resistance pathogenic bacteria thereby being severely dangerous and difficult to combat. Further research about the effect of anthill soils to plants and animals including humans are advanced, this is for clearer and better understanding about the effect of pathogenic bacteria present in anthill soils.

## REFERENCES

- Adesina, A and Chianu, A. (2002). Farmers' use and adaptation of alley farming in Nigeria. *Soil Ecology*, **8**:51-64.
- Ali, M. H., Hoque, M. R., Hassan, A. A. and Khair, M. A. (2007). Effects of deficit irrigation on yield, water productivity, and economic returns of wheat. *Agric. Water Manage* 92, 151-161.
- Andrianjaka, Z., Bally, R., Lepage, M., Thioulouse, J., Comte, G., Kisa, M. and Duponnois, R. (2007). Biological control of *Striga hermonthica* by *Cubitermes* termite mound powder amendment in sorghum culture. *Applied Soil Ecology* **37**:175-183.
- Black, H. I. J. and Okwakol, M. J. N. (1997). Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of termites. *Applied Soil Ecology* **6**: 37-53.
- Brossard, M., López-Hernández, D., Lepage, M. and Leprun, J. C. (2007). Nutrient storage in soils and nests of mound-building *Trinervitermes* termites in Central Burkina Faso: Consequences for soil fertility. *Biology and Fertility of Soils* **43**: 437-447.
- Brune, A. and Kuhl, M. (1996). pH profiles of the extremely alkaline hind guts of soil-feeding termites (Isoptera: Termitidae) determined by micro pH electrodes. *Journal of Insect Physiology* **42**: 1121-1127.
- Brune, A., Miambi, E. and Breznak, J. A. (1995). Roles of oxygen and intestinal micro flora in the metabolism of lignin derived phenyl propanoids and other monoaromatic compounds by termites. *Applied Environmental Microbiology* **61**: 2688-2695.
- Cadet, P., Guichaoua, L. and Spaull, V. W. (2004). Nematodes, bacterial activity, soil characteristics and plant growth associated with anthill and termitaria in a sugarcane field in South Africa. *Applied Soil Ecology* **25**: 193-206.
- Cammeraat, L. H., Willott, S. J., Compton, S. G. and Incoll, L. D. (2002). The effects of ants nests on physical, chemical and hydrological properties of a rangeland soil in semiarid Spain. *Geoderma* **105**: 1-20.

- Cerda, A. and Jurgensen, M. F. (2008). The influence of ants on soil and water losses from an orange orchard in eastern Spain. *Journal of Applied Entomology* **132**: 306-314.
- Chikuvire, T. J., Mpepereki, S. and Foti, R. (2007). Soil fertility variability in sandy soils and implications for nutrient management by smallholder farmers in Zimbabwe. *Journal of Sustainable Agriculture* **30**: 69-87.
- Chisanga, K., Mbega, E. and Ndakidemi, P.A. (2019). Socio-economic factors for anthill soil utilization by smallholder farmers in Zambia. *Sustainability*, **11**(18):4849.
- Clay, D. C., Kelly, V., Mpyisi, E. and Reardon, T. (2002). Input use and conservation investments among farm households in Rwanda: patterns and determinants. *In Barrett* **2002b**: 103-114.
- Cooper, P. J. M. (1972). Arylsulphatase activity in Northern Nigerian soils. *Soil Biology and Biochemistry* **4**: 333-337.
- Copeland, V. N. E. (2007). Anthills touch the sky: Sembène's topographical aesthetic. In Compan. M and Pieprzak K, editors. *Land and landscape in francographic literature: Remapping uncertain territories*. Cambridge Scholars Publishing, Newcastle, UK p. 10-24.
- Culliney, T.W., (2013). Role of arthropods in maintaining soil fertility. *Agriculture*, **3**(4):629-659.
- Dangerfield, J. M., McCarthy, T. S. and Ellery, W. N. (1998). The mound-building termite *Macrotermes michaelseni* as an ecosystem engineer. *Journal of Tropical Ecology* **14**: 507-520.
- Dashtban, M. Schraft-Wensheng, H. Q. (2009). Fungal bioconversion of ligno cellulosic residues: opportunities and perspectives. *International Journal of Biological Sciences* **5**: 578-595.
- Dauber, J. and Wolters, V. (2000). Microbial activity and functional diversity in the mounds of three different ant species. *Soil Biology and Biochemistry*, **32**(1): 93-99.

- De Jager, A., Kariuku, I., Matiri, M., Odendo, M. and Wanyama, J. M. (1998). Monitoring nutrient flows and economic performance in African farming systems (NUTMON). IV. Linking nutrient balances and economic performance in three districts in Kenya. *Agriculture, Ecosystems and Environment* **71**: 81-92.
- Dean, W. R. J., Milton, S. J and Klotz, S. (1997). The role of ant nest mounds in maintaining small- scale patchiness in dry grassland in Central Germany. *Biodiversity and Conservation* **6**: 1293-1307..
- Dhembare, A. J. (2013). Physico-chemical properties of termite mound soil. *Archives of Applied Science Research* **5**: 123 -126.
- Diamond, S.E., Sorger, D.M., Hulcr, J., Pelini, S.L., Toro, I.D., Hirsch, C., Oberg, E. and Dunn, R.R., (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Global Change Biology* **18** (2) 448-456.
- Dostál, P., Březnová, M., Kozlíčková, V., Herben, T. and Kovář, P. (2005). Ant-induced soil modification and its effect on plant below-ground biomass. *Pedobiologia*, **49**(2):127-137.
- Dostál, P., Březnová, M., Kozlíčková, V., Herben, T. and Kovář, P. (2005). Ant-induced soil modification and its effect on plant below-ground biomass. *Pedobiologia*, **49**(2): 127-137.
- Gachimbi, L.N., Van Keulen, H., Thurani, E.G., Karuku, A.M., De Jager, A., Nguluu, S., Ikombo, B.M., Kinama, J.M., Itabari, J.K. and Nandwa, S.M. (2005). Nutrient balances at farm level in Machakos (Kenya), using a participatory nutrient monitoring (NUTMON) approach. *Land Use Policy*, **22**(1):13-22.
- Gonzalez-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M. S., Malouin, F. and Bouarab, K. (2009) Plant antimicrobial agents and their effects on plant and human pathogens. *International Journal of Molecular Science* **10**: 3400–3419. .
- Hatcher, M.J., Dick, J.T. and Dunn, A.M. (2012). Diverse effects of parasites in ecosystems: linking interdependent processes. *Frontiers in Ecology and the Environment*, **10**(4):186-194.

- Henao, J. and Baanante, C. (2001). Nutrient depletion in the agricultural soils of Africa. In: Pinstrup-Andersen, P., Pandya-Lorch, R. (Eds.), *The Unfinished Agenda: Overcoming Hunger, Poverty, and Environmental Degradation*. IFPRI, Washington p. 159-163.
- Holt, J. A. and Lepage, M. (2000). Termites and soil properties. In: Abe T, Bignell D.E, and Higashi M, Ed. *Termites: evolution, sociality, symbioses, ecology*. Kluwer Academic Publishers, Dordrecht, The Netherlands p389-407.
- Holt, J. A., Coventry, R. J. and Sinclair, D. F. (1998). Some aspects of the biology and pedological significance of mound-building termites in a red and yellow earth landscape near charters towers, north Queensland. *Australian Journal of Soil Research* **18**: 97-109.
- Horlings, L.G. and Marsden, T.K., (2011). Towards the real green revolution? Exploring the conceptual dimensions of a new ecological modernisation of agriculture that could 'feed the world'. *Global environmental change*, **21**(2): 441-452.
- Johnson, R. A. (1998). "Foundress survival and brood production in the desert seed-harvester ants *Pogonomyrmex rugosus* and *P. barbatus* (Hymenoptera, Formicidae)." *Insectes Sociaux*, 45(3):255-266.
- Jones, J. A. (1990). Termites, soil fertility and carbon cycling in dry tropical Africa: a hypothesis. *Journal of Tropical Ecology* **6**: 291-305.
- Jönsson, H., Johnsson, P., Höglund, P., Alling, C. and Blomquist, S. (2000). Elimination of S100B and renal function after cardiac surgery. *Journal of cardiothoracic and vascular anaesthesia*, **14**(6): 698-701
- Joseph, G. S., Seymour, C. L., Cumming, G. S., Cumming, D. H. M. and Mahlanguss, Z. (2002). Termite mounds as islands: woody plant assemblages relative to termitarium size and soil properties, *Journal of Vegetation Science* **130**: 1654-1103.
- Jouquet, P., Lepage, M. and Velde, B. (2002). Termite soil preferences and particle selections: strategies related to ecological requirements. *Insectes Sociaux* **49**: 1-7.
- Jungerius, P. D., Van den Ancker, J. A. M. and Múcher, H. J. (1999). The contribution of termites to the microgranular structure of soils on the Uasin Gishu Plateau, Kenya. *Catena* **34**: 349-363.

- Kaschuk, G., Santos, J. C. P. and Almeida, J. A. (2006). Termite's activity in relation to natural grassland soil attributes. *Science Agriculture (Piracicaba, Braz)* **63**(6): 583-588.
- Kheyrodin, H. (2014). Important soil quality and soil agriculture indicators. *Academia Journal of Agricultural Research* **2**: 231-238.
- King, T. J. (1977). The plant ecology of ant-hills in calcareous grasslands- I. Succession on the mounds. *Journal of Ecology* **65**: 257-278.
- Kotova, A. A., Golichenkov, M. V., Umarov, M. M., Putyatina, T. S., Zenova, G. M. and Dobrovol' skaya, T. G. (2013). Microbiological activity in the anthills of fallow lands (Ryazan Region). *Pochvovedenie* **2**: 31-34
- Kovář, P., Kovářová, M., Dostál, P. and Herben, T., (2001). Vegetation of ant-hills in a mountain grassland: effects of mound history and of dominant ant species. *Plant Ecology*, **156**(2): 215-227.
- Menta, C. (2012). Soil fauna diversity-function, soil degradation, biological indices, soil restoration. *Biodiversity and conservation*, **6**:59-94.
- Nyamangara, J. and Nyagumbo, I. (2011). Interactive effects of selected nutrient resources and tied-ridging on plant growth performance in a semi-arid smallholder farming environment in central Zimbabwe. In *Innovations as Key to the Green Revolution in Africa*. Springer, Dordrecht. pp357-363.
- Pokarzhevskij, A. D. (1981). The distribution and accumulation of nutrients in nests of ants *Formica polyctena* (Hymenoptera, Formicidae). *Pedobiologia* **21**: 17-124.
- Rizk, R. and Slimane, M.B. (2018). Modelling the relationship between poverty, environment, and institutions: a panel data study. *Environmental science and pollution research*, **25**(31): 31459-31473.
- Russell, J.A., Funaro, C.F., Giraldo, Y.M., Goldman-Huertas, B., Suh, D., Kronauer, D.J., Moreau, C.S. and Pierce, N.E., (2012). A veritable menagerie of heritable bacteria from ants, butterflies, and beyond: broad molecular surveys and a systematic review. *Plos One*, **7**(12):510-527.

- Senula, Sarah F., Joseph T. Scavetta, Joshua A. Banta, Ulrich G. Mueller, Jon N. Seal, and Katrin Kellner. (2019) Potential distribution of six north American higher-attine fungus-farming ant (Hymenoptera: Formicidae) species. *Journal of Insect Science* **19**(6): 24.
- Shawale, J. G. and Sadana, J. (1981). Purification, characterization and properties of  $\beta$ -glucosidase enzyme from *Sclerotium rolfsii*. *Archives of Biochemistry and Biophysics* **207**: 185-196.
- Siame, J. A. (2005). Termite mounds as fertilizer. *LEISA India* **7**(2): 27-40.
- Sofo, A., Mininni, A. N., & Ricciuti, P. (2020). Soil macrofauna: A key factor for increasing soil fertility and promoting sustainable soil use in fruit orchard agrosystems. *Agronomy*, **10**(4), 456.
- Steinke, T. D. and Nell, N. O. (1989). Some effects of termitaria on veld in the Eastern Cape. *Tydskrif Weidingsveren* **6**: 152-155.
- Tittonell, P. and Giller, K.E. (2013). When yield gaps are poverty traps: The paradigm of ecological intensification in African smallholder agriculture. *Field Crops Research*, **143**: 76-90.
- Vanlauwe, B., Aihou, K., Aman, S., Iwuafor, E. N. O., Tossah, B. K., Diels, J., Sanginga, N., Merckx, R. and Deckers, S. (2002b). Maize yield as affected by organic inputs and urea in the West-African moist savanna. *Agronomy Journal* **93**(6): 1191-1199
- Vanlauwe, B., Descheemaeker, K., Giller, K. E., Huisling, J., Merckx, R., Nziguheba, G., Wendt, J. and Zingore, S. (2015). Integrated soil fertility management in sub-Saharan Africa: unravelling local adaptation. *SOIL* **1**: 491-508.
- Vanlauwe, B., Diels, J., Sanginga, N. and Merckx, R. (2002a). Integrated Plant Nutrient Management in sub-Saharan Africa: From Concept to Practice. CABI, Wallingford, UK p. 352.
- Vasconcelos, H.L. and Davidson, D.W., (2000). Relationship between Plant Size and Ant Associates in Two Amazonian Ant-Plants 1. *Biotropica*, **32**(1):100-111.

- Wagner, U., Gais, S., Haider, H. and Born, J. (2004). Sleep inspires insight. *Nature*, **42**(69): 352-355.
- Watson, J. P. (1977). The use of mounds of the termite *Macrotermes falciger* (Gerrstäcker) as a soil amendment. *Journal of Soil Science* **28**: 664-672.
- Wood, T.G. (1988). Termites and the soil environment. *Biology and fertility of soils*, **6**(3): 228-236.