ASSESSMENT OF TERMITE SPECIES DIVERSITY, SOIL CHEMICAL CHARACTERISTICS, AND EVALUATION OF PHYTOCHEMICALS AS EDIBLE TERMITE ATTRACTANTS

AMEKA CALEB MIKHALI

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SCHOOL OF AGRICULTURAL AND FOOD SCIENCES

JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE AND TECHNOLOGY

DECLARATION AND RECOMMENDATION

"This thesis is my original work and has not en presented for an award o egree in any other university or institution."

Signature …….Date…….

Ameka Caleb Mikhali

A461/4273/2018

Supervisors

Signature 

…….Date……15/12/2022…….

Prof. Bernard Muok
School of biological and physical sciences
Jaramogi Oginga Odinga University of Science and Technology

Signature 

…….Date……15/12/2022…….

Dr. Helida Oyieke
National Museums of Kenya.

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ABSTRACT

Termites are social insects that live in colonies underground. Globally, there are 3000 species, of which 39% are found in Africa. Termites are used as food and feed in most communities of the world. In Kenya, termites are consumed by many communities, especially in the western region. Termite species diversity across different parts of the Luanda sub-County needs to be established as there are many edible and non-edible species in the area. This study envisaged to assess the species diversity of termites in Luanda sub-County, analyse their habitat, soil chemical characteristics and evaluate plant derived phytochemicals used as termite attractants. Termites were sampled in Luanda sub-county using the line transect method. The collected soldier termites were preserved in tubes containing 70% Ethanol. The preserved samples were taken to the National Museums of Kenya for morphological and molecular identification up to species level. The soils in each termite habitat were collected and analysed to determine each habitat's soil chemical characteristics. Eucalyptus stems, bamboo, *Grevillea robusta*, Maize and sugarcane were evaluated for the presence of phytochemicals that attract termites to feed. Choice test bioassays were done to determine the response of termites to different crude phytochemicals extracted from these plants. Species richness of each habitat was analyzed for diversity (Shannon-Wiener) index and Simpson index using Vegan package version 1.16-32 in R. The differences in species composition and diversity of termites in different counties and soil chemical characteristics as well as termite phytochemical attraction bioassay was analyzed using one-way ANOVA. Morphological identification recorded seven species, while molecular identification recorded four termite species in the study area. The results of this study showed that the Shannon diversity index **H** was 0.3606 while Simpson index **D** was 0.20644, which implied a high species diversity of termites in Luanda sub-County. There was a significant difference in the soil Zinc levels for all the termite species excluding *Macrotermes sp1*. Feed choice bioassay revealed that termites were attracted to various feed substrates $p<0.05$. Termite attraction to crude plant extracts was highly significant $p<0.05$. This study will inform food security experts on the status of termite's species richness and evenness in their natural ecosystem and identify plants, which attracts termites to forage, thus increase their productivity during mass harvesting for food and feed.

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ACRONYMS

ANOVA	Analysis of variance
COII	Cytochrome oxidase II
DF	Degree of freedom
DDH_20	Double distilled water
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetate
FAO	Food and Agriculture Organization
HCI	Hydrochloric acid
K	Potassium
NACOSTI	National Commission of Science Technology and Innovation
PCR	Polymerase chain reaction
pH	Potential of hydrogen
RNA	Ribonucleic acid
Zn	Zinc

CHAPTER ONE
INTRODUCTION

1.1 Background

One of the Sustainable Development Goals of the United Nations is to achieve food security and improved nutrition among the population of the world FAO, (2016) Bourguignon *et al*., (2015). Success in sustainably feeding the rapidly growing population requires the development of innovative techniques in food production. Insects, especially termites, provide alternative sources of food and feed Govorushko, (2019) as various species are found worldwide, have short life cycles, and are highly nutritious. Termites (Order: Isoptera) constitute an integral component of various ecosystems and habitats as they are ecosystem valuable engineers. In Africa. Termites are also amongst the most challenging and unique insects to study because of their cryptic behavior and natural nesting habitat Ahmed Shiday, (2014).

There are around 3000 species of termites in 280 genera that have been documented, and archived worldwide, and about 39% of the total termite species are found in Africa according to research by Eggleton and Parr, (2012). Identification of termites is valuable to understanding their ecological distribution and abundance, Buczkowski & Bertelsmeier, (2017) and their relationship to climate change and food security. Termites are important in Africa as many rural folks greatly utilize them as food and feed for their livestock. However, a few species are crop pests, while 99% of the more than 2000 termite species offer beneficial ecosystem services. Despite their importance, termites specifically soldier and workers are largely understudied, and most ecological studies in tropical ecosystems concentrate on charismatic mound builders, while comparatively little is documented about hypogeal termite species Korb *et al.,* (2019). The taxonomy of African termites is yet to be established, and many new species are yet to be fully described (Ahmed Shiday, 2011). Termites used as food and feed have excellent nutritional qualities, proximate composition indicates that termites have a long shelf-life, and are a good source of crude protein of 45.85%Kinyuru, *et al*., (2015), and other micro- and macro-nutrients. Consumption of termites should thus be encouraged in Africa and the rest of the world. Termites have a low phytic acid value and hydrocyanide content which implies that they are less toxic Ikipe *et al* (2006).

Termites colonize different habitats such as grasslands, forests, lowlands, and hilly areas, which makes them have variations in their nutrient composition. This is due to variations in feed sources of termites that grow at different altitudes. Subterranean termites specifically the worker and solder caste are guided to their feed by chemical cues which are produced by the plants they feed. There are a variety of plants that termites feed on, while there are those that termites detest. The plants that termite feed on include Eucalyptus, Grevillea, Bamboo, Maize, Sugarcane, Mango, Avocado, and Blue citronella grass. Soldier and worker termites are attracted to plants through the phytochemicals produced by these plants. Some plants have a strong attraction, while others are not Senanayake, *et al*., (2016). These attractants act as lures or guides for termites to the food source.

This study envisaged characterizing termites morphologically and genetically and determining species diversity based on the soil's chemical characteristics. Further, the study evaluated the feed choice preference of edible termites on selected plants and their phytochemical composition.

1.2 Statement of the Problem

In the western Kenya region, soldier and worker termites have been used as food and feed Kinyuru *et al*., (2015). There are many termite species across different habitats in Luanda sub County such as hills and the low lands. Currently, there is scanty information on termite species diversity in Luanda Sub-County a leading producer of termites for sale as food and feed. Soldier termites are used to identify termites morphologically due to the visible features they have. Morphological identification and molecular characterization can assist to identify the species. In Luanda Termites soil chemical characteristics contribute to the diversity of termites Wakung'oli *et al*., (2020) and this has not been determined in Luanda sub-County. Edible termites are seasonal and are greatly influenced by the phytochemicals present in plants that attract them to feed (FAO, 2013). These phytochemicals need to be isolated and identified so they can be used as an attractant for the soldier and worker termites in large quantities for food or feed in the future. The proposed research will assess the species diversity, characterize them both morphologically and genetically, and finally, evaluate phytochemicals that attract termites.

1.3 Overall Objective

To assess termite species diversity, soil chemical characteristics, and evaluation of Phytochemicals as termite attractants.

1.3.1 Specific Objectives

1. To determine species diversity of termites in the Luanda sub-County.
2. To analyze the effect of soil chemical characteristics on the diversity of termites.
3. To evaluate selected crude plants' phytochemical extracts as edible termite attractants.

1.4 Hypotheses

1. There is no diversity of termite species in the Luanda sub-County.
2. Soil Chemical characteristics do not affect the diversity of termites.
3. Crude plants Phytochemicals extracts from selected plants do not affect the attracting of termites.

1.5 Justification

Some edible termite species in the Luanda sub-county have not been characterized morphologically and genetically based on soil chemical characteristics and altitude. The characterization of termites will give a basis on which the species diversity will be determined. Once the species have been determined, they will be documented and preserved in the National Museum of Kenya repository. One major process of trapping termites in large quantities for use as feed is by using Phytochemicals derived from these plants. Phytochemicals are organic substances that plants produce as food sources, and they elicit a behavioral response in termites. Termites are seasonal hence their yield needs to be maximized to supply enough food and feed. To enhance the maximum trapping of termites for feed, there is a need to use Phytochemicals to lure them out of their nests. This will optimize their production to sustain their utilization as food and feed.

1.6 Scope of the Study

The proposed study was conducted in 47 sites in Luanda Sub-County of western Kenya. A sampling of termites was done from low lands near the river valleys up to the hills. This assisted in obtaining the different species with the increase in altitudes. The study was conducted over two seasons, the dry season January to March, and the wet season September to November 2020. The line transect method was used as described by (Eggleton & Parr, 2012). This design collected more information for the proposed study. Morphological identification and molecular characterization of soldier termites were done at the National Museums of Kenya in Nairobi which has specialized identification equipment. Phytochemical analysis was done at the Photochemistry laboratory at the National Museum of Kenya laboratory. The plants selected for use in the bioassays were bamboo, sugarcane, maize, and Grevillea. The choice of these plants was informed by the presence of termites on dry woody parts of these plants in the Luanda sub-County. In this study, data on the species diversity of soldier termites were collected as well as data on soil chemical characteristics and the termite species.

1.7 Limitations of the Study

In Luanda Sub County, there is heavy use of pesticides to kill termites due to their destructive nature to buildings. The use of pesticides has resulted in the death of termites. There is growing interference of termite habitats by man occasioned by expanding agricultural land and room for house construction. To address these challenges termite sampling was done on road reserves and farm forests that are undisturbed. Human settlements were not sampled due to this interference.

1.8 Assumptions of the Study

This study assumes that there are diverse species of termites in selected counties of the Luanda sub-County. The habitats are also diverse, and the wide geographical area has many termite species present. Termites are also attracted to different plants by specific chemical signals, which are stronger in some plants than in others.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

Termites belong to the order Isoptera. They are classified as dry wood termites, subterranean and Damp wood termites. Species identification is done using both morphological and molecular methods. The solder termites are used for morphological identification as they have many features to use in developing identification keys. This study sought to identify solder termites in Luanda sub-County to determine species diversity.

2.2 Termite Colony

Pranesh & Harini (2015), documented that termites are eusocial insects and have a caste system in their habitats. Each caste can be visually differentiated by several key traits. The division of labor in termite colonies is based on different castes. The caste system may vary depending on the termite species. In *Termitidae bifurcation* division of labor occurs after the first stage of development. An apterous line gives rise to the worker caste after one or two larval instars. Subsequently, the worker caste gives rise to soldiers. Studies carried out by Neoh & Lee, (2009), identified that a nymph line, after five nymphal instars with wing buds, gives rise to alates which are adults. Termites have four castes namely, worker castes, which are composed of true workers, and false Workers of lower termites specifically have no colour. They have soft-bodied and possess hard mouthparts, which are adapted to foraging on wood. Worker termites are functionally sterile male and female individuals which are responsible for all the work required in the nest.

In his work, Miller, (2010) found out that young workers perform domestic duties like feeding, grooming, and caring for the young ones in the nest, while the older workers are recruited on the tough duties of foraging and mound building. Miller's views are also shared with (Smith, 2014), suggesting that subterranean termite soldiers function as defenders of the colony and provide protection against all intruders. Soldier termites are yellow-brown, sometimes pale red, and are bigger than workers. They have enlarged heads and large, powerful mandibles, which they use to bite enemies. Yaguchi, *et al*., (2016) views that soldiers are a much more interestingly specialized caste, and are about 10% of

the termite colony. The alates occupy the top caste in the termite colony, and the whole colony is constructed around them. In the first few years, the termite king and queen produce workers and soldier termites, although some species produce alates that can replace the role of king or queen after one dies. After 4-5 years of existence, the queen produces new reproductive termites, as described by Hari & College, (2018), that will become alates, swarm in the thousands, and go on to form other colonies. In the reproductive form, the king and queen have black colour, with two identical pairs of wings which facilitate swarming. After mating, the alate forms shed all their wings and move to the deeper end of the colony nest to start a new colony.

2.3 Taxonomy of Termites

According to Kanzaki *et al.,* (2012), the scientific classification of termites follows the pattern, Kingdom: Animalia Phylum: Arthropoda Class: Insecta Subclass: Pterygota Infraclass: Neoptera Superorder: Dictyoptera Order: Isoptera. In general, termites are classified into 280 genera and over 2600 species within seven families and 14 subfamilies. Engel, (2011) further stated that isopteran are phylogenetically classified into lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, and Serritermitidae) and higher termites (Termitidae). The (Termitidae) family is the largest and represents over 85% of all termite genera and 70% of all termite species in the world. According to Aldrich *et al*., (2007) infrared reflectance spectroscopy was used in the characterization of Zootermopsis species and subspecies.

2.4 Functional Roles of Termites in the Ecosystem

In Africa, termites play significant roles within the habitats in which they are found. Termites (Blattodea: Termitidoidae) are considered major ecosystem engineers in tropical and sub-tropical systems, they are significant for the bioturbation of soil particles and change the mineral and organic composition of soils, their hydrology, structure, drainage, and infiltration rates. Alves, *et al*., (2011) documented that termites are the dominant arthropod decomposer in tropical systems and hence affect nutrient regimes and distribution. As the practice of using insects for food, entomophagy has played a leading role in the history of human nutrition in Africa, Asia, and Latin America van Huis, (2017).

Despite their use as decomposers, Buczkowski & Bertelsmeier, (2017) documented that termites pose serious threats to wooden structures and crops. Another important use of insects by humans is medicinal, a practice is known as entomotherapy Esther *et al*., (2015). Termites are used in the treatment of various ailments that affect humans, such as influenza, asthma, bronchitis, headache, whooping cough, sinusitis, tonsillitis, and hoarse voice as described by Alves *et al*., (2011). Termites produce rare edible mushrooms in their nests such as *Termitomyces species* most popularly found during the rainy seasons (i.e., April-July), and have unique and delicious flavors Zeleke *et al*., (2013).

In West Africa, termites are harvested from termite mounds or by trapping using inverted pots or containers filled with organic matter and placed near the termite nests. These systems do not allow the harvesting of a large number of termites and, thus, these are often fed to chicks and ducks. Availability and accessibility of termites as food and feed to farmers in different regions and different seasons vary. During certain seasons, farmers walk for long distances to harvest termites.

In a study conducted in Benin, Baofo *et al*., (2019.), farmers reported that some species of termites were poisonous to birds and thus unsuitable as a feed source. *Noditermes* species caused mortalities in poultry while *Trinervitermes* species were safe to use. In Burkina Faso, it was recorded that some species of Cubitermes were toxic to chicks of poultry but not to guinea fowl and ducks (Kenis *et al*., 2014). In Eastern Africa, particularly Kenya, Ayieko, *et al*., (2010) demonstrated that many communities in the Lake Victoria basin consume roasted termites as a delicacy.

2.5 Species Diversity of Termites

There are many termite species across the world, (Pranesh & Harini, 2015) documented that termites are classified into nine families namely, Termitidae is notably the largest family, which consists14 subfamilies, 280 genera, and over 2600 species identified in the world.

In his work (Jamil *et al*., 2017) found out that there are approximately 175 species of termites from 42 genera and three families (Kalotermitidae, Rhinotermitidae, and Termitidae) all originating from Peninsular Malaysia. In research conducted in India,

(Jouquet *et al*., 2015) identified termites of the genus *Odontotermes*sp. The most abundant species were *Odontotermes feae*, *Odontotermes obesus*, and *Odontotermes feoides.* Northern Africa: termite species diversity is low and thus has less than 15 species in total. This is due to the arid and semi-arid conditions as reported by Paridah *et al*., (2016). In East Africa, it was found that less than 143 termite species were present in the habitats. In Djibouti, Eritrea, Ethiopia, Somalia, and Sudan to the North, and Malawi, Mozambique, Zambia Zimbabwe, and South Africa. Southern Africa accounted for more than 165 species. Despite the numerous efforts to identify termites, the taxonomy of African termites is challenging, as new species have not been characterized Nkunika & Sileshi, (2010).

Species diversity is assessed using Simpson and Shannon indices Simpson's Diversity Index is a measure of diversity that takes into account the number of species present, as and also the relative abundance of each species. As species richness and evenness increase, diversity increases. A study by Sulvan *et al* (1998), documented that there were two species of termites in the study area in Pakistan. The simpson diversity scale was 48% while the Shannon scale was 97%. On the other hand, the species richness was 0.5917. In a study done in Indonesia Mohamed, (2018) documented that the lowest termite species richness was 62.5%. A complete or near far-reaching canopy would end in higher termite species richness in West Africa and Cameroon, even though the area is disturbed.

2.6 Morphological Characterization of Soldier Termites

According to Parween *et al.*, (2016), termite species differ in their morphology and ecology, including colony size, nesting, feeding, grooming, swarming, and reproductive behavior. Accurate identification of termite species and information on their distribution is key to developing environmentally sustainable pest control strategies. In their study, Arif *et al*., (2019) recorded that Termite species were identified based on the morphological features of soldiers. Each of the collected specimens was separately put in a bottle containing 70% ethanol. Soldiers were observed keenly and measured for their morphological features under a microscope stereomicroscope STEMI 2000 with phototube camera ERC 5S (Olympus, USA). The measurement and identification technique was based on previous descriptions by Entomologist *et al.*, (2014). In his study, nine species were found, including two families (Termitidae and Rhinotermitidae), five sub-families

(Macrotamatinae, Termitinae, Rhinotermitidae, Coptotermitinae, and Nasutitermitinae). The six genera were (Odontotermes, Microcerotermes, Nasutitermes, Coptotermes, Coptotermes, and Havilanditermes).

According to Norsyarizan & Wan Nurainie, (2016), termite identification, especially, Coptotermes species is very challenging as the morphological identification of this species cannot provide detailed taxonomic status. The over-dependence on the soldier morphological features presents a major challenge to species taxonomy because of the intraspecific variation in morphological characters in soldiers thus molecular identification is needed to give clear information.

According to Loko *et al*., (2019) over 4,300 samples of termite were used in the studies in Thailand. Termites were identified using the morphological features of soldiers. The characteristics used in the identification of termite genera were the shape and size of the head, fontanelle, labrum, clypeus, mandible, and pronotum. Additionally, to mandible characteristics, the location of teeth and number of antennae were also used. Measurements were generally taken in alcohol-preserved specimens which were dissected out and placed on the Petri dishes. During measurement, the body was kept flat and measured under a binocular stereo microscope with the aid of an ocular micrometer. All measurements of the parts were recorded in millimeters. Almeida *et al*., (2018) in their research documented that eight species of termite noted in the study area belonged to the family *Termitidae*. The dominance of the genus *Macrotermes* could be due to their character as generalist feeders that eat organic material. *M. bellicosus* was dominant in fresh grasslands while *M. subhyalinus* which existed in both fresh grassland and dry wood unlike *Macrotermes spp1* which was collected on the ground *Odontotermes spp1*, *Macrotermes spp1* and *Pseudocanthotermes spp1* were identified as crop pests.

2.7 Molecular Characterization of Termites

Kotilingam, (2020) in his work recorded that the use of molecular techniques to identify species involves the use of the polymerase chain reaction (PCR) to amplify short sections of DNA, which are then characterized using sequencing technology. Molecular characterization can address the limitations posed by morphological identification and has been adopted for termite identification and inferring phylogenies. In molecular

identification, sequencing of gene fragments (DNA) barcoding is now an important molecular tool widely used to explain phylogenic relationships between taxa and identify species Zachariah *et al*., (2017). The molecular technique uses nucleotide sequence similarity to identify species from genomic DNA; therefore, identification can be done regardless of the sampled caste.

Inward *et al*, (2007)) in his study reported that the most detailed termite phylogenetic studies have used morphological traits. Many of these traits suffer from a lack of independence, both from each other and from their functional roles. Sequenced data from molecular characterization, on the other hand, provide a wealth of characters that are free of such non-heritable variation and can be produced unambiguously. According to Husen *et al*., (2006), DNA sequences of the mitochondrial genes cytochrome oxidase subunit II, Micro-morphological and Molecular Characterization of Termites 249 (COIl), ribosomal RNA (rRNA) large subunit (16S), and the rRNA small subunit (12S) have been widely used for molecular characterization and to do comparative phylogenetic analyses of subterranean termite genus *Reticulitermes* and also to study taxonomy, gene flow, and termite colony introduction dynamics of the eastern subterranean termite, *R. flavipe.*

2.8 Challenges Facing Current Termite Species Diversity in Africa

Termites are very destructive, are highly voracious, and cause considerable damage to human settlements and other wooden structures in our environment. In severe infestation, the structural integrity of a building and the safety of the occupiers may be threatened and this calls for sustainable control measures to be undertaken. Chemical termite control has gained much popularity due to its effectiveness in controlling the termite population. Despite the use of insecticides Organochloride insecticides such as Heptachlor and Dieldrin has been used in the past but due to long residual effect, these are harmful to beneficial insects and create a serious problem for the environment Handru & Herwina, (2017). Data for termites (*Reticulitermes flavipes* (Kollar)) reported mortality and feeding inhibition for termites observed up to 21 days in different soils treated with imidacloprid insecticide. Studying termites is hampered by inadequate taxonomic knowledge because termites have few diagnostic morphological traits korb, (2019).

2.9 Termite Habitat Ecosystem

Landscape structural changes affect the population dynamics and composition of species or communities where they are found. Habitat loss is a major threat to global biodiversity, leading to an increased extinction rate of termite species in most ecosystems as described by Pranesh & Harini, (2015). Termite biodiversity loss is generally intense in the tropics, where many tropical forests are replaced with agricultural and forest systems. Habitat conversion has an overall effect on natural communities, resulting in the promotion of species that are resilient to changing environments like termites. Clement *et al.*, (2021) documented that When termites tunnel and build mounds, they increase soil bioturbation and nutrient levels, which can last for many years. Termites differ from fungi in the speed at which they break down wood and the pathways by which they release carbon from wood. In some tropical rainforests, termites are responsible for most insect decomposition, but the absence of termite activity in Australian rainforests could slow dead wood decomposition as compared with other tropical rainforests in the world.

2.9.1 Soil Chemical Characteristics of Termite Soil Habitats.

Dhembare (2013) in his work found that termites and ants play a crucial role in nutrient recycling, movement, and transportation of soil material. Termites are ecosystem engineers such that they built mounds, boosting the content of organic carbon, clay, and nutrients. The mound soil is dispersed by erosion, affecting soil microstructure and fertility, thus contributing to overall habitat richness. Jouquet *et al*., (2015) highlight that termites are also major soil pulverizers and bioturbators, thus creating biogenic structures that greatly influence the physical and chemical characteristics of soils.

Kalaichelvi and Ezhili., (2017) documented that termites (Isoptera) are social insects that live in communities having over 3000 documented species, 75% are soil feeders, and 28 species are pests. Termites mainly forage on non-cellular organic matter mixed with clay minerals. Literature cited by Tathiane *(*2019.) highlighted that the gut of termites is modified and adapted for high pH, oxygen, and hydrogen, which are vital for soil biological, chemical, and physical parameters.

Li *et al* (2017) documented that termites colonized acidic and weakly alkaline soils, with surrounding soil's pH values of between 3.3 and 8.6. In a study site of 117 sets of data, 84% showed surrounding soil's pH values were lower than 7, with an average of 5.7. pH values from 4.5 to 5.5 documented the highest value in surrounding soils, covering 38%. These results supported that most termites inhabit an acidic soil environment. ABE *et al*., (2017) recorded that, X-ray analysis of soil showed that termite pulverized and mixed soils containing high amounts of kaolinite as their main clay mineral than the surrounding non-reworked soils. The research illustrates possible mineralogical alterations of the soils mediated by termites.

According to Obi *et al*., (2019), documented in their work that one of the strongest associations established between the pH of termite mounds and the soil properties was encountered in available phosphorus, showing that the capacity of termites to significantly decrease the availability of phosphorus while increasing the incidence of formation of a recalcitrant form of phosphorus through fixation. This was evident in the amount of available phosphorus found in the termite mounds compared to the surface and subsurface soils. The importance of termite activities in the modification of soil pH is confirmed in the very strong relationship between the pH of termite mounds and that of the subsurface soil.

Cheik *et al.*, (2019) documented that, termite foraging activity is associated largely with the production of soil sheeting, these biogenic structures had similar soil properties to the nearby topsoil, except for their organic Carbon content. Particle size distribution between soil sheeting and their surrounding topsoil can be explained by the relatively homogeneous organization of the upper layer of Vertisols. Termites also prefer an acidic environment and their activities increase the pH of termite mound soil compared with adjacent soil. Considering the differences in the distribution areas, termite species, and properties of termite mounds and adjacent soils.

The study documents evidence that the activities of *Odontotermes spp* and *Reticulitermes spp* significantly influence the soil pH and that a soil pH increase may lead to termite inactivity resulting in death. de Lima *et al*., (2018) in their study revealed that Soil physical characteristics showed significant variations between land uses. The rubber plantations

presented the lowest values of soil physical quality, while the natural vegetation showed high soil physical quality. These changes affected the termite community and lead to changes in its composition with disproportionate loss of some species; however, some can acclimate well to the decline in the soil's physical quality.

2.9.2 Soil microbial Carbon and Nitrogen influence on termites

Moura, *et al* (2018) highlighted that Soil microbial biomass, excluding plant roots and animals larger than 5 μm^3 consists on average, 2 to 5% of soil organic matter. According to Makarov *et al*., (2016), soil organisms control the amount of carbon and nitrogen in the biosphere and perform important processes of their cycle. Some of the activities of the microorganisms are the mineralization of organic matter, the immobilization of elements in microbial biomass, nitrogen fixation, nitrification, and denitrification. Therefore, the microbial reservoir of carbon and nitrogen and their spatial and temporal dynamics are important parameters in the study of the nutrient cycles of these elements.

2.9.3 Chemical ecology of termites.

Plants produce numerous diverse bioactive compounds called Phytochemicals Altemimi et al., (2017) and Koche *et al.,* (2016) in their work recorded that phytochemicals have been classified as primary or secondary metabolites, based on their function in plant metabolism. Primary metabolites are the sugars, amino acids, proteins, purines, and pyrimidines of nucleic acids, and chlorophyll. Secondary metabolites contain alkaloids, terpenes, flavonoids, lignin, plant steroids, Saponins, phenolic, and glucosides. Wood is a natural organic material that contains mainly two groups of organic compounds: carbohydrates mainly (hemicelluloses and cellulose) and phenols (lignin), in larger quantities and smaller quantities, respectively as elucidated by Nascimeto *et al.,* (2013). Wood also consists of minor amounts of organic materials, mainly as organic extracts together with inorganic minerals (ash), mainly calcium, potassium, and magnesium.

Blassioli *et al*., (2016) define Semiochemicals as substances that elicit behavioral and physiological responses in the receiver which results in the interaction and attraction between them. Semiochemicals from plants and insects are important because they can be used for behavioral and ecological studies as elucidated by Chouvenc, *et al.*, (2015).

Semiochemicals produce different responses such as attraction, repulsion, detteration, and stimulation as recorded by Ahmad & Razaq, (2014). Trail pheromones produced by certain insects play an important role by influencing foragers to feed in certain areas Carolina, *et al*., (1994).

2.9.4 Termite pheromone mediated behavior.

Termite pheromones are secreted by sternal glands that are present in all species of termites. The sternal glands also produce sex pheromones which guide reproduction processes in termites. Termites place pheromone trails while searching for food sources so they can later recruit their nest mates to the location to forage. Foraging termites especially workers deposit trail- the following pheromone from sternal glands while returning to the nest. The pheromones elicit trail-following behavior in nest mates, leading them to a food source. Lubes & Cabrera, (2018) recorded that a trail pheromone mimic. After the trail pheromones termites also produce aggregation pheromones which ensures that the feeding (3Z,6Z,8E)-dodeca-3, 6, 8-train-1-of induced high trail following behavior is very at extremely low concentrations.

Aggregation pheromones elicit an aggregation behavior that involves the attraction of individual termites from a distance followed by the arrest of the termites at the pheromone source so that they can feed. Mitaka *et al*., (2017) in a study isolated aggregation pheromones in worker *Reticulitermes* termites. When foraging termites discover a new food source, they assemble their nest mates to that area for feeding and subsequent colonization of the new location. Other termite species produce Phagostimulant pheromone which also influences feeding behavior.

Phagostimulant pheromone is a hydroquinone present in the labial glands of worker termites. This compound acts as a Phagostimulant pheromone which elicits worker-feeding behavior in various termites. According to (Reinhard *et al*., 2003) *Mastotermes darwiniensis* workers, produced a phagostimulant hydroquinone (1,4-dihydroxybenzene) in small amounts but highly influenced termite feeding.

2.9.5 Plants foraged on by termites.

2.9.5.1 Grevillea robusta

C et al., (2009) recorded that *Grevillea robusta* is a deciduous tree that grows 15-50m tall. *G. robusta* grows naturally in many diverse habitats and is associated with either Eucalyptus or other Agroforestry tree species grown on farms. *G. robusta* grows well in soils rich in nutrients and well-distributed rain throughout the year. Mugunga, (2009) revealed that *G. robusta* seeds that are used for local planting are mainly collected from on-farm trees found on farmers` fields. Such trees are s thinned during harvesting. Despite its usefulness, *G. robusta* is affected by fungal diseases, particularly young stems. Termite attack has also caused considerable damage to the grey bark of the plant. Attack by termites can be a problem when planted on dry sites in Africa. Chetana & Ganesh, (2012) documented that *G. robusta* was intercropped with coffee as the two have ecological benefits to each other.

2.9.5.2 Eucalyptus

Bayle, (2019) in his work recorded that Eucalyptus is the most commonly grown tree species in forest plantations as well as community programs and farm woodlots. Eucalyptus grows well on poor soil and grows faster compared to indigenous tree species. The eucalyptus tree is useful for timber, wood fuel, and constructing houses and fences as recorded by Zhang & Wang, (2021). Additionally, Eucalyptus has great medicinal value in some countries. Termites also cause considerable damage to Eucalyptus both the seedlings and old trees.

2.9.5.3 Bamboo

Bamboo originates from the grass family. The bamboo plant is fast growing and evergreen plant which has internodes on the stem which are usually hollow. Bamboo plants possess a rhizome-dependent system thus suitable for many reclamation projects. Bamboo is regarded as a good tree for environmental conservation, and climate_change_adaptation as illustrated by Jijeesh & Seethalakshmi, (2009).

Bamboos are of immense economic and cultural significance in Asia. In East Asia and Africa, bamboo is used for building houses and as a food source as recorded by Nongdam & Tikendra, (2014). In their study Emamverdian *et al*., (2020) documented that 80% of bamboo forests are in Asia, 10% in Africa, and 10% in Latin America. Yang *et al*., (2014) reported that bamboo species, which dominate in habitats with contrasting light conditions, have a strong plasticity in morphological, anatomical, and photosynthetic properties. This high structural and functional plasticity may partially explain its ecological success in both open and shaded habitats. In a study conducted by Subekti *et al.*, (2015) on *Coptotermes formosanus* termites as pests of bamboo concluded that termites fed on bamboo and thus pose a threat to buildings constructed using bamboo.

2.9.5.4 Cypress

Cypress trees grow to over 30 meters tall and have a cone-shaped canopy. They have soft smooth bark which exudes gummy substances. The stem and leaves produce a characteristic smell. Asgary *et al*., (2013) revealed that cypress species containing essential oils such as from the branches and fruits of *Cupressus sempervirens* were found to possess promising inhibitory activity against hemoglobin and insulin glycation. Cypress is used for medicinal purposes in many parts of the world. In the Cypress plant tiny male and female reproductive structures are borne on the same tree, specifically at the tips of different branches as described by Liao *et al*., (2014). Baldi *et al* (2011) in their work recorded that Cypress was introduced in places that extend far beyond its natural distribution, and the tree is widely grown throughout the world. The cypress plant is however attacked with scales, aphids, and termites thus reducing their quality.

2.9.5.5 Maize

Huang *et al.*, (2020) described Maize as a staple food in many parts of the world, with total production exceeding that of rice and wheat. In addition to being consumed by humans, maize is also used to produce, livestock feed and other maize products, such as corn starch and corn syrup. There are six types of maize grown worldwide which include, dent corn, flint corn, pod corn, popcorn, flour corn, and sweet corn. Some maize varieties are rich in sugars and are usually grown for human consumption as kernels, while field

corn varieties are used for animal feed, various corn-based human food uses, pressing into corn oil, and fermentation and distillation into alcoholic beverages and as chemical feed stocks.

Maize production requires enough rainfall and in dry areas irrigation is supplemented to increase the yields according to Gadédjisso-Tossou *et al*., (2020). Maize pollen is anemophilous as described by Hofmann *et al.,* (2014), and because of its large settling velocity, most pollen falls within a few meters of the tassel thus success in fertilization. Sekamatte *et al*., (2003) documented that termites (Isoptera: Termitidae) are one of the most important maize production constraints in Uganda. Feeding damage on roots, stem bases and leaves frequently results in plant lodging and damage to cobs causing heavy yield losses in different parts of the country.

2.9.5.6 Avocado

The avocado tree is grown in tropical and temperate climates of many countries, with Mexico as the leading producer of avocados. Avocado is also grown in Africa where its fruit and wood are of great value. Termites were found to attack avocados in a study conducted by Wekhe *et al*., (2019). Avocado is grown in many parts of Africa. According to George *et al.*, (2018) Avocado in Kenya is widely grown in Western, Nyanza which are key edible termite-producing area.

2.9.5.7 Mango

Mango is a fruit tree that is grown worldwide, there are several varieties of mango and some are cultivated in Africa as documented by Litz, (2009). Mango fruit varies in size, shape, sweetness, skin color, and flesh color which can be yellow, purple, green, or orange. The Mango tree has dark green leaves and a thick stem. The stem of the Mango is white and is also attacked by insects. Novita *et al.*, (2020) noted that termites were serious pests of mango as they build their arboreal nests on Mango trees.

2.9.5.8 Blue citronella grass.

Blue Citronella is a grass that grows in many parts of Africa. According to Pinheiro *et al.*, (2013), blue citronella grass had termite-repellent properties. In some countries, it is

planted on large scale for commercial uses such as the production of citronella oil and antiseptics as revealed by Bibwe *et al.*, (2019).

2.9.5.9 Sugarcane

Sugarcane belongs to the grass family, as described by Cheavegatti-Gianotto *et al.*, (2011), an economically important family that includes maize, wheat, rice, sorghum, and many fodder crops. Sugarcane has ratooning potential and thus can be harvested over many seasons without planting new cuttings as described by Nadeem *et al*., (2020). Sugarcane is rich in sugar and is used to sweeten beverages, decorative finish for cakes, as a raw material in the food industry, or fermented to produce ethanol as revealed by Shintani, (2019). The stem produces bagasse which is used as fuel in many factories. Many sugarcane farms have a problem with moles and termites which leads to reduced yields as described by Miranda *et al*., (2004).

2.9.5.9.1 Neem

Ogbuewu, *et al.,* (2011)in their study documented that, Neem is a fast-growing plant of the Meliaceae family that originates from the Indian subcontinent and to dry areas throughout Asia. Neem was introduced to parts of Africa, the Caribbean, and many counties in South and Central America. Jodi *et al*., (2012) recorded that Neem trees grow to a height of 20-24 meters and have round canopies and thick-furrowed bark. According to Shahin-uz-zaman *et al*., (2008), new technologies for propagating Neem such as tissue culture are in use. Neem has very important uses and is commonly used in shampoos for treating skin diseases and in soaps or creams for the skin. Boadu *et al*., (2011) recorded that among the various herbs, neem plant-based insecticides have been the most used botanical pesticides, because of the presence of phytochemicals such as limonoids in neem plant extracts and oil that provides a sustainable pest control mechanism as reported by Harit *et al*., 2017). Fajar *et al*., (2021) also documented that Neem oil extracted from seed was repellant to termites.

2.9.6 Phytochemicals

There are approximately 4,000 phytochemicals that have been documented and are classified into protective function and physical and chemical properties. About 150 phytochemicals have been isolated and characterized in plants and are biologically active natural and thus provide health benefits for humans. Phytochemicals are classified as primary or secondary constituents, based on their role in plant metabolism. Primary constituents contain the sugars, amino acids, proteins, purines, and pyrimidines of nucleic acids, chlorophyll. Secondary phytochemicals include plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids, and glucosides. Literature surveys indicate that phenolics are the most numerous and structurally diverse. According to Larayetan *et al*., (2019) found that a phytochemical study of the ethyl acetate and methanol plant extracts revealed the presence of different bioactive compounds such as alkaloids, saponins, steroids, triterpenoids, fats and oils, flavonoids, phenols, and tannins.

Kurmukov, (2013) in his study revealed that standard preliminary phytochemical qualitative analysis of the extract was carried out for various plant constituents and screened for the presence or absence of biologically active compounds or secondary metabolites using standard procedures. In this study, the major phytochemical constituents isolated were alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins, and terpenoids in methanol extracts.

Belay & Sisay, (2014) describes Phytochemicals as compounds produced by plants. They include Alkaloids, tannins, triterpenoids, phenols, carbohydrates, and reducing sugars. Alkaloids contain neuroactive molecules, such as caffeine and nicotine, as well as important medicines such as emetine used to fight oral intoxication. Alkaloids can act as plant defense compounds, and thus are efficient against disease-causing organisms and predators due to their toxicity. Plants have developed excellent defense mechanisms such as efficient and specific signal transduction for triggering alkaloid accumulation.

Fraga-Corral *et al*., (2020) in their work documented that Tannins are polyphenolic compounds naturally found in green plants. The presence of phytochemicals in plants and vegetation has necessitated their historical use in different ways. The change in traditional

uses has made it easier for them to be modified. Natural tannins have been replaced by synthetic organic compounds that pose danger to human and animal health and damage to the environment. In their study, Mierziak *et al*., (2014) documented that, Flavonoids are small secondary metabolite molecules synthesized by plants with various biological activities. Due to their physical and biochemical properties, they are capable of participating in plants' interactions with microorganisms, animals, and other plants alongside their reactions to environmental stresses. The uses of tannins can be obtained from their strong antioxidative properties. Although an increasing number of studies focus on the application of flavonoids in medicine or the food industry much is needed to exploit other areas that might be useful.

In their work Sandjo & Kuete, (2013) noted that Triterpenoids are a biologically interesting group of terpenoids that consists of a large structural diversity of natural secondary plant metabolites. Bishayee *et al* (2011) documented that Triterpenoids are secondary metabolites of isopentenyl pyrophosphate oligomers and represent the largest group of phytochemicals. Approximately 20,000 terpenoids are found to exist in plants. They are found in various plants including seaweeds as well as in wax-like coatings of various fruits and medicinal herbs, apples, and cranberries.

2.9.7 Termite foraging behavior

In their study on termites Materu *et al.*, (2013) documented that termites forage mainly for food during the wet season and are highly reduced during the dry season. Exposure of termites on the soil surface during the dry season may lead to desiccation. The common food storage is feces and fungal comb in Microtermitinae subfamily or as cartons in other species. According to Korb *et al.*, (2019), the distribution and abundance of termites were found to be significantly correlated with a set of environmental variables such as Fire presence, Herbaceous Species Richness, Plant Species Richness, Plant Diversity, plant Families Diversity, and soil Organic Carbon. Rao *et al*., (2012) documented that termites of the genus Macrotermes were found actively feeding on only a twig litter or forage inside dead trees, and trunks, and were found to forage on live trees consuming only dead tissues. However, *C. hemi* and *H. indicola* were observed foraging on decomposing parts of the living trees and dead tree trunks. Thus termites play a very important beneficial role in

agrosilvicultural systems. Ravan *et al*., (2015) in his work recorded that termites usually feed on a wide variety of food sources like trees, dead wood, humus fungi, etc. Feeding preferences of the dry wood termite Cryptotermes brevis were studied and found that only pine was consumed in significantly less quantity both by volume and weight as compared to Balsa Western Red Cedar.

Poissonnier *et al.*, (2018) recorded that the optimal macronutrient balance in termites was mainly carbohydrates and this indicates the nutritional composition of wood. Termites have evolved to rely on their gut bacteria to supply nitrogen from their low- protein feed and thus may even survive on a pure cellulose diet. *Reticulitermes flavipes* were recorded to have stayed for more than 4 months on cellulose. This ability relies on their association with a large community of gut symbionts, which helps to break down plant tissues. Additionally, termites depend on the biochemical capacities of the symbionts as a nutritional resource. Wood- feeding termites make use of nitrogen from the atmosphere with the aid of Nitrogen-fixing bacteria in the gut to balance the low nitrogen content in their feed sources. The research recorded that termites could also depend on pure cellulose for a short time but can survive better on Eucalyptus wood, their natural diet.

According to Ali *et al*., (2021), Termite foraging always depends on the moisture of the feed substrate. In another study on *Reticulitermes spp.* with sugars and amino acids, Rosales & Isidro, (2006) found significant consumption differences in choice tests. Uric acid was preferred at 0.001 M, and some amino acids stopped feeding at 0.1 M. However, no compound stimulated feeding in no-choice tests, which agrees with the results herein. In another test with two alternatives, filter papers treated with aspartic acid at several concentrations were not more consumed by C. formosanus than the control. Results of the study by Suhara, (2020) indicate that *C. formosanus* preferred filter paper containing phosphatic fertilizers. The results suggest that *C. formosanus* may feed preferentially on food sources high in phosphate and take a proactive role in supplying the colony with needed phosphorus.

Peterson & Gerard, (2007) recorded that It was difficult to compare termite feeding between choice and no-choice tests because termites may consume a non-preferred food option in the absence of a more preferred option. In a choice test, termites may avoid a

certain wood when a more preferred wood is available, yet feed on the same wood in a no-choice situation out of necessity. Choice tests are limited in their ability to distinguish between food sources that are conditionally consumed and those that are unpalatable and may overestimate resistance. No-choice tests, on the other hand, do not differentiate between food sources that are readily consumed and conditionally consumed and may overestimate susceptibility.

2.9.8 Termite Attractivity to feed substrates.

In their work Indrayani *et al*., (2017) recorded that termites follow a concentration gradient of the attractant material to find food sources in the bait. The attractants used in the bait system consist of synthetic chemicals. In their experiment, a significantly faster termite movement to the bio-based attractant chamber was recorded the termite movement time in the clove leaf treatment was significantly lower than that of all other leaves. In treatments other than clove leaf, the termite moving moment was noted after some time for the concentration of 10% and 4 to 11 minutes for the concentration of 50%, except that of the cinnamon leaf treatment which was at 1.4 minutes. These results showed that termites can detect chemicals in the feed substrates and move toward chambers containing bio-based attractants. Additionally, these results also indicated that eugenol compounds in the leaf extracts have the potential to attract termites to find the bait matrix.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

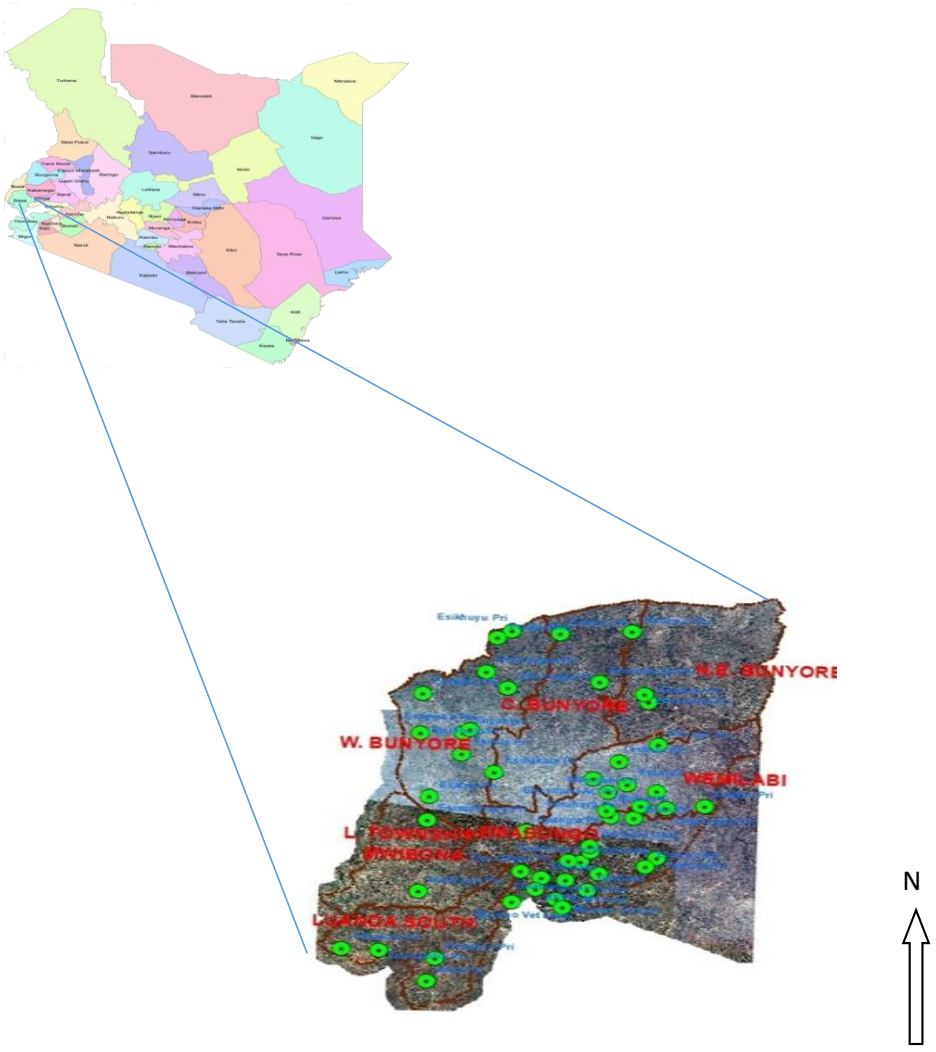

Figure 1: Map showing the study site. Source. Vihiga county Geospartial laboratory.

3.1.1 Study Site Location

Luanda sub-County is located 0◦, 30’ 34◦ 35’ E it has a population of 134,202 (2019 Census) and occupies an area of 1200km2. The agro-ecological zone is Lower midland 1. The climate is mainly tropical with variations due to altitude. Luanda Sub County is mainly warm with a mean temperature of 21º C and wets most of the year. The dominant soil type is sandy loam which is a mixture of clay and sand soil. The soil is usually fertile, and well-drained, with moderate moisture retention capabilities and varied particle size.

3.2. Economic Activities and Food Security

The main economic activity is farming, majorly, sugarcane and maize on large scale. Some farmers practice small-scale farming by growing soybeans, cassava, beans, tea, millet, and sorghum. Dairy farming is also practiced, specifically, zero grazing. The staple diet of the region is maize. Due to diversified farming, the study area is averagely food secure. In addition to the food crops, farmers in the Luanda sub-County of the western region also consume a variety of edible insects Kinyuru, (2015).

3.3 Sampling of soldier termites to determine the species composition

Solder termites were sampled in Luanda sub-County. This is because they have a high prevalence of termites. The sampling technique used was the belt transect method as described by Eggleton (2000) with minor modifications. The habitats that were sampled included grasslands, forests, farms, and hills. Sampling was done in the Agroecological zones as per the soil map of Vihiga County.

3.3.1 Determination of species composition of Solder termites in each habitat

The solder and worker termites were sampled as they possess the morphological features that guide in identification. A belt transect was set measuring 100 m long and 2 m wide and divided into 20 successive quadrate sections of 5 m x 2 Eggleton & Parr, (2012). Solder termites were collected by sampling wood, leaf litter, and surface soil down to 10–15 cm deep, visible nests, and galleries up to the height of 2 m in tree mounds close to the transect. Termite sampling was done randomly along a line transect of 500 to 1000 meters long in

natural vegetated land or grassland. Sampling was done from the lowland areas near Maseno up to the highlands in Emmatsi.

Solder termites and workers were sampled in a grid, 20cm below the ground, and 1 meter on trees. Each grid in the study site was thoroughly searched for any termite mud trails, tree trunks, fallen tree trunks, rotting vegetation, and termite mounds. The solder termites were sorted by hand using forceps according to different castes and kept in tubes containing 70% ethanol then taken to the National Museums of Kenya laboratory for identification. Information on the GPRS of the sampled site was recorded and the procedure was repeated in grasslands, forested areas, and hilly sites.

3.4 Morphological identification of termites.

The collected termites were examined at the National Museum of Kenya under real–time imaging dissecting microscope. Identification of termites was based on the description from Kaur & Abbasi, (2018) and Ackerman *et al*., (2009). The identification was done up to the species level. The features included the shape of mandibles, length of head, width, and height, pro – and mesonotum length and width, body length, shape and segment number of antennae, mandible length, and distance among marginal teeth of mandibles. The other features used in morphological identification were the length of the tibia, femur length, labrum, fontanelle, width and length, postmentum length and width, and nasus length. The termite species were sorted in each habitat and labeled.

3.5 Molecular Characterization of Termites.

The termite samples were further subjected to molecular identification to a certain their true identity or close relatives.

3.5. 1 Genomic DNA extraction

The genomic DNA was extracted from soldier termite heads since the head does not harbor microbes like the gut which could present a different source of DNA. The phenol: chloroform extraction method was adopted as described by Sambrook, Fritsch, and Maniatis (1989) with minor modifications as described below. One head of each soldier termite with complete features was sterilized and homogenized in 200 µℓ of TE (Tris EDTA pH 8) and crushed using a mortar and pestle.

The tissues were broken down by taking, 500 µℓ of lysis buffer (400 µℓ of TE and 100 µℓ of 5% SDS) and adding it to the homogenate followed by 20 µℓ of Proteinase K.

The mixture was incubated at 65°C for one hour. A mixture of 120 µℓ of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and the tubes were carefully vortexed for 30 seconds, and then centrifuged for 10 minutes at 10,000xg. The upper aqueous layer was carefully transferred to a fresh sterile Eppendorf tube, without destabilizing the protein layer at the interphase. To precipitate DNA 700 µℓ of ice-chilled isopropanol was added followed by Ammonium acetate and incubated at -20 °C overnight and then centrifuged at 12,000xg for 10 minutes. The supernatant was decanted out and discarded and the pellet was washed using 70 % ethanol followed by absolute ethanol. The washed pellet was air-dried for 30 minutes and eluted in 70 µℓ of TE buffer. The extracted gDNA was then stored at a temperature of -4 °C ready for subsequent use.

3.5.2 Determination of DNA quantity and quality

Agarose gel electrophoresis was performed to check for the presence and quantity of genomic DNA by running 4 µℓ of the extracted DNA on separate lanes of 0.8% Agarose gel stained with Ethidium bromide. The concentration of genomic DNA was determined by the optical density/absorbance generated automatically using a spectrophotometer.

3.5.3 Amplification and sequencing of COII gene

The extracted gDNA was used as a template for PCR amplification of the mtCOII gene fragment. A set of primers, A tLeu (5’ CAG ATA AGT GCA TTG GAT TT 3’) for the forward direction and B tLys (5’ GTT TAA GAG ACC AGT ACT TG 3’) for the reverse direction was used according to Miura *et al* (1998). A reaction mixture of 25 µℓ was made up of 2.5 µℓ of 10X PCR buffer, 2.0 µℓ MgCl2 (2.5 mM), 2.0 µℓ dNTPs (200 µM), 0.25 µℓ of *Taq* Polymerase (5U/ µℓ), 1 µℓ of each forward and reverse primer sequences, (5 Pico moles) 0.5 µℓ of DNA, and 15.75 µℓ of PCR water. A control reaction tube was set up with all the reagents except the template. The thermal cycling program was set as follows; initial denaturation at 95 o C for 5 minutes, 35 cycles of (denaturation at 95 o C for 30 seconds, annealing at 52 o C for 45 seconds, and extension at 72 o C for 1 minute), and a final at 72 o C for 7 minutes. 5 µℓ of each sample of the PCR products were loaded onto separate lanes of a 1 % agarose gel and gel electrophoresis was run for 30 minutes at

90 mA (150 V). The electrophoresis result was then visualized under UV illumination and photographed using a digital camera. The fragments were excised from the gel, solubilized in sodium iodide solution then bound to the (silica) column in the gene clean procedure. As described in the QIAquick PCR purification Kit protocol (Qiagen, Germany). DNA was eluted in 30 μℓ nuclease-free double distilled water (ddH2O).

3.5.4 Sequencing

-X version suite (Tamura et al., Gene cleaned DNA of the amplified fragments were sequenced at the Molecular and Infectious Diseases Research Lab of the University of Nairobi Kenya, using the Applied Biosystems Sanger's dye terminator method. Each of the analyzed pure samples was independently sequenced three times and the raw sequences with non-ambiguous consensus were selected.

3.5.5 Blast

BLAST in NCBI online application was used to find out the similarities of the obtained mtCOII gene sequences compared with other known termite species genes sequenced and submitted to the gene Bank NCBI (2013).

3.5.6 Phylogenetic analysis

Mitochondrial DNA sequences derived from this study were combined with related sequences obtained from NCBI's nucleotide database.

The sequences were then aligned using the Clustal-W program in Bio Edit (Version 7.05) and the phylogenetic relationships inferred from the aligned nucleotide sequences by the Neighbour-Joining method at Bootstrap 1000 replicates using Phylip program Felsenstein, (1985) as implemented in the MEGA2018).

3.6 Determination of soil chemical characteristics

In the Solder termite sampling area, one soil block measuring 25 x 25 x 30 cm was collected by digging a trench to 30cm deep around a quadrat of 25 x25 cm. The soil was scooped and the ground, was divided into 3 layers of 10cm depth (0- 9.9) (10- 19.9) (20-30) cm and mixed. Soil samples were packed in bags labeled and transported to Egerton university laboratories for analysis. About 200g of soil was collected per study site. The soil sample was also dried in an oven and then ground in the laboratory. Soil pH analysis, Available

Nitrogen, available phosphorus, Zinc, and Potassium w done as described by (Sarcinelli *et al*., 2009).

3.6.1 Soil pH analysis

The electrometrical method was used. About 20g of dry soil was weighed into a plastic bottle. 50mls of 1N Potassium chloride and distilled water in a ratio of 1: 2.5. The mixture was shaken using an electric shaker for 30 minutes. The suspension was allowed to settle for 10 minutes and the pH was determined using a pH meter.

3.6.2 Determination of organic carbon

About 0.5g of 0.5mm sieved dry soil was weighed into a 500ml conical flask. 10ml of Potassium dichromate was added into the flask with burette. 20ml of conc. Dilute sulphuric acid was added and swirled gently in a fume hood until the soil and reagents were mixed vigorously for one minute. The mixture was allowed to stand for 30 minutes. 200ml of distilled water and 10mls of concentrated orthophosphoric acid and a few drops of diphenylamine indicator were added slowly. A blank titration with 10ml of Potassium dichromate and 20ml of concentrated sulphuric acid without soil was done. Titration of the samples and the blank with 0.5N ferrous ammonium sulphate.

Calculations were done as follows,

1. Calculate the % C and % 0.M using the formula below

i) % C = $\frac{(B-T) \times 0.3 \times V}{Wt\ (g) \times B}$

Where: -

B = Blank titre

T = Sample titre

V = Volume of 1N $K_2Cr_2O_7$

0.3 = 1ml of 1N $K_2Cr_2O_7$ = 0.003g of C x 100%

ii) % 0.M = % C x 1.33

3.6.3 Determination of available Phosphorus in the soil (Mehlich and Olsen method)

The Mehlich method was developed in North Carolina and was first used to extract phosphorus from some strong phosphate-fixing North Canadian soils. This method is widely used and was introduced in Kenya by Mehlich when working at the National Agriculture Laboratories (N.A.L) Nairobi. The extractant is a general-purpose extractant designed to extract several Nutrient-elements in a single shaking. The extractant is used to determine both phosphorous and extractable, magnesium, potassium, sodium, and manganese.

The extracting solution is recommended by Mehlich and is a mixture of 0.01N HCl and 0.025N H2SO4. The phosphorus levels obtained by the Mehlich method are classified as follows: -

Level Classification

0 – 20ppm Deficient

20-80ppm Sufficient

More than 80ppm High

A: Extraction.

5g of air-dry sieved soil was weighed into a 50 ml plastic bottle. One spoonful of P-free charcoal was added. 25ml extraction solution was added and the mixture was shaken with the electric shaker for 30 minutes. The mixture was filtered into a 100ml flask.

B: Colour Development by Vanadium Yellow Method.

5 ml of the filtered extractant was measured in a clean test tube or colorimeter tube. 1ml of Ammonium vanadate/Ammonium molybdate solution mixture was added. The density of the colour developed was read at 430NM after 30 minutes for the samples.

C: Standard curve

5mls of the standard solutions were pipetted into test tubes and 1ml of the developing reagent was added. The colour density of the standard was read with a spectrophotometer set at 430NM.

3.6.4 Determination of available Nitrogen

Analysis of total nitrogen in soil and plant tissues required the complete oxidation of organic matter in the soil. This was done through ashing or acid mixture digestion. Wet

acid oxidation was based on Kjeldahl oxidation using a digestion block set at 360oC. The main advantage of this method was that single digestion was required [for either soil or plant samples] to remove all nutrients N and P into a solution for analysis.

The digestion mixture consisted of 0.42g selenium powder and 14g lithium sulphate to 350ml 30% Hydrogen peroxide was added and mixed well.

To the mixture,420ml of concentrated H2SO4 was slowly added while cooling in an ice bath.

The mixed indicator was prepared by mixing 0.1g of bromocreasol green and 0.066g of methyl red and dissolved in 100 ml absolute ethanol.

0.3g of sieved soil or plant tissue sample was weighed and placed into a digestion tube and 5 ml of digestion mixture was added. The digestion tube was placed into a digestion block which was set at 360oC.

The sample was digested for 2 hours and cooled for 30 minutes. The digested sample was transferred into a 100ml volumetric flask and diluted with distilled water to the mark and shaken. The blank was run [digestion mixture) as described above. 10mls of the digested sample was weighed into a distillation tube and 10mls of 40%

sodium hydroxide added. The receiver was prepared in a 650ml conical flask by measuring 10mls of 1% boric acid and 2mls of a mixed indicator. The sample was distilled for 4 minutes until the volume of the distillate was 150ml. The procedure was repeated the same with the blank. The samples were titrated with 0.1N HCl until the color turned from pink to green as the endpoint.

Calculation of the %N in the soil and the plant samples was done as follows.

Calculations

$$\%N = \frac{(S-B) \times NHCl \times 0.014 \times 100 \times 100\%}{Wt\ (g) \times 10mls\ (aliquot)}$$

Where: -

S = Sample titre

B = Blank titre

Wt = Weight of sample taken

10mls = Sample volume taken for distillation

3.6.5 Determination of Potassium

Available potassium present in the soil was extracted with neutral ammonium acetate of 1M. The level of Potassium was determined using a flame photometer. The apparatus consisted of multiple dispensers or automatic pipettes (25 ml); flasks, beakers (100 ml); and a flame photometer.

The reagents used were prepared as follows. About 77 g of ammonium acetate was dissolved in 1 litre of water. The pH was checked with a pH meter. If not neutral, ammonium hydroxide or acetic acid was added depending on the volume used to neutralize it to pH 7.0.

Standard potassium solution: 1.908 g of pure potassium chloride was dissolved in 1 litre of distilled water. This solution contained 1 mg K/ml. 100 ml of the solution was diluted to 1 litre with ammonium acetate solution. This gave 0.1 mg K/ml as a stock solution.
Working potassium standard solutions were made as follows: About 0, 5, 10, 15, and 20 ml of the stock solution was taken and diluted each volume separately to 100 ml with the molar ammonium acetate solution.

Preparation of the standard curve was done by setting the flame photometer and atomizing 0 and 20 μg K/ml solutions alternatively to readings of 0 and 100. Working standard solutions were atomized immediately and readings were recorded. Readings were plotted against the respective potassium contents and points connected with a straight line to obtain a standard curve.

Extraction: 25 ml of the ammonium acetate extractant was added to a conical flask fixed in the rack containing 5 g of soil sample. The mixture was shaken for 5 minutes and filtered. Potash in the filtrate was determined with the flame photometer.

3.6.6 Determination of Magnesium

Magnesium was determined by the versenate (EDTA) method. The soil sample containing Magnesium solution was titrated with 0.01N EDTA using EBT dye as an indicator at pH 10 using ammonium chloride and ammonium hydroxide buffer. At the endpoint, the colour changed from wine-red to blue or green. The apparatus required consisted of: a shaker; a porcelain dish; a beaker; a volumetric/conical flask. The reagents required were: EDTA or

versenate solution (0.01N). Ammonium chloride – ammonium hydroxide buffer solution. EBT indicator: 100 ml of ethanol was dissolved in 4.5 g of hydroxylamine hydrochloride. 0.5 g of the indicator was added and prepare the solution prepared. Hydroxylamine hydrochloride was used to remove the interference of Mn by keeping it in a lower valency state (Mn2+).

About 5 g of air-dried soil was put in a 150-ml flask, add 25 ml of neutral normal ammonium acetate solution and shaken on a mechanical shaker for 5 minutes, then filtered through No. 1 filter paper. Approximately 5 ml of aliquot was pipetted containing about 0.1 me of Ca plus Mg. About 2–5 crystals of carbamate were added and 5 ml of ammonium chloride – ammonium hydroxide buffer solution.3–4 drops of EBT indicator were added. This solution was titrated with 0.01N versenate until the colour changed to bright blue or green and no tinge of wine-red colour remained. For the calculation, if N1 and V1 were normality (concentration of Ca2+ + Mg2+) and volume of aliquot taken, and N2V2 were the normality and volume of EDTA used, respectively, then, N1V1 = N2V2; or N1 = ml of aliquot taken Normality of EDTA Vol. of EDTA 1 2 2 x = V N V Here, N1 (normality) = equivalents of Ca2+ plus Mg2+ present in 1 litre of aliquot.

3.6.7 Determination of Zinc

The preparation of the standard curve for zinc was done as described below. The reagents required were: Standard Zn solution: 1.0 g of pure zinc metal was weighed in a beaker. 20 ml of HCl (1:1) was added and kept for a few hours, allowing the metal to dissolve completely. The solution was transferred to a 1-litre volumetric flask and made up to the volume with glass-distilled water. This was 1 000 µg/ml Zn solution. For preparing the standard curve, refer to the 1 000 µg/ml solution as a solution. 1 ml of standard A was diluted to 100 ml to obtain a 10 µg/ml solution, which was designated standard B. Glass-distilled or demineralized acidified water of pH 2.5 ± 0.5 was made by diluting1 ml of 10 percent sulphuric acid to 1 litre with glass-distilled or mineralized water and adjusted the pH to 2.5 with a pH meter using 10 percent sulphuric acid or sodium hydroxide. This solution was acidified water.

Working Zn standard solutions were as described. 1, 2, 4, 6, 8, and 10 ml of standard B solution was pipetted in 50-ml numbered volumetric flasks and the volume was made up

with DTPA solution to obtain 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 µg/ml zinc. The flasks were stoppered and shaken well. Fresh standards should be prepared whenever a fresh lot of acidified water is prepared.

The procedure was:

Flaming the solutions: The standards were atomized on an AAS at a wavelength of 213.8 nm (Zn line of the instrument). A standard curve of known concentrations of Zn solution was prepared by plotting the absorbance values on the y-axis against their respective Zn concentration on the x-axis.

3.7 (Objective 3) Determination of feed preferences of worker termites on selected plants (Uncrushed and crushed) in an open field bioassay.

Test plants were selected from termite habitats in the Luanda sub-county. The design of the study used was a randomized complete block design. The plants were Sugarcane, Bamboo, Grevillea, Blue citronella grass, maize stems, Eucalyptus, Cypress, Mango, Avocado, Neem, and a mixture of all the test plants. The control had no feed material.

A field measuring 100m by 40m was used. A randomized complete design was used. Rectangular holes measuring 25cm by 15 cm were dug at a spacing of 3m apart. There were eleven treatments replicated four times. About 200g of chopped dry materials of the test plants were assigned randomly in the dug holes and covered with grass turf at 6 pm. The feed traps were checked the following day at 6 am before termites move out. The number of termites foraging on the plant materials was removed and placed into a plastic basin, counted using forceps. This procedure was repeated in four different locations spread 500m apart. The test plants that showed high feeding preference were selected for laboratory phytochemical screening. This procedure was repeated with crushed test plants.

3.7.1 Collection of test plant material.

The bark and stem of fresh mature *Grevillea robusta* were collected from the Luanda Sub-county of Vihiga County. The stem was dried at 60°C in a shade for 72 h. The resulting dried sample was ground using a mill (Jehmlich, Germany) and placed in bags, and transported to the National Museum of Kenya for further experiments. The same procedure was repeated for

Eucalyptus grandis, Sugarcane, Maize, Bamboo, and Cypress.

3.7.2 Preparation of plant extracts

About 200g of *Grevillea robusta* powder was soaked in Methanol and Dichloromethane at a sample: solvent ratio of 1:20 (w/v) for 48 h at 24°C. The mixture was filtered in glass containers over cotton wool. The mixtures were homogenized at 60°C for 4 h using a homogenizer (IKA, Germany). The extracts were filtered using filter paper, concentrated at 60°C using a rotary evaporator (Polylab, India), and freeze-dried for 24 h. All freeze-dried extracts were stored at 4°C before further experiments. All experiments were repeated in triplicate. The same procedure was repeated using sugarcane, maize, bamboo, Cypress, and Eucalyptus extract.

3.7.3 Detection of alkaloids

Alkaloids were detected according to (T *et al*., 2015), the Dragendorff's test. 2 mg of the methanolic extract was placed in a test tube and 5 ml of distilled water was added. 2M Hydrochloric acid was added until an acid reaction occurred. To the mixture, 1 ml of Dragendorff's reagent was added. The formation of orange or orange-red precipitate indicated the presence of alkaloids.

3.7.4 Detection of reducing sugars

About 2 mg of methanolic extract was shaken with 10 ml of water in a test tube, filtered, and the filtrate was concentrated. To the concentrate 5 ml of Benedict's solution was added and boiled for 5 minutes. The formation of brick red colored precipitate indicated the presence of carbohydrates.

3.7.5 Detection of carbohydrates

Approximately 2 mg of methanolic extract was shaken with 10ml of water in a test tube, filtered and the filtrate was concentrated. To the filtrate 2 drops of freshly prepared 20% alcoholic solution of α- naphthol was added. 2 ml of conc. sulphuric acid was added to form a layer below the mixture. A Red-violet ring appeared, indicating the presence of carbohydrates which disappeared with the addition of an excess of alkali.

3.7.6 Detection of monosaccharides

About 1ml of the sample was placed in a dry test tube.1ml of distilled water was placed in another tube as control. 2ml of Barfoed's reagent was added to all the tubes and Kept in a boiling water bath. Time taken to develop a brick-red color was observed.

3.7.7 Detection of flavonoids.

Flavonoids were detected according to the method described (Banu & Cathrine, 2015) the Shinoda test. A bout2 mg of methanolic extract was dissolved in 5ml of ethanol in a test tube and the mixture of 10 drops of dilute hydrochloric acid was added followed by a small piece of magnesium. The formation of pink, reddish or brown color indicated the presence of flavonoids.

3.7.8 Detection of Glycosides

Approximately 2 mg of ethanolic extract was shaken with 10 ml of water in a test tube, filtered, and the filtrate concentrated. To the filtrate, 2-3 drops of Molisch's reagent were added together with 2ml of concentrated sulphuric acid, carefully through the side of the test tube. A reddish-violet ring appears, indicating the presence of glycosides.

3.7.9 Detection of Triterpenoids

About 2 mg of dry extract was dissolved in acetic anhydride in a test tube, heated to boiling, and cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. The Formation of pink color indicated the presence of Triterpenoids.

3.7.9.1 Detection of resins

Resins were detected according to the method described by Brunni et al., (2019)
. About 1 ml of methanolic extract was dissolved in acetone in a test tube and distilled water was added to the mixture. Turbidity indicated the presence of resins.

3.7.9.2 Detection of Saponins

A drop of sodium bicarbonate solution was added to a test tube containing about 5 ml of methanolic extract in a test tube. The formation of honeycomb-like froth indicates the presence of Saponins.

3.7.9.3 Detection of Steroids

Approximately 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled, and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. The formation of green color indicated the presence of steroids.

3.7.9.4 Detection of tannins

Tannins were detected according to the method described by Galdo et al., (2006). About 1-2 ml of the ethanolic extract and a few drops of 5% w/v Iron(III) chloride solution was added to a test tube. The green color indicated the presence of gallotannins, while the brown color indicated the presence of pseudo-tannins.

Note

These tests were repeated on samples of Eucalyptus, Sugarcane, Maize, Bamboo, and Cypress.

3.8 Evaluation of selected plant extracts as termite attractants.

The extracts that were used in this procedure were obtained from Grevillea, Eucalyptus, Maize, Sugarcane, Bamboo, and Cypress. A Y-shaped metallic tube measuring 60cm and each arm 20cm. An entrance and outlet were placed on the metallic tube for easy placement and monitoring of termites' movement. The experiment was done in a room in the dark between 7 pm and 2 am. Ten soldier termites and ten worker termites were used in this procedure; they were placed at the entrance of the Y-shaped metal tube. 20g of the solid extract was placed in a small container inside the end of the metal tube while the other arm was the control i.e. no substance was placed. The movement towards the two arms was recorded. The soldier and worker termites that moved to the extract were counted. The procedure was repeated by rotating the metal container to face in a different direction. Each time fresh termites and the extract was used and the metal tube was washed in acetone. The metal tube was rotated three times. This experiment was repeated using Eucalyptus, Sugarcane, Maize, Bamboo, and Cypress,

3.9 Data Analysis

The species richness of each habitat was analyzed for diversity (Shannon-Wiener) index and Simpson index by using Vegan package version 1.16-32 in R.

Shannon diversity indices

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

Where,

pi is often the proportion of individuals belonging to the species in the dataset of interest.

Simpson diversity indices

$$D = 1 - \left(\frac{\Sigma\, n(n-1)}{N(N-1)} \right)$$

Where

n = the total number of organisms of a particular species

N = the total number of organisms of all species

The differences in species composition and diversity of termites in different collection sites in the Luanda sub-County were analyzed using one-way ANOVA and the Least Significance Difference test (LSD) will be performed. chemical parameters were compared using the LSD test while termite feed preference bioassay was analyzed using one-way ANOVA and LSD test.

In summary data for each objective was analyzed as follows.

Objective 1. Species diversity using Shannon and Simpson diversity indices.

Objective 2. One- way ANOVA

Objective 3. One-way ANOVA and Post Hoc test.

3.9.1 Ethical clearance

Permission to carry out this research in the local communities and farms was obtained from the Ethical review committee of Jaramogi Oginga Odinga University of Science and Technology, and National Commission of Science, Technology, and Innovation (NACOSTI license number NACOSTI/P/21/5797) (Appendix 1

CHAPTER FOUR

RESULTS

4.1 (OBJECTIVE 1) To determine the species diversity of termites in Luanda sub county.

Determination of species diversity was achieved by first characterizing the soldier termites morphologically. The species diversity was calculated using Simpsons and Shannon diversity indices on a scale of 0 to 1.

4.1.1 Identification of termite species in Luanda Sub County

Soldier termites were identified morphologically at the National Museums of Kenya and the species listed below were identified in the 47 sites within Luanda sub county.

Table 1. Table showing *Pseudocanthotermes militaris* and site where it was collected.

Pseudocanthotermes militaris was collected in six sites namely Mulwanda, Ilonje, Elukunza, Ebiba, Ebukanga 2, and Ekwanda. The highest species richness was at Ekwanda 2 (6.91440) while lowest species richness was at Mulwanda 95.95014). The major features used to distinguish termite genera are shape of the mandibles, position of the mandibular tooth, shape and size of the head, labrum, fontanelle and shape of postmentum and pronotum (Kalleshwara, *et al* 2013).

Table 1: ***Pseudocanthotermes militaris*** and site where it was collected

Site code	Site Name	Species richness	s.d	Longitude	Latitude	Species
19	Mulwanda	5.95014	0.68727	34.63194 E	0.04083 N	*Pseudo militaris*
21	Ilonje	6.09741	0.66292	34.5800 E	0.04827 N	*Pseudo militaris*
24	Elukunza	6.28064	0.62274	34.63622 E	0.04009 N	*Pseudo militaris*
26	Ebiba	6.38323	0.59425	34.60236 E	0.04128 N	*Pseudo militaris*
37	Ebukanga 2	6.77241	0.41882	34.59143 E	0.09946 N	*Pseudo militaris*
43	Ekwanda 2	6.91440	0.27865	34.56234 E	0.0347 N	*Pseudo militaris*

The major distinguishing feature of Pseudocanthotermes militaris is the
Distal end of leaf mandible slightly incurved, not bent like a beak as shown in **Fig 2**

Figure 2: Photograph of Head of soldier of *Pseudocanthotermes militaris*

4.1.4 *Pseudocanthotermes grandicepes* and site where it was collected.

Pseudocanthotermes grandiceps was collected in three sites namely Esibakala, Esianda, and Ibubi. Esibakala had the highest species richness of (5.86704) while Ibubi had the lowest species richness.

Table 2. *Pseudocanthotermes grandicepes* and site where it was collected.

CODE	SITE	Species richness	s.d	LONGITUDE	LATITUDE	SPECIES
18	Esibakala	5.86704	0.69846	34.6333 E	0.03935 N	*Pseudo grandicepes*
15	Esianda	5.56884	0.72625	34.62626 E	0.04427 N	*Pseudo grandicepes*
12	Ibubi	5.16922	0.73911	34.6333 E	0.03935 N	*Pseudo grandicepes*

The key features for P. grandiceps identification are one or both mandibles with crenulations or serrations or teeth on the inner margin. The mandible are also closed as illustrated in **Fig 3**

Figure 3: Photograph of head of soldier of *Pseudocanthotermes grandicepes*

Macrotermes bellicosus was collected in 9 sites. The site with the highest species richness was Ebukanga 1 (6.95745) while Maseno 2 had the lowest species richness (5.31655).

Table 3: Sites *Macrotermes bellicosus* and site where it was collected.

Code	Site	Species Richnesss.	s.d	Longitude	Latitude	Species
30	Mwikunga 2	6.55318	0.53450	34.61007 E	0.01562 N	*Macrotermes bellicosus*
31	Maseno 3	6.58971	0.51904	34.6027E	0.00312 N	*Macrotermes bellicosus*
33	Maseno 4	6.65694	0.48736	34.60213 E	0.00709 N	*Macrotermes bellicosus*
22	Esalwa	6.16296	0.64994	34.6333 E	0.03935 N	*Macrotermes bellicosus*
45	Ebukanga 1	6.95745	0.20185	34.6012 E	0.09980 N	*Macrotermes bellicosus*
41	Essumba	6.86987	0.33442	34.5124 E	0.05278 N	*Macrotermes bellicosus*
14	Emmaloba	5.44908	0.73278	34.56514 E	0.02802 S	*Macrotermes bellicosus*
13	Maseno 2	5.31655	0.73727	34.60175 E	0.00597 N	*Macrotermes bellicosus*
48	Khwiliba	5.54328	0.72156	34.57106 E	0.01122 S	*Macrotermes bellicosus*

Macrotermes bellicosus is characterised by Inner margin of mandibles having one prominent tooth and the left mandible with crenulations in basal half and right mandible is lacking them as shown in Fig 4.

Figure 4: Photograph showing head of soldier of *Macrotermes bellicosus*

4.1.6 *Macrotermes herus* and site where it was collected.

Macrotermes herus was collected in 18 sites. Waluka had the highest species richness (6.93605) while Ekwanda had the lowest species richness of (2.98703). Waluka is found in the hilly parts of Luanda while Ekwanda is found in the lowlands.*M. herus* soldiers are characterised by monomorphic, labrum without hyaline tip **fig. 5**

Figure 5: Photograph showing head of soldier of Macrotermes *herus*

Table 4: showing *Macrotermes herus* and site where it was collected.

CODE	Name	Species richness	s.d	Longitude	Latitude	Species
4	Ekwanda	2.98703	0.47022	34.57178 E	0.02261 S	*Macrotermes herus*
5	Maseno 1	3.42188	0.55501	34.60248 E	0.00748 N	*Macrotermes herus*
6	Emmunwa	3.78888	0.61898	34.61972 E	0.023925 N	*Macrotermes herus*
7	Emmatsi	4.10279	0.66523	34.61411 E	0.00436 N	*Macrotermes herus*
9	Emabungo	4.61137	0.71833	34.60926 E	0.00083 N	*Macrotermes herus*
10	Eliang'oma	4.81994	0.73102	34.63891 E	0.02558 N	*Macrotermes herus*
16	Emmabwi	5.67750	0.71816	34.58273 E	0.09307 N	*Macrotermes herus*
17	Emusenjeli	5.77650	0.70881	34.64576E	0.04178 N	*Macrotermes herus*
23	Asiongo	6.22388	0.63653	34.60520 E	0.00324 N	*Macrotermes herus*
25	Emutsa	6.33364	0.60864	34.60613 E	0.02418 N	*Macrotermes herus*
32	Ebusiratsi 2	6.62424	0.50335	34.6120 E	0.05989 N	*Macrotermes herus*
34	Ebusiratsi 1	6.68796	0.47102	34.62829 E	0.05866 N	*Macrotermes herus*
35	Emwatsi	6.71746	0.45424	34.57604 E	0.007560 N	*Macrotermes herus*
38	Esibila hill	6.79814	0.39983	34.5813 E	0.06144 N	*Macrotermes herus*
39	Emukhuya	6.82288	0.37967	34.62588 E	0.02243 N	*Macrotermes herus*
40	Esibila down hill	6.84675	0.35802	34.5946 E	0.06300 N	*Macrotermes herus*
44	Waluka	6.93605	0.24408	34.64212 E	0.02248 N	*Macrotermes herus*
47	Ebukolo	7.000	0.23243	34.58730 E	0.006255 N	*Macrotermes herus*

4.1.7 sites *Macrotermes spp1* and site where it was collected.

*Macrotermes spp*1 was collected at Ebulako 34.61169 E 0.00194N. *Macrotermes spp*1 had species richness of 6.47339. Ebulako was at the foot hill of Emmatsi hill with plenty of rock outcrops.

Table 5: Sites *Macrotermes spp1* and site where it was collected.

Code	Site	Species richness	s.d	Latitude	Longitude	Species
28	Ebulako	6.47339	0.56479	34.61169E	0.00194 N	*Macrotermes spp1*

Macrotermes spp1 is characterised with one or both mandibles with crenulations or serrations or teeth on the inner margin only left mandibles has either prominent tooth or crenulations on inner margin.

Figure 6: Photograph showing *Macrotermes spp1*

4.1.8 *Macrotermessp1* and site where it was collected.

Macrotermes sp1 was collected in 8 sites namely Mwikunga, Esibuye, Esiamatete, Murram, Enyahera, Essongolo, Mukhunzulu, and Esibuye 2. The species highest richness was Mukhunzulu at 6.97872 while the lowest was Mwikunga at 1.82239.

Table 6: *Macrotermessp1* and site where it was collected.

Code	Site	Species richness	s.d	Longitude	Latitude	Species
2	Mwikunga	1.82239	0.24459	34.62037 E	0.02418 N	*Macrotermes sp1*
3	Esibuye 1	2.46364	0.36405	34.63854 E	0.08564 N	*Macrotermes sp1*
27	Esiamatete	6.42972	0.57963	34.59099 E	0.1113 N	*Macrotermes sp1*
29	Murram	6.51447	0.54974	34.62862 E	0.04084 N	*Macrotermes sp1*
36	Enyahera	6.74556	0.43689	34.4216 E	0.05287 N	*Macrotermes sp1*
42	Essongolo	6.89238	0.30828	34.6332 E	0.03962 N	*Macrotermes sp1*
`46	Mukhunzulu	6.97872	0.14430	34.6023 E	0.1267 N	*Macrotermes sp1*
11	Esibuye 2	5.00467	0.73742	34.63954 E	0.09524 N	*Macrotermes sp1*

Macrotermes sp1 is characterised by Inner margin of mandibles with one prominent tooth. Left mandible with crenulations in basal half and right mandible lacking them **Fig 7**

Figure 7: Photograph the head of the soldier of *Macrotermes sp1*

4.1.9 *Macrotermes spp2* and site where it was collected.

Macrotermes spp2 was collected in two sites, Itabalia and Ebukanga 3. The species richness was highest at Itabalia (5.86704) while the lowest was (5.56884) at Ebukanga 3.

Table 7: *Macrotermes spp2* and site where it was collected.

Code	site	Species richness	s.d	latitude	longitude	species
1	Itabalia	5.86704	0.69846	34.6333 E	0.03735 N	*Macrotermes Spp2*
8	Ebukanga 3	5.56884	0.72625	34.58623 E	0.09424 N	*Macrotermes Spp2*

Macrotermes spp2 is characterised by having Mandibles twisted and largely asymmetrical**.** The antennae is serrated with 15 segments

Figure 8; Photograph of head of soldier of *Macrotermes spp2*

4.2 Molecular Identification of termite species in Luanda sub county

Soldier termites were identified using molecular identification procedures at the National Museums of Kenya and the species listed below were identified in the 47 sites within Luanda sub county**.**

4.2.1 Macrotermes bellicosus

Macrotermes bellicosus was identified in samples collected in 6 sites**,Table 8**.The areas sampled are Itabalia, Esikhuyu, Esiamatete, Murram, Essongolo and Mukhunzulu. Analysis of the soldier termite samples through the PCR process showed the DNA of the Macrotermes bellicosus.

Table 8: *Macrotermes bellicosus* and sites.

Code	site	Longitude	latitude	species
1	Itabalia	34.6333 E	0.03735 N	*Macrotermes bellicosus*
20	Esikhuyu	34.55087 E	0.1231 N	*Macrotermes bellicosus*
27	Esiamatete	34.59099 E	0.1113 N	*Macrotermes bellicosus*
29	murram	34.62862 E	0.04084 N	*Macrotermes bellicosus*
42	Essongolo	34.6332 E	0.03962 N	*Macrotermes bellicosus*
46	Mukhunzulu	34.6023 E	0.1267 N	*Macrotermes bellicosus*

4.2.2 Macrotermes herus

Macrotermes herus was identified in 38 sites,**Table 9**. *Macrotermes herus* was the most dorminant species identified in the 47 sites within Luanda sub County.

Table 9: *Macrotermes herus* and sites

code	name	longitude	latitude	Species
3	Esibuye 1	34.63854 E	0.08564 N	*Macrotermes herus*
11	Esibuye 2	34.63954 E	0.09524 N	*Macrotermes herus*
14	Emmaloba	34.56514 E	0.02802 S	*Macrotermes herus*
17	Emusenjeli	34.64576E	0.04178 N	*Macrotermes herus*
21	Ilonje	34.5800 E	0.04827 N	*Macrotermes herus*
23	Asiongo	34.60520 E	0.00324 N	*Macrotermes herus*
25	Emmutsa	34.60613 E	0.02418 N	*Macrotermes herus*
28	Ebulako	34.61169E	0.00194 N	*Macrotermes herus*
30	Mwikunga 2	34.61007 E	0.01562 N	*Macrotermes herus*
31	Maseno 3	34.6027E	0.00312 N	*Macrotermes herus*
34	Ebusiratsi 1	34.62829 E	0.05866 N	*Macrotermes herus*
35	Emwatsi	34.57604 E	0.007560 N	*Macrotermes herus*
38	Esibila hill	34.5813 E	0.06144 N	*Macrotermes herus*
39	Emukhuya	34.62588 E	0.02243 N	*Macrotermes herus*
40	Esibila down hill	34.5946 E	0.06300 N	*Macrotermes herus*
41	Essumba	34.5124 E	0.05278 N	*Macrotermes herus*
44	Waluka	34.64212 E	0.02248 N	*Macrotermes herus*
45	Ebukanga 1	34.6012 E	0.09980 N	*Macrotermes herus*
47	Ebukolo	34.58730 E	0.006255 N	*Macrotermes herus*
48	Khwiliba	34.57106 E	0.01122 S	*Macrotermes herus*
2	Mwikunga 1	34.62037 E	0.02418 N	*Macrotermes subhyalinus/herus*
6	Emmunwa	34.61972 E	0.023925 N	*Macrotermes subhyalinus/herus*
7	Emmatsi	34.61411 E	0.00436 N	*Macrotermes subhyalinus/herus*
9	Emabungo	34.60926 E	0.00083 N	*Macrotermes subhyalinus/herus*
10	Eliang'oma	34.63891 E	0.02558 N	*Macrotermes subhyalinus/herus*
13	Maseno 2	34.60175 E	0.00597 N	*Macrotermes subhyalinus/herus*
16	Emmabwi	34.58273 E	0.09307 N	*Macrotermes subhyalinus/herus*
33	Maseno 4	34.60213 E	0.00709 N	*Macrotermes subhyalinus/herus*
37	Ebukanga2	34.59143 E	0.09946 N	*Macrotermes subhyalinus/herus*
4	Ekwanda	34.57178 E	0.02261 S	*Macrotermes herus*
5	Maseno 1	34.60248 E	0.00748 N	*Macrotermes herus*
6	Emmunwa	34.61972 E	0.023925 N	*Macrotermes herus*

7	Emmatsi	34.61411 E	0.00436 N	*Macrotermes herus*
9	Emabungo	34.60926 E	0.00083 N	*Macrotermes herus*
10	Eliang'oma	34.63891 E	0.02558 N	*Macrotermes herus*
16	Emmabwi	34.58273 E	0.09307 N	*Macrotermes herus*
17	Emusenjeli	34.64576E	0.04178 N	*Macrotermes herus*
23	Asiongo	34.60520 E	0.00324 N	*Macrotermes herus*
25	Emutsa	34.60613 E	0.02418 N	*Macrotermes herus*
32	Ebusiratsi 2	34.6120 E	0.05989 N	*Macrotermes herus*
34	Ebusiratsi 1	34.62829 E	0.05866 N	*Macrotermes herus*
35	Emwatsi	34.57604 E	0.007560 N	*Macrotermes herus*
38	Esibila hill	34.5813 E	0.06144 N	*Macrotermes herus*
39	Emukhuya	34.62588 E	0.02243 N	*Macrotermes herus*
40	Esibila down hill	34.5946 E	0.06300 N	*Macrotermes herus*
44	Waluka	34.64212 E	0.02248 N	*Macrotermes herus*
47	Ebukolo	34.58730 E	0.006255 N	*Macrotermes herus*

4.2.3 Macrotermes subhyalinus

Macrotermes subhyalinus was identified in 6 sites namely Ekwanda, Maseno 1, Ebukanga 2, Esalwa, Ebusiratsi, and Enyahera. **Table 10**.

Table 10: *Macrotermes subhyalinus* and sites.

CODE	NAME	Longitude	latitude	species
4	Ekwanda	34.57178 E	0.02261 S	*Macrotermes subhyalinus*
5	Maseno 1	34.60248 E	0.00748 N	*Macrotermes subhyalinus*
8	Ebukanga 2	34.59143 E	0.09946 N	*Macrotermes subhyalinus*
22	Esalwa	34.6333 E	0.03935 N	*Macrotermes subhyalinus*
32	Ebusiratsi 2	34.6120 E	0.05989 N	*Macrotermes subhyalinus*
36	Enyahera	34.4216 E	0.05287 N	*Macrotermes subhyalinus*

4.2.4 Psudocanthotermes militaris

Pseudocanthotermes militaris was identified in 7 sites in Luanda sub county, they include Ibubi, Esianda, Esibakala, Mulwanda, Elukunza, Ebiba, and Ekwanda Table 11.

Table 11. *Pseudocanthotermes militaris* and sites.

Code	Name	longitude	Latitude	species
12	Ibubi	34.6333 E	0.03935 N	*Pseudocanthotermes militaris*
15	Esianda	34.62626 E	0.04427 N	*Pseudocanthotermes militaris*
18	Esibakala	34.6333 E	0.04427 N	*Pseudocanthotermes militaris*
19	Mulwanda	34.63194 E	0.04083 N	*Pseudocanthotermes militaris*
24	Elukunza	34.63622 E	0.04009 N	*Pseudocanthotermes militaris*
26	Ebiba	34.60236 E	0.04128 N	*Pseudocanthotermes militaris*
43	Ekwanda 2	34.56 .234 E	0.0347 S	*Pseudocanthotermes militaris*

4.3 Analysis of results for morphological and molecular identification of termites.

4.3. 1 Species diversity of termites in Luanda sub county.

Soldier and worker termites were sampled in Luanda sub-County, in 47 sites. The Shannon diversity index was **H** = 0.3606685, while Simpson index was **D** = 0.2064429

4.4 Species richness

The total number of species identified in Luanda sub county was 7 and the species accumulation curve is shown in Fig 1. Showing the abundance of species in the 47 sites

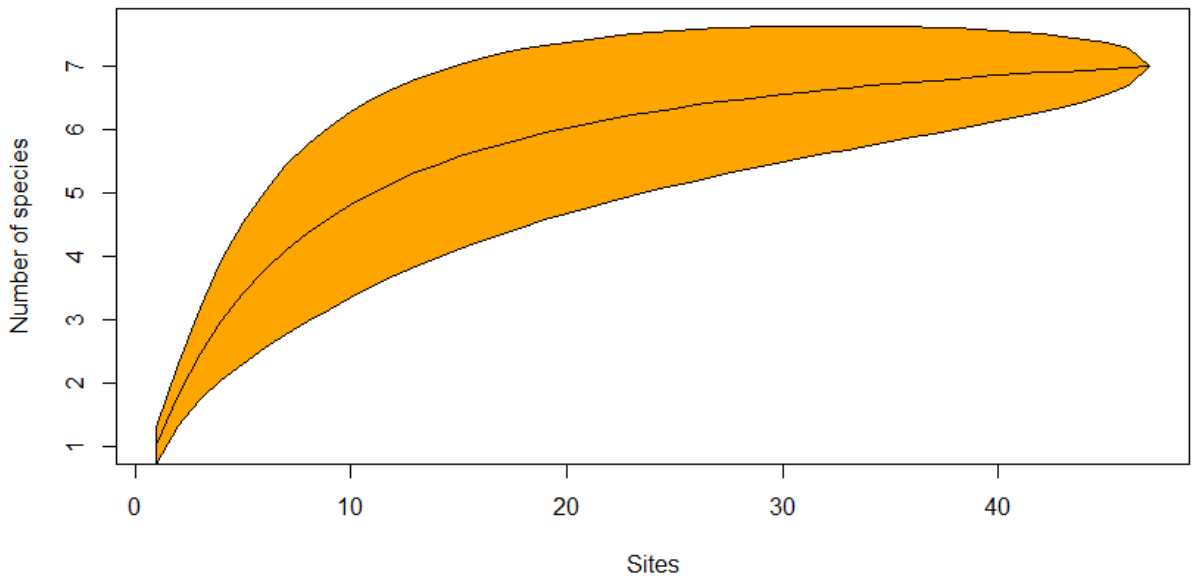

Figure 9:The species accumulation curve for species diversity data of termites in Luanda using the exact method.

4.5 Analysis of variance of termite species

The results from ANOVA show that the difference between Soldier s and workers was significant at **p < 0.05 . Appendix iii**. Comparison between the number of soldiers and workers. Post hoc test was done showed that there was no significant difference between the number of soldiers and workers. A box plot was developed, figure **11** to show comparison of sampled workers and solder termites in Luanda sub county

Figure 10: Boxplot of termite species count across the workers and soldiers.

Table 12: Mean summary of the termites collected, soldier and workers.

Comparisons between number of workers and solders sampled in Luanda sub county table **12**. The mean for workers was higher (177.250) and 84.625 for soldier termites collected .

Termite	**Mean ± SE**
Workers	177.250 ± 10.52[a]
Soldiers	84.625 ± 16.64[b]

Different letters indicate significant difference (least significant difference at 5%)

4.6 Comparison between different termite species.

A total of 7 species were identified out of which six species had no significant difference (a) while there was significant difference in *Macrotermes spp1* (a b)

Table 13. Table showing mean termites of different species

Mean termite species identified morphologically in Luanda sub county Table **16.** The mean for Macrotermes herus was higher in Luanda (192.63889) while Macrotermes species 2 has low mean of 30.5000.

Macrotermes herus	192.63889[a]
Macrotermes spp1	140.50000[ab]
Macrotermes sp1	134.93750 [b]
Pseudo militaris	92.75000[b]
Pseudo grandicepes	85.16667 [b]
Macrotermes bellicosus	77.11111[b]
Macrotermes spp2	30.50000[b]

4.7 Molecular identification of termites and their phylogeny.

Samples of termites were processed and characterized genetically. The procedure gave the results illustrated in Fig 3 and 4.

4.7.1 Polymesare chain reaction amplified products of CO11 gene.

The PCR procedure was done and the samples subjected to gel electrophoresis. The results of the gel electrophoresis are shown in fig 11. The numbers 1 to 11 were the samples collected from 1 to 11. The bp was below 500bp

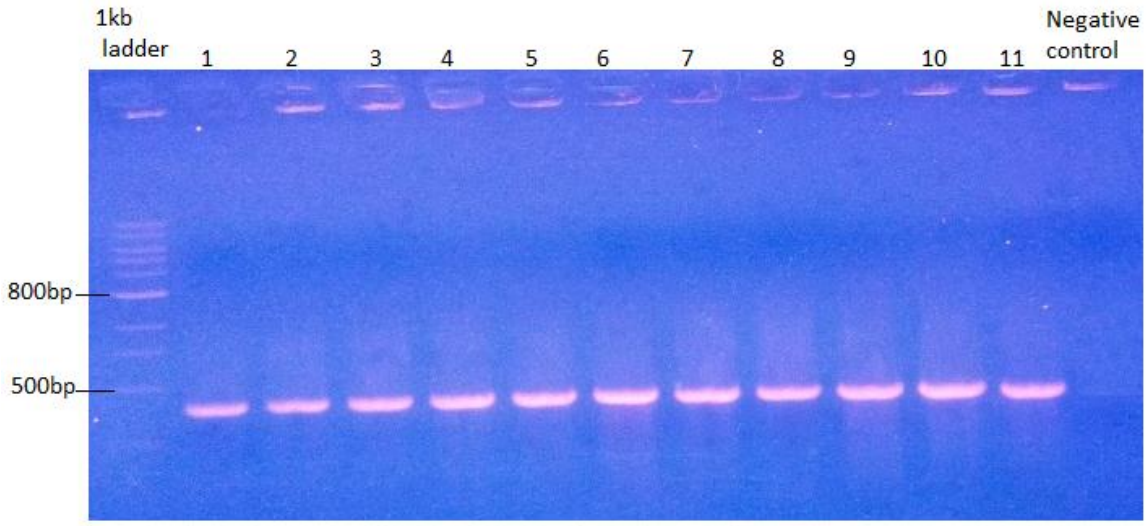

Figure 11: PCR amplified products of COII gene of various termite population.

4.7.2 Molecular identification of termites.

Termites were identified using molecular techniques and the results showed the following species. *Macrotermes bellicosus, Macrotermes herus, Macrotermes subhyalinus Macrotermes subhyalinus/herus* and *Pseudocanthotermes militaris*as shown in the phylogenetic tree Figure 12. The DNA sequences that were used in determining the closeness of the species identified are in appendix II.

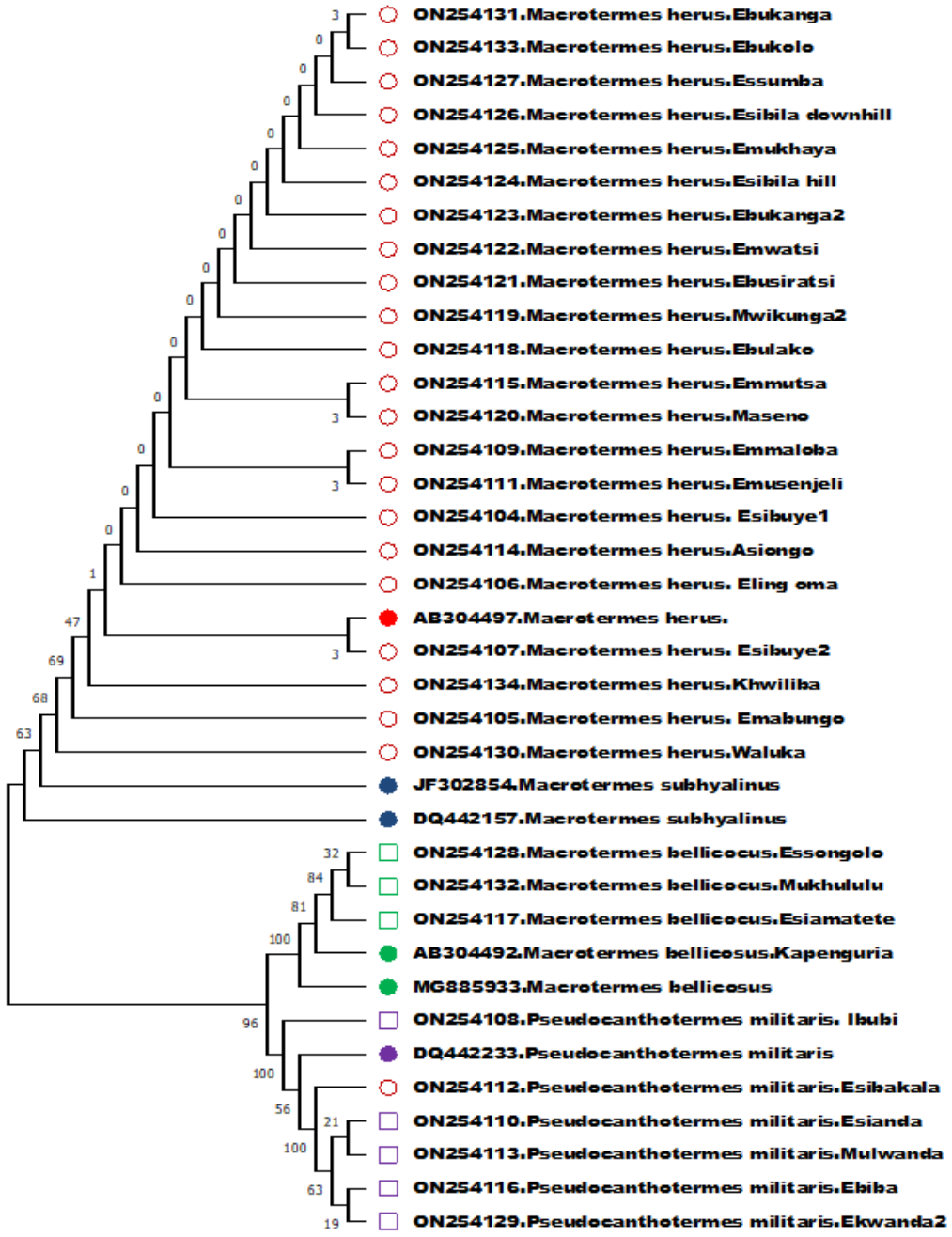

Figure 12: The phylogenic relationships of termites in Luanda sub county.

4.7.3. DNA sequencing of termites in Luanda sub-County.

The PCR products were subjected to sequencing at the university of Nairobi and the results in table xxx

Table 14: Showing the DNA sequences of termites in Luanda sub-County.
The PCR products were subjected to sequencing at the university of Nairobi and the results in table 14.

4.7.4 NCBI Gene Bank accession numbers for termite nucleotide sequences.

The sequence data was submitted to NCBI and the accession numbers in **table 14** were generated.

Table 14: Showing gene bank accession numbers of termites in Luanda sub-County.

S.N	Species(NCBI Blast result)	Site	Longitude/ latitude	Genebank Acc. No
1	*M. herus*	Esibuye 1	34.63 E 0.08 N	ON254104
2	*M. subhyalinus*	Emabungo	34.620 E 0.024N	ON254105
3	*M. subhyalinus*	Eliangoma	34.6389 E 0.025N	ON254106
4	*M .herus*	Esibuye 2	34.639 E 0.095N	ON254107
5	*P. militaris*	Ibubi	34.633E 0.039N	ON254108
6	*M. herus*	Emaloba	34.565E 0.028 N	ON254109
7	*P. militaris*	Esianda	34.626E 0.044N	ON254110
8	*M. herus*	Emusenjeli	34.645E 0.0417 N	ON254111
9	*P. militaris*	Esibakala	34.633 E 0.044N	ON254112
10	*P. militaris*	Mulwanda	34.631E 0.0408N	ON254113
11	*M. herus*	Asiongo	34.605E 0.00324N	ON254114
12	*M. herus*	Emmutsa	34.606E 0.0241N	ON254115
13	*P .militaris*	Ebiba	34.561E 0.034N	ON254116
14	*M. bellicosus*	Esiamatete	34.590 E 0.0113N	ON254117
15	*M. herus*	Ebulako	34.611E 0.00194N	ON254118
16	*M. herus*	Mwikunga 2	34.610E 0.015 N	ON254119
17	*M. herus*	Maseno 3	34.602E 0.00312N	ON254120
18	*M. herus*	Ebusiratsi 1	34.628E 0.0586 N	ON254121
19	*M. herus*	Emwatsi	34.576E 0.00756N	ON254122
20	*M. subhyalinus*	Ebukanga 2	34.591E 0.099N	ON254123
21	*M. herus*	Esibila hill	34.581E 0.061N	ON254124
22	*M. herus*	Emukhuya	34.625E 0.0224N	ON254125
23	*M. herus*	Esibila down	34.594E 0.063N	ON254126
24	*M. herus*	Essumba	34.512E 0.0527 N	ON254127
25	*M. bellicosus*	Essongolo	34.631E 0.039N	ON254128
26	*P. militaris*	Ekwanda 2	34.562E 0.0347N	ON254129
27	*M. herus*	Waluka	34.642 E 0.022N	ON254130
28	*M. herus*	Ebukanga 1	34.601E 0.099N	ON254131
29	*M.bellicosus*	Mukhunzulu	34.6023E 0.113N	ON254132
30	*M. herus*	Ebukolo	34.587E 0.0062N	ON254133
31	*M. herus*	Khwiliba	34.571E 0.011N	ON254134

4.8 (Objective 2) Effect of soil physiochemical characteristics on termite species diversity in Luanda sub County.

4.8.1 Soil pH

Soil pH was determined at Egerton university soil laboratories and results as shown in table 15. On a PH scale of 1-14 *M. bellicosus* had a PH of 5.89 while the lowest was *M.herus* had 5.29 Table 15.

Table 15. Table showing mean pH values of soils habitats with termite species

Macrotermes bellicosus	5.890000[a]
Macrotermes spp1	5.860000[a]
Pseudo grandicepes	5.586667[a]
Macrotermes spp2	5.433333[a]
Pseudo militaris	5.383333[a]
Macrotermes sp1	5.343750[a]
Macrotermes herus	5.290000[a]

Same letter means pH has no significant difference on the diversity of termites.

The highest pH was 5.89000 while the lowest pH was 5.29000

4.8.2 Organic carbon

Organic carbon analysis was done and results as in table 16. The organic carbon has no significant difference on the diversity of termites Table 16.

Table 16. Table showing the mean Organic carbon in ppm

Pseudocanthotermes militaris	47.00000[a]
Macrotermes herus	45.25000[a]
Macrotermes sp1	44.93875[a]
Macrotermes spp2	44.75000[a]
Pseudo grandicepes	44.03333[a]
Macrotermes spp1	42.60000[a]
Macrotermes bellicosus	39.50000[a]

The organic carbon has no significant difference on the diversity of termites

The highest organic carbon was 47.000 where *Pseudocanthotermes militaris* was collected, and the lowest was 39.5000 where *Macrotermes bellicosus.*

4.8.3 Nitrogen

Nitrogen levels in the sampled soil was analyzed and results recorded in **table 17**. *Pseudocanthotermes grandiceps* had the highest Nitrogen levels 0f 0.14 % while *Macrotermes spp1* were associated with low Nitrogen levels of 0.05%.

Table 17. Table showing percentage Nitrogen levels and termite species

Pseudo grandicepes	0.14733333[a]
Pseudo militaris	0.13150000[a]
Macrotermes bellicosus	0.13000000[a]
Macrotermes sp1	0.11475000[a]
Macrotermes herus	0.09923529[a]
Macrotermes spp2	0.09300000[a]
Macrotermes spp1	0.05500000[a]

The nitrogen content across all the sites with the termite species was not significantly different.

4.8.4 Phosphorus

Determination of potassium done and results indicated in table 18. The highest Phosphorus content was 47.00ppm with termite *Pseudocanthotermes militaris* while the lowest was *Macrotermes spp1* at 42.60ppm.

The Phosphorus content across all the sites with the termite species was not significantly different

Table 19. Mean Phosphorus in ppm and termite species

Pseudo militaris	47.00000[a]
Macrotermes herus	45.25000[a]
Macrotermes sp1	44.93875[a]
Macrotermes spp2	44.75000[a]
Macrotermes bellicosus	44.21000[a]
Pseudo grandicepes	44.03333[a]
Macrotermes spp1	42.60000[a]

4.8.5 Potassium

Determination of potassium was done and results recorded in table **20.** The highest Potassium content was 29.02667 with termite *Pseudocanthotermes grandicepes* while the lowest was *Macrotermes spp1* at 21.48000. The Potassium content across all the sites with the termite species was not significantly different

Table 20. Mean potassium in ppm and termite species

Pseudo grandicepes	29.02667 [a]
Macrotermes sp1	26.50375 [a]
Macrotermes herus	25.75824 [a]
Macrotermes spp2	24.58333 [a]
Pseudo militaris	24.36833 [a]
Macrotermes bellicosus	23.71857 [a]
Macrotermes spp1	21.48000 [a]

4.8.6 Zinc

Determination of Zinc was done and results recorded in table 21.

Table 21. Mean Zinc in soil and termite species.

Macrotermes sp1 was found under 46.66875 ppm the highest while *Macrotermes spp1* was found at 32. 022000 ppm the lowest. There was a significant difference in the Zinc levels for all the termite species apart from *Macrotermes sp1*. Mean followed by same alphabet letter show same level of nutrient content

Table 21. Mean Zinc in soil and termite species

Macrotermes sp1	46.66875[a]
Macrotermes bellicosus	41.34429[ab]
Pseudo grandicepes	41.07000[ab]
Macrotermes herus	40.71000[ab]
Pseudo militaris	40.05833[b]
Macrotermes spp2	39.13000[b]
Macrotermes spp1	32.02000 [b]

Table 22. Mean Magnesium in soil in ppm in comparison to soldier termite species
Soil magnesium level was determined and results recorded in table 22. *Macrotermes sp1* inhabiting soils recorded high levels of 110.86ppm while *Macrotermes spp1* soil phosphorus levels was 103.37ppm.

Table 22. Mean Magnesium in soil in ppm in comparison to soldier termite species

Macrotermes sp1	110.867[a]
Macrotermes bellicosus	109.3622[a]
Pseudo grandicepes	106.7667[a]
Macrotermes herus	106.1145[a]
Pseudo militaris	105.4833[a]
Macrotermes spp2	104.2314[a]
Macrotermes spp1	103.3729 [a]

4.9 (Objective 3) Evaluation of various termite feed substrate on attraction of termites

4.9.1 Uncrushed termite feed substrate

This study aimed to find out the levels different plant attract termites ie could Avocado attaract termites differently than Grevilea, Eucalyptus, Cypress, Neem, Mango, Maize, Sugarcane, Bamboo, Blue citronella grass and what would be the effect of having no substrate?

Analysis of variance table for uncrushed feed substrates
analysis for one-way ANOVA is as shown in Appendix III b. The termites attracted to the variousfeed substrates were highly significant $p<0.05$. The means and SE values for crushed feed substrates illustrated in **Table 23.** Sugarcane, *Grevillea robusta* and maize are not different they had the same level of attraction (b, b c) While *Grevillea, robusta* bamboo, and maize had same level of attraction (bc, cd). Maize and cypress had the same level of attractiveness (cd, de). Cypress and Eucalyptus had same level of attractiveness (de, e,). Neem, avocado and Mango had same level of attractiveness (f, fg,). Avocado, Mango, blue citronella grass and control had the same level of attractiveness. Mean values followed by different letters in the same row are significantly different at $p < 0.05$**;** Mean and SE with the same alphabet letter show the same level of attractiveness

Table 23. Means and SE for uncrushed feed substrates.

Substrate	Mean ±SE
Mixture	676.500± 41.7 [a]
Sugarcane	552.500±33.7[b]
Grevilea	499.625±34.2[bc]
Bamboo	475.500±29.2[bc]
Maize	425.000±34.5[cd]
Cypress	326.625±24.6[de]
Eucalyptus	312.375±33.3[e]
Neem	312.375±11.6[e]
Avocado	141.875±10.5[f]
Mango	77.500±12.8[fg]
Blue citronella	18.500±0.26[g]
Control	0.625±0.09[g]

4.9.2 Evaluation of Crushed feed substrate on attractiveness of termites.

Crushed feed substrates were subjected to one way ANOVA test to determine the level of termite attractiveness (appendix ii a). Termite attraction to crushed feed substrates was highly significant $p< 0.05$. **Table 24** illustrates the mean termite count that were attracted to the feed substrates. The mixture had the highest level of attraction (772.250), while blue citronella had the least attraction (4.375). Maize, Grevillea robusta and sugarcane had the same level of attractiveness by termites (ab, b c, bcd). Maize and mixture had the same effects (a, ab). Sugarcane, Bamboo, and Eucalyptus have the same effects on termite attractiveness (b c, bcd, and c d e). Eucalyptus, Bamboo and cypress have the same effects (cde, de, e). Mango Avocado and neem have the same level of attraction but much lower than the rest. Blue citronella and control had the same effects. Mean and SE with the same alphabet letter show the same level of attractiveness.

Table 24. Mean termite count and SE of crushed feed substrates

Substrate	**Mean ± SE**
Mixture	772.25 ± 41.7 [a]
Maize	668.62 ± 34.5[a b]
Grevillea	622.37 ± 34.2 [b c]
Sugarcane	591.00 ± 33.7 [b c d]
Eucalyptus	534.87 ± 33.3 [c d e]
Bamboo	469.12 ± 29.2 [d e]
Cypress	411.12 ± 24.6[e]
Mango	218.00 ± 12.8 [f]
Avocado	195.25 ± 10.5 f
Neem	184.87 ± 11.6[f]
Blue citronella	4.37 ± 0.26[g]
Control	1.00 ± 0.09[g]

4.10 (Objective 3) Phytochemical analysis of plants used as termite feed.

Phytochemicals present in the termite feed substrates, Grevillea, sugarcane, Bamboo, cypress, Eucalyptus and maize were as documented in table 25.

Table 25. Phytochemical screening of plant metabolites from selected edible termite feed substrates.

The results for phytochemical analysis of plants were as documented in table 25. Qualitative analysis of the secondary plant metabolites revealed the presence of different phytochemicals.

		Plant sample					
No	**Phytochemical Test**	Bamboo	Sugarcane	Cypress	Grevilea	*Maize*	Eucalyptus
1.	**Saponins**	++	+	++	-	+	+++
2.	**Tannins**	-	+++	-	+++	_	+++
3.	**Alkaloids**	+	-	-	+++	-	+++
4.	**Flavonoids**	-	-	-	-	-	+++
5.	**Sterols**	+++	+++	-	-	+++	-
6	**Resins**	++	++	++	+++	++	+++
7.	**Triterpenoids**	_	_	+++	-	-	-
8	**Cardiac Glycosides**	+++	-	+	-	-	+++
9.	**Carbohydrates**	++	+++	++	+++	+++	+
10.	**Reducing sugars**	+	+++	_	+	+++	-
11.	**Flavones**	-	-	-	-	-	-
12	**Phenols**	-	++	+	+++	+++	+++

Key +++ means present in large quantities
++ means present in moderate quantities
+ means present in small quantities

Percentage phytochemical present in the feed substrates was as follows,
Eucalyptus 58.33%
Sugarcane 58.33%
Bamboo 58.33%
Grevilea 50%
Maize 50%
Cypress 50%

Flavonoids were present in Eucalyptus alone while Triterpenoids were present in Cypress while Flavonoids were absent in all. On the other hand, resins and carbohydrates were present in all the plant species tested.

4.10.1 Crude plant extracts from plants and termite attractiveness.

Crude plant extracts from Eucalyptus, Grevilea, Cypress, Maize, Sugarcane, and Bamboo were tested for attractiveness on Solder termites. Analysis of Variance table for crude plant extracts and termite attraction Appendix IIIc. The ANOVA table was computed for crude plant extracts and their degree of attractiveness. The attraction of termites to different crude extracts were highly significant at $p<0.05$**.**

4.10.2 Termite Attractively to crude extracts from plants

Sugarcane had the highest level of attractiveness while bamboo had the lowest level of attractiveness. Sugarcane, and maize had the same level of attractiveness, they were not significantly different. Maize, Eucalyptus, Cypress and *G. robusta*, had the same level of attractiveness, thus were not significantly different. Bamboo and the control had same level of attractiveness. Mean and SE with the same alphabet letter show the same level of attractiveness and there is a significant difference as per **Table 26**.

Table 26. Means and SE for termite attractiveness.

SUBSTRATE	MEAN±SE
Sugarcane	7.750 ± 0.248 [a]
Maize	6.625 ± 0.283[ab]
Eucalyptus	6.375 ± 0.221[b]
Cypress	6.250 ± 0.281[b]
Grevilea	6.250 ± 0.283[b]
Bamboo	4.750 ± 0.281[c]
Control	1.375± 0.115[d]

CHAPTER FIVE

DISCUSSION

5.1 Species diversity of termites in Luanda sub-County.

This study envisaged identifying termite species in the Luanda sub-county to document the species diversity of termites in the area. Termites were identified at the National Museums of Kenya; using termite identification keys as referred to by Scheffrahn *et al*., 2006) Specimens were identified to species level using soldier castes. Termite identification done on the termites collected in the Luanda sub-county revealed that there were 7 species identified collected in 47 sites. The identified species include *M. herus, Macrotermes spp1, Macrotermes sp1, Pseudocanthotermes grandiceps, Macrotermes bellicosus, Macrotermes spp2,* and *Pseudocanthotermes militaris.* Termites are widely distributed in tropical and sub-tropical savannas as elucidated by (Muvengwi *et al*., 2017). In western Kenya, several species have also been identified and this illustrates the high species diversity of termites.

This study focused on 47 locations where the soldier and worker termites were sampled, Shannon diversity index was H = 0.3606685, while the Simpson index was D = 0.2064429, from January to March. This indicated a high species diversity of termites in the Luanda sub-county. Out of the 47 sites, there were seven termite species identified. Seasonal variations of the termite species indicated that the Shannon indices were H=0.1 while the Simpson index was D=0.12, this showed that there was a high diversity of termites during the rainy season in April to June than in the dry season where H = 0.3606685 D = 0.2064429. The higher the value the lower the diversity, the indices are between 0-1. During the rainy season, there are numerous plants on which the termites can forage as opposed to the dry season when the foraging plants are scarce. *M. herus* had the largest species richness of 7.0 which was obtained at Ebukolo. *Macrotermes sp1* had the lowest species richness among all the termites identified. The species richness was 1.8239 at Mwikunga.

Comparative studies on termite diversity conducted in West Islamabad, Pakistan by Nazir *et al*., (2016) revealed that Simpson and Shannon's diversity indices reflected that on

Simpson's index, the overall diversity (D=0.7034) was 70.34% and it was 99% in the case of the Shannon scale. *Odontotermes horai* was the most dominant species with a value of 0.4332 on Simpson's index.

5.1.2 Termite species Richness in Luanda sub-county

Species richness was assessed in all 47 collection sites and the results show that *P. militaris* had a high species richness of 6.9140 at Ekwanda 2 while Mulwanda had the lowest species richness of 5.95014. Table 1.*M. bellicosus* on the hand had the highest species richness at Ebukanga 1 while the lowest species richness at Maseno 2.*M. herus* was collected in 18 locations and Ebukolo had the highest species richness of 7.000 while Ekwanda had the lowest species richness of 2.98703Caleb *et al.,* (2022). Comparatively, Ekwanda *M. herus* had a lower species richness but on the other hand, had the highest species richness for *P. militaris*. From these results, the two species do not coexist fully in their natural habitats.

Macrotermes sp1 had the lowest species richness among all the six species, at Mwikunga it had a species richness of 1.822. In a similar study Koné *et al.,* (2018) recorded that the lowest species richness of termites observed in the grassy savanna could mainly be explained by the annual disturbance. Fire behavior intensity, the rate of spread, flame height, residence time, and surface temperature are influenced by a wide range of variables such as fuel characteristics, burning season, and weather conditions.

5.1.3 Molecular identification of termites

A total of 47 termite samples were used for phylogenic analysis. The phylogenetic tree was built using *Macrotermes bellicosus, Pseudocanthotermes militaris, Macrotermes subhyalinus, and Macrotermes herus.* The tree was divided into one major cluster and two smaller clusters. The first major cluster consisted of *M. herus* and *M. subhyalinus* with a bootstrap score of 100, 63, and 2. This indicates the close relationship between the two

species as identified and collected in 22 sites. Morphological identification did not show close relationships thus the need for molecular identification. *P. militaris* strain had a bootstrap score of 100. This cluster was subdivided into 6 small sub-clusters each with a score of 100. This species was the most harvested in western and Nyanza as food and feed. *M. bellicosus* and *M. bellicosus*. Kapenguria was divided into 3 small sub-clusters with a bootstrap of 100, 95, 68, and 68 (Figure 12).

Molecular identification found that four species were closely related. From the results the species identified genetically were, *Macrotermes bellicosus, Macrotermes herus, Macrotermes subhyalinus, Macrotermes subhyalinus/herus,* and *Pseudocanthotermes militaris. M. subhyalinus* and *M. herus* were found to be very close genetically. They were differentiated using the morphological approach and they were found to be *M. subhyalinus*. Phylogenetically, *M. subhyalinus* came first then *M. herus* followed through mutation. some termite species exhibited similarities in their morphology and thus were difficult to identify. Molecular characterization provided a clear identity where the species was not properly identified.

The species richness curve Fig 2 showed a higher species richness. Most termite species belong to the family Macrotamatinae of which *Macrotermes herus* (25.56%) was the most abundant species in most sites in Luanda county while *Macrotermes sp 1* was (4%). There was also no significant difference in the number of workers and soldiers collected in the Luanda sub-County $p>0.05$. Termites are one of the soil macrofaunae that have sensitivity to microhabitat variation as reported by Allan, (2016). Luanda sub-county has diverse features of termite habitats thus the presence of different species which contribute to food and feed for the local community. Luanda sub-county is characterized by farm forests. Grasslands, swamps, hilly terrain with rock outcrops. Termite sampling was done using the belt transect method which ensured that most locations were searched for the presence of termites. (Darlington et al., 1997) in his study noted that although the geographical ranges of two Macrotermes species largely overlap, *Macrotermes michaelseni* prefer higher elevations. At the Ebulako site, Macrotermes sp1 was predominantly found in 18% of all the termite species collected. Ebulako is found on latitude 34.61169E 34◦ 36' 42.078" and longitude 0.00194 N 0◦ 0' 6.978". Ebulako is characterized by rock crops hilly terrain

and shrubs with little grass. (Ayieko e*t al.*, 2010) found out that weather conditions precipitated by climate change favour the increased emergence of insects specifically termites. Moisture and temperature play a significant role in insect ecology. Climate changes influence insect populations by influencing benthic fauna and its biodiversity that supports the insects. In Luanda sub-County a large proportion of termites were collected during the wet months, unlike the dry months. During the wet months, vegetation was abundant thus a food source for the termites to forage.

In a similar study in Ethiopia Wale & Nega, (2019) a total of over 16,000 termite individuals representing one family (Termitidae), two subfamilies, i.e., Macrotermitinae and Termitinae, and five genera (Macrotermes, Odontotermes, Microtermes, Amitermes, and Microcerotermes) were found. More Microtermes and Macrotermes termite individuals were found than in other genera. Microtermes and Macrotermes were more abundant. Shannon's diversity index and Simpson's index of diversity values appeared to be higher in the protected vegetation.

Soldier termites were used for identification in this study, the isolated genomic DNA of termites from various locations in Luanda Sub-County was characterized through COII gene fragment (648-656 bp size) was successfully sequenced for all the specimens, and the alignment of all specimens considered in this study lacked any insertion or deletion. A total of 33 species were sequenced over COII regions and the BLAST was done with the NCBI database. Such studies were earlier carried out by Kotilingam, (2020)

The sequence data was obtained and submitted to Genbank for generating the accession numbers. Chromatograms were edited to remove the ambiguous bases and then aligned using the Basic Local Alignment Search Tool (BLAST), with the sequences of the same or related genera retrieved from the nucleotide database (PUBMED) of the National Centre for Biological Information (NCBI). The COII nucleotide sequences of the termite species included in our present study were aligned and compared with the species obtained from PUBMED, using CLUSTAL. Molecular sequence information from NCBI revealed relatedness in all the collected termites, accurately as revealed by their morphological characters.

5.2 Effect of soil chemical characteristics on termite species diversity in Luanda sub-County.

This study shows that the soil pH had no influence on the diversity of termites in Luanda Sub County. All seven termite species were found in a range of (5.890000- 5.290000). In a similar study Arinana *et al*., (2016) found out that termites thrived in neutral pH. *P. militaris* was the species that had a large count of 47 while *Macrotermes bellicosus* had the least count (39.5000). In another study, Chisanga *et al.*, (2020) showed that soil pH values in different termite mounds were alkaline to moderate levels. This differs from the study in Luanda where the soils associated with termite habitats are slightly acidic.

The soil samples in the Luanda sub-county were collected from termite habitats and nearby mounds. The area is covered by rock outcrops with trees and shrubs. Many farming activities might influence the soil pH owing to the use of inorganic fertilizers and other agrochemicals. In other places, termites inhabit soils with a pH of between 4.0- 4.2 as described by Bourguignon *et al.*, (2015b). In some cases, termites also inhabit neutral soils with a pH of 7.28 as described in the work by Echezona *et al*., (2012). Soil pH has also a great influence on the availability of other minerals, in their study Dahlsjö*et al*., (2020) reported that soil pH above 5 and heightened levels of elements such as Zinc, copper, Magnesium, and Calcium, have been shown to cause a decline in termite diversity. Organic carbon content had no significance on the termite diversity. From this study, the highest organic carbon was 47.0000 ppm while the lowest was 39.50000 ppm. *P. Militaris* were associated with high organic carbon while the lowest organic carbon was recorded where there was *Macrotermes bellicosus.*

A study by Siebers *et al*., (2015) recorded that termite diversity and abundance were low in soils with low organic carbon levels. Another study by Eze *et al*., (2020) recorded that termite mounds had less organic matter than the surrounding soils, but concluded that they had higher aluminum, iron, and titanium content as well as their oxides which are effective in the absorption of materials. The soils in Luanda sub-County are characterized by farm forests and grasslands which contribute to organic matter as well as feed sources for the termites. Nitrogen levels in soils within Luanda sub-County had no significant impact on

the termite diversity. The highest Nitrogen content was 0.1473333 ppm with termite *P. grandicepes* while the lowest was *Macrotermes spp1* at 0.0550000 ppm. The nitrogen content across all the sites with the termite species was not significantly different. Nitrogen is a major element in termites. West, (2015) recorded that high levels of C and N in termite mounds were due to the feeding and building habits of termites that concentrate organic matter in termite mounds.

Potassium levels also were not statistically significant in the Luanda sub-county. The highest level was 29.02667 ppm while the lowest was 21.48000 ppm. The values were very close to each other implying that potassium was abundant in the soils in the Luanda sub-county. A study by Janzow & Judd, (2015) recorded that K levels in the termites were not found to be significantly different among all the treatment groups. Potassium being more abundant in most soils, Abe *et al*., (2011) found out that *Macrotermes bellicosus* accumulated exchangeable K and Mg in mound structures. Botch & Judd, (2011) in their work also noted that decomposing logs had a high concentration of K than non-decomposing. He also concluded that Potassium is released rapidly from decomposing woody debris and is considered highly leachable. Potassium is abundant in most soils in Kenya and East Africa.

Phosphorus levels across the sites in the Luanda sub-county were not significant. The highest Phosphorus content was 47.0000 ppm and was inhabited by *P. militaris*. The lowest Phosphorus content was 42,6000 ppm. In a study by López-Hernández *et al*., (2006), he recorded that the values of Phosphorus were low in *Macrotermes bellicosus* mounds and associated soils. Apori *et al*., (2020) recorded that soil phosphorus levels were moderate. In his study, he found out that when termite mound soil was compared with the surface soil of the surrounding soils, the available Phosphorus content of the termite mound was significantly lower compared to the surrounding soils. Alves *et al*., (2011) noted that Phosphorus content was higher in termite mounds that feed on grasses. From this study, it is evident that Phosphorus levels are generally lower in Termite inhabited soils in Luanda sub-County.

Macrotermes sp1 was found under 46.66875 ppm of Zinc the highest while *Macrotermes spp1* was found at 32. 022000 ppm is the lowest. There was a significant difference in the Zinc levels in the soils associated with the termite species collected from Luanda sub-County, table 21. Janzow & Judd, (2015) recorded that termite micronutrient levels were not found to be significantly different. Another study by Idowu *et al*., (2014), noted that higher soil zinc levels were found in Soils associated with fertilizer and pesticide residues. He also concluded that there were higher concentrations of Zinc in soldiers than in workers in *Nasulitermes spp.*

5.3 Evaluation of selected plants as termite attractants

5.3.1 Evaluation of uncrushed feed substrates

This study envisaged finding out how termites respond to different plant substrates as food. The termites attracted to the various feed substrates were highly significant $p<0.05$. Luanda sub-County has many tree species among them *G. robusta*, cypress, Eucalyptus, Neem, Mango, and Avocado, Ameka *et al.*, (2022). A mixture of all the plant species gave the highest termite attraction (Mean ±SE, 676.500± 41.7 a) followed by sugarcane (552.500±33.7b), while the blue Citronella grass had the lowest attraction (18.500±0.26g). Termites visited the feed traps at night. Variation in wood chemistry is known to affect termite feeding behavior. In their study Evans *et al*., (2005) recorded that termites prefer small blocks of wood to large blocks. In this study, the selected plants were chopped into small pieces and put in the termite traps. Among the tree species used in this study, *G. robusta* had the highest attraction followed by Eucalyptus and Cypress respectively. Fajar *et al*., (2021), reported that termites fed on Eucalyptus in Indonesia.Nakabonge & Matovu, (2021) documented that Eucalyptus species are highly susceptible to termites.

Worker termites were found in large numbers than soldiers. The workers feed while the soldiers keep guard. Worker termites produce a loud noise when chewing. This loud chewing generates acoustic emissions that attract more workers to feed in the dark, as elucidated by Wang *et al*., (2019).

5.3.2 Evaluation Crushed feed substrates

In the crushed feed bioassay, the mixture had the highest mean of termite attraction (772,25±41.7a) while blue citronella grass had the lowest (4.37 ±0.26g) the control had (1,00±0.09g). The termites were attracted to the crushed feed substrate p-value was very significant $p<0.05$. When crushed feed is used, the feed attractiveness was higher (772,25±41.7a) compared to uncrushed (676.500± 41.7 a). Tarayre *et al*., (2015) recorded that wood-feeding termites can digest up to 85 and 83 of glucosyl and Xylosyl residues from lignocellulose, respectively. Predigested diets will give termites ample time to forage faster than uncrushed feed substrates as recorded by Menezes *et al*., (2018).

5.3.3 Phytochemical analysis of termite feed

Test plants, including Grevillea, Eucalyptus, Bamboo, Sugarcane, Maize, and Cypress were evaluated for the presence of phytochemicals. Saponins were present in all termite feed substrates, excluding Grevillea. Eucalyptus had the highest quantities (+++) while sugarcane had the least (+). Negri & Tabach, (2013) isolated Saponins in *Periandra dulcis* roots as evidence of the presence of Saponins in plants.

Tannins were present in large quantities in sugarcane, Grevillea, and Eucalyptus (+++), while Bamboo, cypress, and maize were absent.

A study by Fraga-Corral *et al*., (2020) recorded that Tannins were found between 20—70% in plants. Ukoha *et al*., (2011) also documented that tannins were present in *Samanea saman* pods.

Alkaloids are low-molecular-weight nitrogen-containing compounds and, due to the presence of a heterocyclic ring containing a nitrogen atom, are typically alkaline as defined by Matsuura & Fett-Neto, (2015). Alkaloids were present in Grevillea and Eucalyptus in large quantities (+++), while in Bamboo, small quantities were present (+). Sugarcane, Cypress, and maize Alkaloids were absent. Jeyasankar *et al*., (2014) in his work documented that some plant species contain high amounts of Alkaloids which can be lethal to insects. Therefore, the use of plants for termite feed with high amounts of Alkaloids should be used sparingly.

Flavonoids were present in Eucalyptus in large quantities (+++) but absent in the rest. In their study, Takahashi *et al.*, (2004) reported that Eucalyptus maculate had three flavonoids isolated namely 2c,6c-dihydroxy-3c-methyl-4c-methoxy-dihydrochalcone, Eucalyptin and 3,8-desmethyl-eucalyptin. Sterols were present in large quantities in Bamboo, Sugarcane, and maize (+++) but absent in Cypress, Grevillea, and Eucalyptus.

Resins were present in all six feed substrates but were found in large quantities in Grevillea and Eucalyptus (+++) but moderate in Bamboo, sugarcane, cypress, and maize. Simoneit *et al*., (2019) documented that Resins were also present in confers.

Triterpenoids were present in cypress only in large quantities (+++) but were absent in the rest. Cardiac Glycosides were present in Bamboo and Eucalyptus in large quantities (+++), while cypress had small quantities (+). Sugarcane, Grevillea, and maize had no Cardiac glycosides. Carbohydrates were present in all feed substrates with sugarcane, Grevillea, and maize having large quantities (+++). A Study conducted by Sandjo & Kuete, (2013) recorded that two Cameroonian medicinal plants, *Duboscia macracarpa*, and *Canarium schweinfurthii* contained Triterpenes Dubosic acid, while Canarene Bamboo and cypress had carbohydrates in moderate quantities (++). Eucalyptus had carbohydrates in small quantities. In their study Alves *et al.*, (2010) analyzed the presence of carbohydrates in Eucalyptus. Reducing sugars were found in maize in large quantities (+++) while bamboo and Grevillea were in small quantities. Cypress and Eucalyptus had none. Pereira *et al*., (2017) showed that fructose and Glucose levels were significantly higher in sugarcane

Flavones were absent in all the feed substrates. Phenols were present in Grevillea, Maize, and Eucalyptus in large quantities (+++), while sugarcane had moderate quantities (++). Cypress had small quantities (+), while bamboo had none Mierziak *et al*., (2014). Flavones play a role in providing colour, Fragrance, and taste in plants which makes them attractants for insects birds, and mammals.

5.3.4 Evaluation of crude plant extracts as termite attractants

Soldier and worker termites were evaluated on their attractiveness to crude extracts of Grevillea, Cypress, Bamboo, Maize, Sugarcane, and Eucalyptus. The results showed that the attraction of termites to different crude extracts was highly significant at $p<0.05$. Sugarcane had the highest level of attractiveness while bamboo had the lowest level of

attractiveness. Sugarcane and maize had the same level of attractiveness (a, ab), They were not significantly different. Maize, Eucalyptus, Cypress, and Grevillea, had the same level of attractiveness (ab, b), and thus were not significantly different Table 24. Bamboo and the control had the same level of attractiveness. This study showed there was a significant difference in the number of termites recruited to the attractant chamber. The crude extract from maize was statistically different by 6.625± 0.283 a b. From phytochemical analysis, maize had high levels of sterols, (+++) carbohydrates (+++), reducing sugars (+++), and phenols (+++). A combination of carbohydrates and reducing sugars gave high levels of Attractivity. There was no significant difference in sugarcane, Eucalyptus, Cypress, Grevillea, Bamboo, and control. These results revealed that termites could detect chemicals in the substrates and move toward containers having the crude extract. Furthermore, these results also indicated that carbohydrates and reducing sugars have the potential to attract termites on a large scale. It was also noted that *G. robusta*, Maize, and Eucalyptus had high amounts of phenols which could also boost the termite attraction. Similar studies by wallet *et a l*(1999) and (Ameka Caleb1, 2022) showed that a greater number of workers were recruited to containers containing sucrose and yeast than those with water as controls.

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CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Introduction to conclusion.

This study has documented four edible termite species in the Luanda sub-county. The study indicated that there was a high species diversity of edible termites in the Luanda sub-county. The species include *Macrotermes herus, Macrotermes subhyalinus, Macrotermes bellicosus, and Pseudocanthotermes militaris*. To fully identify termites two methods were used both morphological and molecular as termites have features that resemble one another and the closeness of the species. The morphological identification revealed 7 species while on confirmation with the molecular tool it was found that there were four edible termite species in the Luanda sub-county. Molecular identification was able to narrow down to all the species and how they are related to one another. The most edible species that is abundant is *M. herus* while the least abundant edible species were Edible termites in the Luanda sub-county inhabit a wide range of habitats ranging from hills, plains, and valleys. Termite being soil inhabitants interact with a wide range of soil minerals. This mineral determines their survival and nutrition. The soils in the Luanda sub-county had moderate pH, which was slightly acidic and all four termite species were found within the pH range of 5.0-6.2. Further chemical determination of minerals revealed that the four termite species were abundant in soils with Nitrogen, Phosphorus, Potassium, Zinc, Magnesium, and calcium.

Edible termite species in the Luanda sub-county were identified in 47 locations. These sites/locations had diverse feed resources onto which termites feed. In the open field bioassay to determine termite attractiveness to feed, Eucalyptus, grevillea, neem, mango, Avocado, cypress, maize stalk, sugarcane stems, and bamboo stems were evaluated as termite feed. High termite counts were obtained in Maize, sugarcane, Eucalyptus, cypress, Bamboo, and Grevillea. High edible termite species diversity was possible due to the large quantities of termite feed present in the study area. This study revealed that termites

invaded maize, sugarcane, and Eucalyptus in high amounts. The mixture of all the feed was the best as more termites were attracted to it.

This study examined the phytochemical component of six plants on which termites feed. They included Eucalyptus, cypress, Maize, Sugarcane, bamboo, and grevillea. The phytochemical components were extracted from this plant and they included, Tannins, Saponins, Reducing sugars, triterpenoids, resins, and flavonoids. alkaloids. Flavones were absent in all the plant samples tested.

Extraction of the phytochemicals was done and the phytochemicals were tested on solder and worker termites to evaluate their attractiveness levels. Results revealed that termites were attracted to sugarcane and Maize due to high carbohydrate and reduced sugar levels. more than the other plants while bamboo had the lowest attraction. Carbohydrates and sugars are rich in energy needed by termites. This is important as termites are active insects and forage for many hours carrying the feed material to store in their colonies. The choice of the feed material is influenced by the level of the phytochemical constituent that is of preference by termites.

6.2 Conclusion

1. The study revealed that there was a high species diversity of edible termites in the Luanda sub-county and the most prominent was from the Macrotamatinae family. The higher species diversity and richness signify available termite diets for food and feed in the Luanda sub-county. Termites are used for food and in Luanda hence data on the species richness and diversity must be known. This data will assist in their conservation for future sustainability to prevent over-exploitation by termite harvesters. The identified termite species will be conserved at the entomology repository of the National Museum of Kenya. This national repository will assist termite taxonomists in identifying new species in the future.
2. Soil physicochemical characteristics have no effect on the species diversity of edible termites in the Luanda sub-county as the four species were found in soils rich in Nitrogen, phosphorus, Potassium, zinc, and Magnesium. All the termite species collected from the Luanda sub-county are soil inhabitants hence the soil's chemical properties directly affect their population. The soils in the Luanda sub-

county have been affected by overreliance on fertilizers. Due to the heavy application of fertilizers, there exist high amounts of minerals in the soil. From this study, it is evident that soil nutrient levels have no effect on termite species diversity which ensures the conservation of the species as termites coexist in environments with rich Phosphorus, Nitrogen, Calcium, and magnesium.

3. Phytochemicals present in selected plants attracted soldier and worker termites. Termites were attracted to some plants which guided them to feed. The isolated phytochemicals such as Alkaloids, triterpenoids, Flavonoids, and Carbohydrates were key determinants in the quantity of plant tissue foraged upon. Therefore, some plants such as Grevillea, Eucalyptus, and sugarcane contain high levels of phytochemicals that attract termites.

6.3 Recommendation

1. This study recommends that the termite species identified should be conserved in local and international repositories so that the species identified can be availed for future studies.
2. Soil's chemical properties do not affect the species of termites that were sampled. This study recommends that should not be destroyed or contaminated with chemical contaminants which will destabilize the mineral balance that will make termites thrive.
3. Further work is still required concerning the effectiveness of the crude extracts of maize, sugarcane, Cypress, and Eucalyptus as attractant compounds for the development of termite baits which can be used to harvest termites for food and feed in the Luanda sub-county. The attractant can be isolated and characterized in the laboratory for future use in the mass harvesting of edible termites in the Luanda sub-county and the rest of Africa.

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APPENDICES

APPENDIX 1 : RESEARCH PERMIT

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **390580**

Date of Issue: **16/February/2021**

RESEARCH LICENSE

This is to Certify that Mr.. CALEB MIKHALI AMEKA of Jaramogi Oginga Odinga University of Science and Technology, has been licensed to conduct research in Vihiga on the topic: ASSESSMENT OF SPECIES DIVERSITY, HABITAT PHYSIO CHEMICAL CHARACTERISTICS AND EVALUATION OF PLANT DERIVED SEMIOCHEMICALS AS EDIBLE TERMITE ATTRACTANT for the period ending : 16/February/2022.

License No: **NACOSTI/P/21/5797**

390580

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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APPENDIX II. Showing the DNA sequences of termites in Luanda sub-County.

The PCR products were subjected to sequencing at the university of Nairobi .

Seq.1_Macrotermes3

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APPENDIX III

Appendix iii a: The analysis of variance table termite species

Source of variation	DF	Sum of Squares	Mean Sum of Squares	F value	P value
Termite	1	205905	205905	30.503	3.34e-07***
Species	6	280240	46707	6.919	4.82e-06***
Residual	88	594025	6750		

*** indicates significance at 5%**

Analysis of variance table for uncrushed feed substrates

Data analysis for one way ANOVA is as shown in table24

Appendix iiib: Analysis of variance table for uncrushed feed substrates.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
substrate	11	4601105	418282	34.42	< 2e-16 ***
termite	1	1072940	1072940	88.29	1.05e-14 ***
Residuals	83	1008683	12153		

Appendix iii c. Analysis of variance table for termite attraction to crushed feed substrates

Source of variation	DF	Sum of Squares	Mean Sum of Squares	F value	P value
Substrate	11	6045179	549562	34.26	<2e-16***
Termite	1	2344063	2344063	146.15	<2e-16***
Residual	83	1331227	16039		

*** indicates significance at 5%**

Appendix iii d. Analysis of Variance table for crude plant extracts and termite attraction.

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Substrate	**6**	**205.50**	**34.25**	**19.53**	**2.22e-11 *****
Termite	**1**	**111.45**	**111.45**	**63.55**	**2.43e-10 *****
Residuals	**48**	**84.18**	**1.75**		