University of Nairobi

EFFECT OF LAND USE ON DIVERSITY OF MACROFUNGI IN KEREITA FOREST, KIKUYU ESCARPMENT AND THE POTENTIAL OF CULTIVATION OF SELECTED SPECIES

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DECLARATION

I hereby declare that this research is my original work and has not been previously submitted in this or any other university or institution of higher education.

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DEDICATION

First and foremost I dedicate this study to God who is the giver of all knowledge and wisdom. Secondly, to my family members for their encouragement, unwavering support and strong belief that I can make it.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFLP	-	Amplified Fragment Length Polymorphism
AWARD	-	African Women in Agricultural Research and Development
DNA	-	Deoxyribonucleic Acid
ECM	-	Ectomycorhiza
GPS	-	Global Positioning System
ITS	-	Internal Transcribed Spacer
JICA	-	Japan International Co-operation Agency
KEF	-	Kikuyu Escarpment Forest
KENVO	-	Kijabe Environment Volunteers
MEA	-	Malt Extract Agar
NCBI	-	National Center for Biotechnology Information
PAST	-	Paleontological Statistics Software Package
PDA	-	Potato Dextrose Agar
PDYA	-	Potato Dextrose Yeast Agar
PFA/TFM	-	Principal Factor Analysis/Total Fatty Matter
RDA	-	Redudancy Analysis
TBA	-	Tropical Biology Association
WEM	-	Wild Edible Macrofungi
WEMS	-	Wild Edible Mushrooms

ABSTRACT

Tropical forests are a haven of biodiversity with the richest macrofungi diversity in the World. However, the rate of forest loss greatly exceeds the rate of species documentation and increased risk of losing biodiversity to extinction. There is also an increasing interest to exploit and domesticate wild edible mushrooms (WEMs) worldwide. The aim of this study was to determine the occurrence and diversity of macrofungi across the indigenous and *Pinus patula* land use systems in Kereita forest block, Kikuyu Escarpment Forest. This study was carried out to determine the diversity of macrofungi in indigenous forest and a 22 year-old *Pinus patula* plantation forest block, Kikuyu escarpment and to determine the suitable culture media for the laboratory cultivation of selected edible species. This was done during the short rains in December, 2014 and long rains in May, 2015 seasons. During the two seasons three transects 1 km apart were laid down and plots measuring 20 by 20m which were 500m apart were established in Kereita block. Sample of macrofungi were collected within each plot and the abundance of macrofungi fruit bodies were recorded by counting. The samples were identified using, reference collections, taxonomic keys and books. The common edible species were further identified using molecular techniques which was based on internal transcribed spacer (ITS) of Ribonucleic acid (RNA) genes 5.8s rRNA. *Macrolepiota dolichaula*, *Auricularia polytrica* and *Pleurotus djamor* were cultured invitro for mycelia growth at 25^0C for 7 days on potato dextrose yeast agar (PDYA), potato dextrose agar (PDA), and MALT extract Agar in a complete randomized design with 5 replicates. Mineral content for the three species was determined using Microwave Digestion Method and analyzed using Atomic Emission spectrometry method. A total of 224 species distributed across 28

families and 76 genera were encountered in Kereita forest across the indigenous and plantation land use types. Macrofungi species from Agaricaceae family (16%), was the commonly represented taxa in the Kereita ecosystem. 90% of the macrofungi fruitbodies were saprophytic followed by both ecto-mycorrhiza and parasitic fungi at 3%. Land use type significantly ($p<0.05$) affected species richness and density and the indigenous forest had 70% macrofungi diversity compared to the plantation forest. The indigenous forest and plantation forest (*Pinus patula*) showed altered species composition, but species diversity was not different. Seasonality also significantly ($p<0.05$) affected the diversity of macro-fungi, with 61% of the total macrofungi species being encountered during the wet season. Molecular characterization successfully identified the species as *Agaricus inoxydabilis, Agaricus volvatulus, Macrolepiota dolichaula, Stropharia rugosoannulata, Fayodia leucophylla, Suillus luteus, Pleurotus djamor, Auricularia polytrica, Agaricus / Hymenagaricus* were identified using molecular techniques. *Macrolepiota dolichaula, Auricularia polytrica* and *Pleurotus djamor* all showed the ability to develop mycelia on PDA, PDYA and Malt extract agar. *M. dolichaula* and *A. polytrica* fully colonized forming everage mycelial diameter of 4.5 by the 7^{th} day on all the culture media (PDA, PDYA and malt extract agar) except *P. djamor* that took more than 7 days to colonize fully. The mineral content for the species was in the range of 0.66-2038 for P, K, Ca, Mg, Na, Fe, Mn, Cu and Zn. From the study, the indigenous forests harbor a wide range of macrofungi species compared to the exotic plantation forest while the wet season had higher diversity of macrofungi compared to the dry season.

Key words: Macrofungi, Characterization, forest type, Season, Diversity, Edible

CHAPTER ONE

INTRODUCTION

Macrofungi also commonly known as mushrooms are fruit bodies visible to the naked eye and a representative of invisible extensive belowground mycelia (Rajaratnam & Thiagarajan, 2012; Enow, *et al*., 2013). Forested ecosystems are a haven for a wide range of these macrofungi (Paz, *et al*., 2015). The Kikuyu Escarpment Forest which is part of Aberdare forest is one of the habitats known to harbour a wide range of flora and fauna (Republic of Kenya, 2015). Since early 1970, Kenya has witnessed a deliberate conversion of indigenous forest to plantation forest, in order to introduce the fast growing exotic *Pinus* and *Eucalyptus* tree species especially for timber (Yihaisi & Clark, 2004). Though both indigenous and plantation forest types offer suitable habitats for diverse macrofungal populations, the conversion poses a threat to biodiversity (Kost, 2002; Goldman, *et al*., 2015). The conversion causes changes in plant communities, organic matter production and quality (C: N ratios of organic matter), which may influence macro- fungi growth and development (Claudia, *et al*., 2015). Additionally, the forests have also been facing serious conservation threats as a result of unsustainable human activities, including charcoal burning, illegal logging and encroachment. Such activities are a major risk to biodiversity loss before proper documentation and utilisation is realised (Kost, 2002; Enow, *et al*., 2013; Malavasi, *et al*., 2016).

Macrofungi are associated with very critical roles in nature and are regarded as one of the necessary components in determining the health of a native forest system through the measure of their diversity and species richness (Ambrosio, *et al*., 2015). They play key roles such as nutrient cycling through decomposition processes (López-Quintero, *et al*., 2012), soil

formation and plant protection from diseases. Most of these macrofungi (saprophytic and ectomycorhizal) make an important contribution to local livelihoods through provision of food as wild edible mushrooms (Thatoi & Singdevsachan, 2014; da Fonseca, *et al*., 2015) and income. Macrofungi have also led to the development and growth of industries involved with dyes, pharmaceuticals, organic acids, hormones, animal feeds and beverage processing (Pushpa & Purushothama, 2012).

Despite the role of macrofungi in both natural and agro-ecosystems, scanty information exists about their interactions within the forest ecosystems (Claudia, *et al*., 2015). Other organisms have received great attention and have been adequately studied (Angelini, *et al*., 2015). It is estimated that there are over 3-5 million species of fungi in the world and more data for the species is expected from the tropical region for an even greater figure (Hawksworth, 2012). This is because the tropics have been less studied for the macrofungi diversity compared to the temperate region (Hawksworth, 2001). Approximately 25,000 and 7,000 of animals and plants biodiversity respectively have been described and documented in Kenya compared to only 2,071 species of fungi (Republic of Kenya, 2015). Yet, 5000 species of fungi have been reported to exist under various habitats in Kenya and need to be studied (Republic of Kenya, 2015).

Forested ecosystems also host a wide range of wild edible macrofungi (WEMs) diversity that has not been fully exploited (Mwai & Muchane, 2016). Little is known about WEMs in Kenya (Musieba, *et al*., 2012; Mbaluto, 2014; Wandati, 2014), yet they offer potential for cultivation and are an under-exploited income source. WEMs are important natural resources which have been utilized from the wild collections since time immemorial as important source of food and medicine (Tibuhwa, 2013; Degreef, *et al*., 2016; Mwai & Muchane,

2016). Over the past years, there has been increased interest in exploiting and domesticating the wild edible mushrooms. Since forest habitat are rapidly being destroyed alongside the germplasm of WEMs, there is need to assess their wild diversity by developing mycelial cultures in the laboratory to encourage their propagation and conservation thus protecting them from extinction *(*Enow, *et al*., 2013).

It is also important to assess their nutritional composition as a way of promoting their consumption both in the wild and from the domesticated forms. To increase number of locally available mushroom germ-plasms, it is vital to establish wild mushroom identities using both morphology and molecular techniques (DNA barcoding and phylogenetic analysis). Molecular tools provide a quick, reliable and accurate method for mushroom identification (Rajaratnam & Thiagarajan, 2012) although this far, only a few mushroom species in Kenya have been identified using molecular techniques (Musieba, 2013; Onyango, *et al*., 2016; Mbaluto, 2014). This study was conducted in Kikuyu Escarpment Forest, Kereita forest block which is part of the world-renowned Aberdare forest. The area was identified because it harbors wide range of flora and fauna diversity and is also suggested to host diverse macrofungi diversity (Kost, 2002) and some species may contribute to the expansion of mushroom industry in Kenya. The forest has also been undergoing various disturbances including conversion of indigenous forest to fast growing and high value forest plantations with species of *Pinus radiata, Pinus patula*, *Pinus taeda*, *Cupressus lusitanica* and *Eucalyptus* species. This study forms the baseline data in understanding the diversity of macrofungi under different land uses.

1.1 Statement of the problem

Forested ecosystems in Kenya harbor diverse macrofungi diversity. However, most of these macrofungi diversity are at risk of extinction following unsustainable human activities (Enow, *et al*., 2013). These activities which include conversion of native forests to fast and short cycle growing plantation species are a threat to fungi biodiversity. Over the past 20 years, more than 30% of forested ecosystems and its associated biodiversity have been lost through anthropogenic activities associated with clearing of forests to make provision for agriculture, illegal timber harvesting and settlements (Gateri, *et al*., 2014). Information on fungi lost during forest conversion from the indigenous to the plantation forest and agricultural land is scanty and knowledge on their distribution and ecology is also lacking (Paz, *et al*., 2015).

Research and conservation efforts over past years has concentrated more in restoration and conservation of plant and wild animal species, but very little has been dedicated to fungi community. Yet fungi in particular macrofungi (mushrooms) play significant role in sustaining plant community by regulating nutrient cycling processes through decomposing dead plant and animal material, supplying nutrients to plants, and providing food to insects, small mammals and soil microbes (O'Hanlon & Harrington, 2012). They are also non-wood forest products associated with immense nutritional, medicinal and economic benefits. The products (wild edible macrofungi) are harvested in wild for food and medicine in western and coastal regions in Kenya (Musieba, 2013), and very few of these species have been domesticated to support mushroom industry in Kenya (Mwai & Muchane, 2016). In-order to conserve and sustainably utilize diverse wild macrofungi community in forested areas in Kenya, knowledge on their morphological diversity as influenced by different land uses as well as genetic variability among edible populations are important (Hussein, *et al*., 2014).

Therefore, the study aimed at assessing the morphological and genetic variation of macrofungi in Kereita forest within the indigenous and *Pinus patula* forest and also to compare the mycelial growth of selected wild edible macro on the artificial media and their mineral content.

1.2 Justification

Kikuyu Escarpment Forest is part of the world-renowned Aberdare forest and an important biodiversity area with flora and fauna of global significance. The forest has been suggested to harbor high fungal biodiversity and a major portion of these biological resources are yet to be documented (Kost, 2002). Over the past years, the forest has undergone diverse land use changes inorder to introduce the fast growing *Pinus* and *Cupressus* tree species associated with immediate economic benefits such as timber leading to changes in the forest biodiversity (Kost, 2002). Macrofungi occurring in native and plantation forest types of Kikuyu escarpment have the potential to contribute positively to the food, health and economic needs of the communities living at the edge of the forest. Thus there is need to determine the diversity loss in the region and conserve the macrofungi biodiversity of Kereita forest. This study contributes to the conservation of biodiversity by determining the diversity in the indigenous and plantation block and the species with potential to be utilized in the mushroom industry.

1.3 Main objective

To determine the effects of land use on the diversity of macrofungi in Kereita forest, Kikuyu Escarpment.

1.3.1 Specific objectives

(i) Determine the diversity of macrofungi in the indigenous and plantation blocks and seasonal occurrence in Kereita forest

(ii) To characterize macrofungi collected from the two land use types using classical methods and the selected species using molecular techniques

(iii) Analyse the mineral content of the common edible species from Kereita forest

(iv) To determine the suitable culture media for the growth of common edible species invitro collected from Kereita forest.

1.4 Hypothesis

H1: There will be a difference on the diversity of macrofungi from different forest types, different genetic variability of the selected edible macrofungi species, difference on the mycelial growth of the selected macrofungi species using different artificial culture media and different mineral content levels of the edible species.

CHAPTER TWO

LITERATURE REVIEW

2.1 Macrofungi characteristics and classification

Macrofungi are macroscopic fruit structures (bodies) visible to the naked eye that are produced by Ascomycota and basidiomyota fungi during their sexual reproduction cycles. They are representative of invisible extensive belowground mycelia (Rajaratnam & Thiagarajan, 2012; Enow, *et al*., 2013). The mycelium aids in the uptake of food nutrients through absorption and when the climatical conditions are favourable it forms the fruit bodies (sporocarp) structure (reproductive phase that bears the spores). The fruit body usually forms when the mycelium of the same species binds together in the sexual stage. The fruit body has a spore producing tissue called the hymenium (Etang, *et al*., 2006). The macrofungi are also known as macromycetes belonging to the Kingdom Fungi. Fungi are heterotrophic organisms which lack chlorophyll and therefore they grow saprophytically on dead decaying organic matter. They lack differentiated organs and belong to a kingdom of their own different from that of animals and plants (Enow, *et al.*, 2013). Most of these macrofungi belong to both Ascomycota and the largest phylum Basidiomycota (Undan, 2016). Basidiomycota division consists of members that have drawn world's attention due to their valued novel metabolites especially *Ganoderma lucidum* and *Trametes versicolor* with potent medicinal properties against cancer (Pushpa & Purushothama, 2012).

The life cycle of a mushroom starts from a spore which generates structures known as primary mycelia (produced from a single spore). When 2 spores mate they produce the secondary mycelium. The secondary mycelium grows vegetatively to form macrofungi/ mushroom fruit body (Stamets & Chilton 2006) as shown in the figure below (Fig 1).

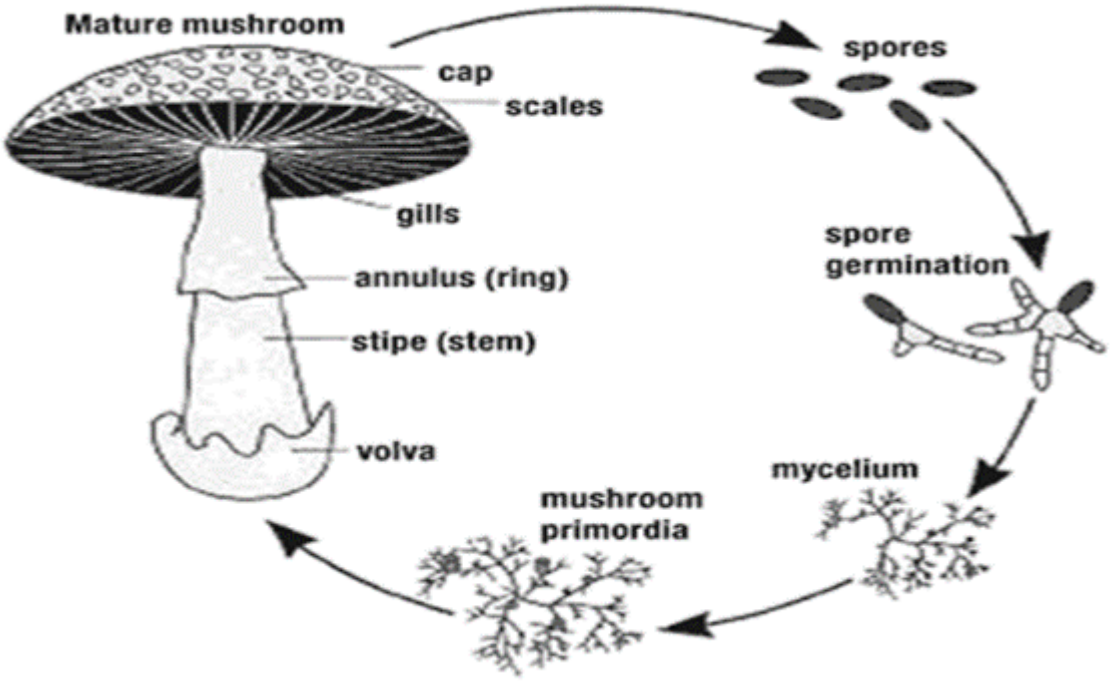

Figure 1: Mushroom life cycle: Adopted from (www.mushroomgrow.com) accessed on 2/11/2017

2.2 Importance of macrofungi

Macrofungi are valuable non-wood forest products and their role in nature is critical. Macrofungi are decomposer of organic matter in form of wood, litter, soil based substrates which eventually enriches the soil through nutrient cycling (Baral *et al*., 2015). The macrofungi are also source of food to the insects and the small mammals especially the edible macrofungi (Schigel, 2009). Since time immemorial, human have had interaction with mushrooms for food and medicine. Currently over 1100 macrofungi species are consumed in over 80 countries world wide (Boa, 2004). Through the measure of diversity and species richness of macrofungi in any forest, it is possible to deduce the health of that ecosystem. Thus, macrofungi serve as an indicator of a healthy forest (Ambrosio, *et al*., 2015). Fungi in the division basidiomycetes have evolved symbiotic relationship with numerous insects by creating suitable habits for them. For example, the scale insects depend on the fungi for security from predators. On the other hand, the fungus benefits by obtaining nutrients

fostered by the insect from parasitized plant and also a means to disburse its spores. The fungal mycelium also covers and gives protection to the insect colonies. One of the macrofungi tht has been found to form an association with over 200 beetles is *Polyporus squamosus* has been found to host over 200 beetles in Europe (Cockle *et al*. 2012).

2.3 Effect of land use, seasonality and diversity of macrofungi

Forested ecosystems are suitable and major habitats for diverse macrofungi (Kost, 2002; Tibuhwa, *et al*., 2011; Goldman, *et al*., 2015). Trees which are the main components in the forest habitats influences fungi development by creating shade that regulates forest temperature and moisture (Gomoryova, 2013). Activities associated with forest land management are critical in determining the macrofungal composition since they alter vegetation communities, tree species composition and soil factors (Baral, 2015). Among the ongoing anthropogenic activities in many countries, includes change of land use types from indigenous forest to plantation forest type (Kost, 2002). This is also coupled by other activities linked with forest clearance to make room for agriculture, timber harvesting and charcoal burning which is increasingly becoming a threat to macrofungi diversity (Enow, *et al*., 2013). The negative effects are associated with reduced specific habitats essential for fungal development such as lack of course dead wood due to the disappearance of old trees. It is estimated that approximately 50% of the macrofungi are wood decomposers which continuously depend on the availability of dead wood to maintain nutrient cycling (Josefsson, *et al*., 2010; Zotti, *et al*., 2013). Macrofungi are seasonal and occupy diverse niches in forest, grasslands and wetlands habitats. Their fruit bodies of macrofungi are observed above the ground during the rainy and wet seasons. The season under which they appear is characterized by favorable conditions such as ample moisture, sunshine and relative humidity (Krishna, 2015). However, during hot and dry seasons, few species are encountered for example the *Microporus*, *Trametes*, *Phellinus*, *Ganoderma* among others (Enow, *et al*., 2013)

2.4 Taxonomy challenges

Despite fungi ranking as the second most diverse component within the ecosystem, it has been given low study consideration compared to insects, plants and animals (Yamatisha, *et al*., 2015). The situation has made taxonomy and interaction of fungi with other organisms to be poorly known compared to the majority of plants and other organisms present in forest ecosystems (Rudolf, *et al*., 2013). In particular, macrofungi taxonomy has been poorly conducted in the African tropical forests which is estimated to be higher compared to the temperate region (Hawksworth, 2001). As a result, status of macrofungi diversity in the tropics has remained unclear (Hawksworth, 2004). However, work on fungi diversity in the tropical regions is on -going and according to the recent estimates of the described and identified fungi, the previous figure of approximately 1.5 million have risen to roughly 5 million (Hawsksworth, 2012). More data from the tropical regions could raise the figures even higher. According to Krishna, *et al*., (2015), macrofungi are represented by approximately 41,000 species and about 850 species have been documented in India most of which are gilled fungi. Research and conservation efforts over past years has concentrated more in restoration and conservation of plant and wild animal species, with very little focus on fungi community (Republic of Kenya, 2015). In addition, scarce information is available on the diverse richness of indigenous fungi species with potential in sustainable afforestation use.

2.5 Morphological characterization of macrofungi

Morphological characterization of fungi mostly relies on the macro morphological features such as; the cap appearance and size, colour, shape, surface texture and surface moisture, gill attachment, gill colour, gill spacing, lamellules, the stem size and attachment, shape, surface texture and surface moisture, presence or absence of partial and universal veils, flesh colour and texture, stem base morphology, habitat/substrate (Kolet, 2013). Microscopic features

includes investigation of the spore print colour, spore sizes, spore shape, texture and chemical reaction of particular macrofungi components such as spores, basidium and the stipe and use of chemicals such as Meltzer reagent and potassium hydroxide (Kuo, 2016).

2.6 Molecular characterization of macrofungi

Molecular techniques offer valuable tools for characterizing fungi (Rajaratnam & Thiagarajan, 2012). The markers targets the barcodes commonly identified as nuclear and ribosomal DNA and it is agreed that the ITS gene region of the ribosomal DNA (rDNA) is the main barcode of fungi (Schoch, *et al*., 2012). They provide more dependable taxonomic identity (Tibuhwa, 2013). Currently, amplification of the Internal Transcribed Spacers region of ribosomal DNA (ITS rDNA) has been proven as a powerful tool for the identification and phylogenetic analysis of mushrooms. It is therefore possible to classify macrofungi and discriminate the species lineages variation that occurs at species level using molecular markers (Onyango, *et al*., 2016). The ITS region (ITS), is possibly the most sequenced due to its high degree of variation compared to the other rDNA regions (Hussein, *et al*., 2014). The region is polymorphic and thus provides sequence variability critical in distinguishing among fungi species (Martin, *et al*., 2004). The prevailing ITS1 and ITS2 gene regions are good in showing disparity between fungal species with variations (Korabecna, *et al*., 2003). Exact identification of wild edible macrofungi is a very important step towards proficient exploitation of macrofungi germplasm obtained from the wild (Undan, 2016). Improper description of species may have negative implication on important programmes such as breeding, domestication and on property rights protection (Onyango, *et al*., 2016). Both morphological and molecular applications are therefore best combination approaches in the identification of macrofungi incase of missing morphological features especially in the young fruitbodies (Parnmen, 2016).

2.7 Mineral composition of wild edible macrofungi

Macrofungi are recognised as delicacies in different parts of the world as therapeutic foods whose chemical composition makes them appreciable as agents of preventing diseases such as diabetes, hypertension, cancer and hypercholesterolemia (Puttaraju, *et al*., 2006). Their nutritional value and the sensory properties is mainly dependent on their chemical composition. Different species of macrofungi differ in the mineral content level which is influenced by prevailing atmospheric conditions, age of the macrofungi fruitbody, the part of the fruitbody and the substratum on which it is growing (Rajarathanam, *et al*., 1998 ; Table 1). Organisms require low quantities of some metals such as copper, iron, chromium, cobalt, manganese and zinc and higher levels of minerals such as potassium and phosphorus (Mallikarjuna, *et al*., 2012). They are nutritious protein rich foods and good sources of major and minor nutrients (Ijioma, 2015). Therefore, knowledge on mineral nutrient composition of wild edible macrofungi is critical because it allows usage of fungi as food, income generation and in promoting conservation actions and better management of these resources.

Table 1: Range of mineral levels in macrofungi reported in literature (mg/100g)

Mineral element	Range of mineral level from literature (mg/100g)	Reference
Phosphorus (P)	120-2000	(Falandysz, *et al*., 2001)
Potassium (K)	2500-4100	(Bakken and Olsen 1990)
Calcium (Ca)	1.8-59	(Falandysz, *et al*., 2001)
Magnesium (Mg)	60-250	(Mallikarjuna, *et al*., 2012)
Sodium (Na)	6-92	(Falandysz, *et al*., 2001)
Iron (Fe)	1.46-83.5	(Mallikarjuna, *et al*., 2012)
Manganese (Mn)	1.81-10.3	(Mallikarjuna, *et al*., 2012)
Copper (Cu)	7.1-9.5	(Mallikarjuna *et al*., 2012)
Zinc (Zn)	2.98-15.8	(Mallikarjuna, *et al*., 2012)

2.8 Domestication of wild edible macrofungi

Wild edible macrofungi are among the most valuable none wood forest products (NWFP) (Tibuhwa, 2013). More than 200 of the species are edible though least explored in the developing countries for their food, economic and health importance (Mwai & Muchane, 2016). The wild harvested truffles, chantelles, porcini are extensively commercialized fetching billions of euros (Donnini, *et al*., 2013) especially in the developed part of the world. Numerous kinds of macrofungi are inedible but are important candidates of pharmacological and medicinal properties and applications (Krishna, et *al*., 2015). For the edible species, it might be difficult to identify some correctly in the field. Some are toxic and have been proved very deadly when eaten (Tibuhwa, *et al*., 2013). Deaths due to consumption of poisonous wild mushrooms are popular among indigenous poor people (Parnmen, *et al*., 2016). To mitigate against such risks, several researchers in Africa have made efforts towards domestication of known wild edible mushrooms such as *Pleurotus Citrinopileatus* (Musieba, 2013) *Auricularia auricula* (Onyango, *et al*., 2011) *Coprinus cinereus, Volvariella volvacea, Pleurotus flabellatus* (Mshandete & Cuff, 2008), *Psathyrella atroumbonata* (Ayodele & Okhuoya, 2009), *Lentinus sajor caju* (Hussein, *et al*., 2016).These macrofungi are among the species that man has gathered since the creation of the world for consumption. Cultivation of saprophytic wild edible mushrooms is currently an economical biotechnology for converting the abundant lignocellulose organic waste into protein rich food which also combines with the reduction of environmental pollution and creates income-generating opportunities (Okoro & Achuba, 2015). However, the world's largest mushroom consumption is still from commercial enterprises (Thawthong, *et al*., 2014). Approximately, 130 macrofungi are reported as domesticated though the world estimates reveal that 650 -700 macrofungi are edible (Thawthong, *et al*., 2014). Inorder to expand the mushroom industry in Kenya, new strains are required for uptake by mushroom farmers other than the two commonly cultivated

species (*Agaricus* and *Pleurotus* sp.). These two species have been associated with low yield compared to similar varieties cultivated in the temperate regions and increased susceptibility to pests and diseases (Mwai & Muchane, 2016). When selecting mushrooms for domestication; it must be palatable, have regional adaptability suitable to the local climate, substrates availability and market acceptance (Ilori, *et al*., 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted in Kikuyu Escarpment Forest (KEF), Aberdare Range Forest. The KEF is considered to be an important biodiversity area, suspected to harbor high diversity of fungi due to the wide range of elevations, habitats and soil types that exist. The forest lies on the southern slopes of Aberdare Forest, 30 km north-west of Nairobi and covers an area of 37,620 ha. It is positioned at 0°56'S, 36°40'E at an altitude of 1,800.2,700 m and mean rainfall of 1500mm per year. The KEF is divided into 6 main blocks namely; Uplands, Kereita, Kieni, Kamae, Kinale, Raggia and Kijabe (Fig 2). This study was conducted in Kereita forest block that covers approximately 4,720 ha of which 75% is the indigenous forest, 8% exotic tree plantation while 13% is characterized by shrub land, Bamboo and agricultural crops. The indigenous forest in Kereita forest consists of bamboo *Ocotea, Podocarpus, Macaranga, Neoboutonia, Strombosia,* and *Juniperus* tree species while exotic tree plantations include *Cupressus lusitanica, Pinus patula, Pinus radiatae* and *Eucalyptus grandis*.

3.2 Experimental design

In this study, six blocks within Kereita forest under the indigenous and *Pinus* plantation forest types (Fig 2; Fig 3; Fig 4) were considered for the inventory of macrofungi. The blocks investigated are shown in Table 2.

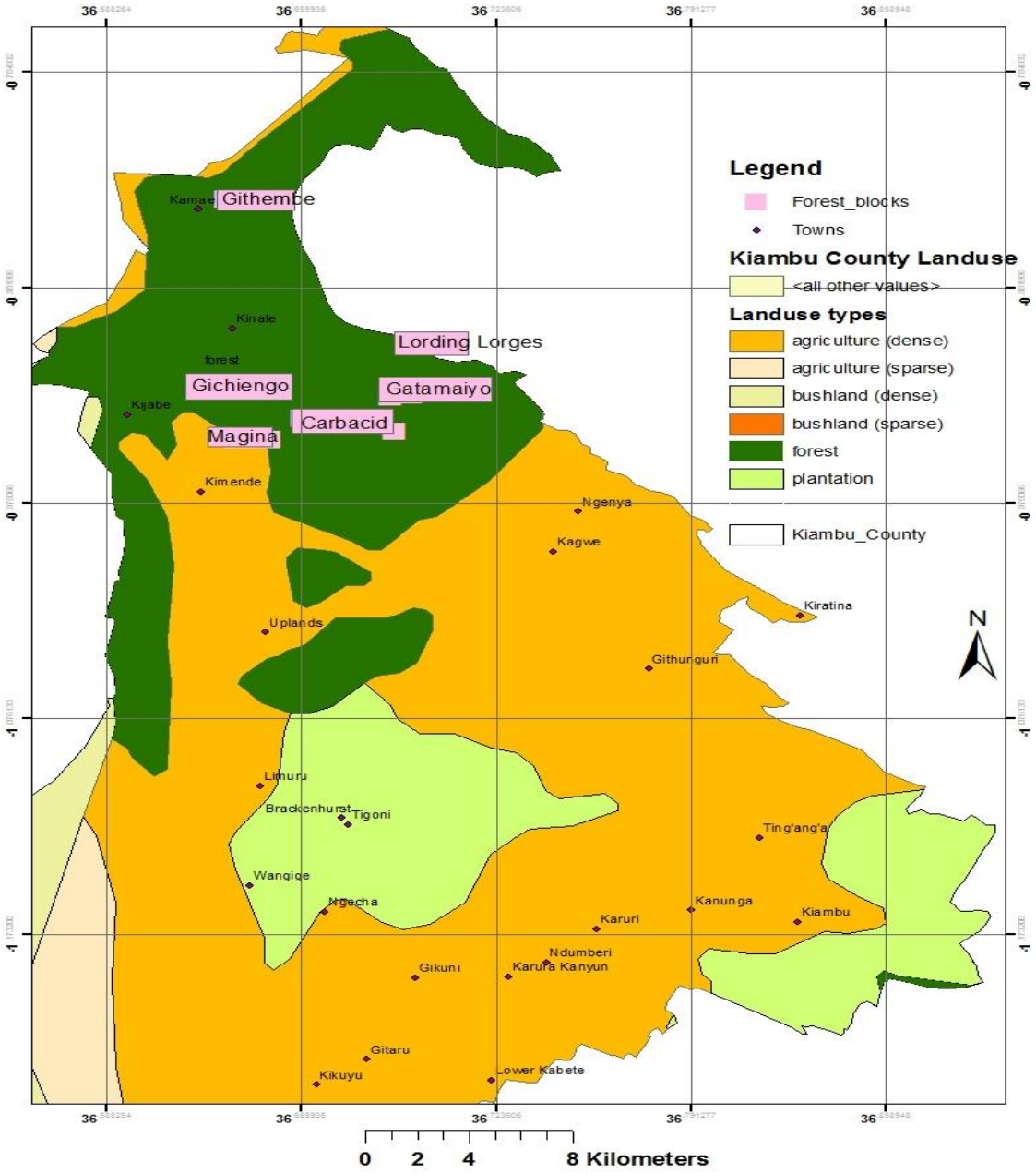

Figure 2: Kereita forest sampling (Source: From 1980 landsat data by the Japan International Co-operation Agency, JICA, National Water Master Plan,Kenya (http//192. 156.137.110/gis/search.asp accessed on 15/11/2017)

In each forest type, 5 plots along 3 transects which were 500m apart were delineated using markers (with their GPS readings).The macrofungi were sampled in 20 m by 20 m sampling plot laid down along the transects. A total of 60 plots in the two vegetation type (indigenous and plantation forest) and two seasons (wet and dry) were sampled .

Table 2: Sample sites in Kereita forest

Land use types	Blocks	Transects	No of plots
Pine plantation	Gichiengo	5	1
	Magina	5	2
	Githembe	5	3
Indigenous	Carbacide	5	1
	Gatamaiyo	5	2
	Lordgings ridges	5	3

Figure 3: Indigenous forest land use (photo by Muchai Muchane, 2014)

Figure 4: Plantation forest land use type (Photo by Muchai Muchane, 2014)

3.3 Collection and documentation of macrofungi

Encountered macrofungi were photographed in-situ counted and recorded for estimation of diversity. Representative fruit bodies of each species were carefully uprooted by holding the stipe carefully but firmly. Features such as phenology, smell, habitat, colour of fresh macrofungi , nature of substrate and associated plant species were recorded to avoid the phenetic changes that would occur after drying. The specimens were then tagged and packaged in separate grease proof papers in order to prevent spore contamination among the different taxa that would eventually interfere with microscopic identification. Collected specimens were carefully labeled before being transported to the Mycology laboratory using plastic food baskets. In the laboratory, spore prints were made using the freshly collected fruiting bodies. The specimens were oven dried at 45°C and the period of drying depended on the thickness of the macrofungi. The specimens were then preserved for later identification.

3.4 Identification of the specimens

3.4.1 Morphological characterization of collected macrofungi

The study used morphological characterization to identify macrofungi species found in natural forests and plantation forests based on both specimen macroscopic and microscopic features (Mueller, *et al* 2004). The information of the various characters was used to identify each specimen by comparison with illustrations in colour field guides and descriptions. Using varieties of field monograph of coloured mushrooms, keys and books (Ryvarden, *et al*., 1994, Westhuizen & Eicker, 1994; Härkönen *et al*., 2003, Philip, 2006; McAdam, 2009) as well as peer reviewed journal articles, the species were identified up to species level and most of them to the genus level. The macroscopic features ranged from: the cap appearance and size, colour, shape, surface texture and surface moisture, gill attachment, gill colour, gill spacing, lamellules, the stem size and attachment, shape, surface texture and surface moisture, presence or absence of partial and universal veils, flesh colour and texture, stem base morphology, habitat/substrate. Microscopic features were examined under the compound microscope using standard microscopic methods (Roy & De, 1996). The Edinburgh Botanic Gardens colour chart was used for the description of specimens and spore print colours. The dried specimen were revived in 10% KOH in order to study further details, Meltzer reagent and cresyl blue were used to study the spores amyloidity and metachromic reactions respectively.

The common edible species were delineated based on the following morphological characteristics: *Agaricus* genus; Cap colour, shape, size, surface appearance and texture and margin shape, lamellae type either free, gills close or crowded, stipe shape, texture and colour, Annular shape, spore print colour, spore shape and reaction to KOH and Meltzer reagent and odour and habitat (Chen, *et al*., 2015). *Macrolepiota* sp: Basidiomata pileus size,

pileus shape, surface appearance and texture, size, shape, lamellae nature (free, crowded and colour), stipe size, base, type (hollow or firm), annulus colour and type, spore colour and type, reaction to meltzer reagent and habitat. *Pleurotus* sp: Cap shape, colour, orientation of the stipe from the substratum, margin, gills (close or distant), colour, stipe size, spore print colour, spore shape and type, odour and habitat (Avin, *et al.*, 2014). *Auricularia* sp: Colour of the external basidiome, Stalk presence or absence, nature of the abhymenial hairs (Onyango, *et al*., 2011). *Stropharia* sp: Cap shape, size, colour, texture, margin, hymenium colour, gill arrangement, stipe colour, texture, size, annulus type and point of placement on the stipe and habitat (Kuo, 2016). *Suillus* sp: Cap colour, texture and appearance, size, margin, hymenial pore type, stipe texture and appearance, annular type and shape, spore print colour and spore type and habitat (Kuo, 2016) (Appendix 5).

On the basis of biotrophic classification the groups were recognized as saprotrophic, symbiotic and parasitic groups.

3.4.2 Molecular characterization

The extraction of total genomic DNA was obtained from dry fruit bodies of the common edible macrofungi samples whose morphological identification matched with species documented as edible. The macrofungi belonging to the genera *Macrolepiota, Auricularia, Pleurotus, Fayodia* and *Agaricus* genera were characterized. CTAB (Cetyl trimethyl-ammonium bromide) (White, *et al*., 1990) was used. Using new sterile blade, approximately 5 g of each species was sliced and transferred into 1.2 ml appendorf tubes which were clearly labelled. The tubes were shaken to mix the contents and then inserted in a prewarmed waterbath for 30 minutes. The samples were then removed and centrifuged for 1 minute at 2000 rpm. The top phase of the mixture was discarded and resultant residue at the bottom of the tube was maintained. 2% of polyvinylpyrrolidone, preheated (65°C) extraction buffer

CTAB [2.5% w/v] (100 mM Tris-HCl [pH 8], 1.4 M NaCl, Dithiol threitol solution (DTT) [1% v/v] 20 mM EDTA,], and polyvinyl pyrolidone (PVP) 1% w/v) were added. The tubes were then homogenized gently by inverting the tubes. The tubes were again incubated in a rocking water bath at 65°C for 45 minutes. 450 µL of chloroform-isoamylalcohol (24:1) was added after the mixture had cooled at room temperature. Each tube was inverted several times to mixing the constituents. This was followed by Centrifugation at 4000 rpm for 20 minutes followed and then the supernatant was transferred into new labelled appendorf tubes. Ice cold 40% isopropanol (600 µl) was added to the mixture which was inverted twice to aid in DNA precipitation. The tubes were then incubated at -20ºC overnight in a freezer. To achieve the formation of DNA pellet, the samples were centrifuged at 4000rpm for 20 minutes. Addition of Ethano and Sodium acetate (25:1) was followed and the mixture was incubated for 45 minutes at -20ºC. The supernatant was poured and the DNA pellet washed with 500 µL of 70% ethanol twice. The recovered DNA was left to air dry for approximately 15 minutes and re-suspended in 100 µL TE 0.1. Two µl of RNAse was put in each tube and the mixture incubated for 30 minutes at 37 ºC. The estimation of DNA quality and concentration was done using NanodropTM Lite Spectrophotometer (Thermo-Scientific Inc, USA) at 260-280 nm. 0.8 % (W/V) of stained agarose gel was used for Horizontal gel electrophoresis (Thistle Scientific Ltd, USA) visualization under UV. The samples were then sent to Inqaba Biotec East Africa Ltd (Africa's genomic company) for full sequencing reactions.

3.4.3 Amplification

Polymerase chain reaction (PCR) amplification of the ITS region (5.8s rRNA gene) was performed in a programmable Mastercycler thermocycler (C1000-BioRad, USA) using the PCR conditions described by Vellinga *et al*., (2003). The primers included; the forward primer ITS 1 (TCCGTAGGTG AACCTGCGG) and reverse ITS 4

(TCCTCCGCTTATTGATATGC) (White *et al*., 1990). The amplified products were separated by horizontal gel electrophoresis on 1.5 % (w/v) agarose gel on 0.5X TBE at 70V for 60 min and visualized under UV after staining with 2 µl GelRedTM (Thermo Scientific).

3.5 Selection of the wild edible macrofungi for culturing and mineral composition analysis

Three macrofungi belonging to the families Agaricaceae, Auriculariaceae and Pleurotaceae families were selected for culturing and mineral analysis based on macro-micro morphological identification and molecular confirmation of the three species. The selection was also based on the ability of the species to colonize on the artificial culture media and potential in colonizing readily available substrates (Mbaluto 2015, Onyango, *et al*., 2011). The species were identified as *Macrolepiota dolichaula, Auricularia polytrica* and *Pleurotus djamor*.

3.6 Media preparation and inoculation on the culture media

Potato infusion was prepared to make PDA and PDYA by boiling 200 g of sliced unpeeled potatoes in 1 liter of distilled water for 30 minutes. The infusion was filtered using a sieve to collect the effluent. 20 grams of agar was added to the contents. The mixture was divided into two 500ml. One 500ml was used to make PDYA (Potato Dextrose Yeast Agar) by adding 10 grams of yeast. The other 500ml content was used to make PDA. 250mg of amoxillin was added to the two media to discourage bacterial contaminants. The contents were boiled to dissolve and finally autoclaved at 121°C for 15 minutes. Malt extract agar was prepared and sterilized as per the manufacturer's instructions by dissolving 39 grams in 1litre of distilled water. After cooling, the media was poured aseptically into sterile petri plates (disposable) and allowed to solidify. The inoculation of the macrofungi was done asceptically on Potato

Dextrose Agar (PDA), Potato Dextrose Yeast Agar (PDYA) and Malt extract agar. The media was used to culture the above mentioned macrofungi species. Tissue culture method was used to inoculate the macrofungi. The dry fruit bodies of the selected specimens were sterilized in 5% sodium hypochlorite and tissue sections approximately 2x2mm^2 were cut with new sterile surgical blades (from inner surfaces of the cap) cleaned using 70% ethanol. The cut fragments were then placed at the middle of the plates of each media. The plates were then sealed with a parafilm and incubated in dark cabinets sterilized with 70 % ethanol at 25 ^{0}C until the mycelia developed. A complete randomized design was used to place the petri plates in the incubator. Sub- culturing of the mycelia was done until pure cultures were obtained. This was followed by culturing each species on each media which was replicated five (5) times. The mycelia of *Pleurotus*, *Macrolepiota dolichala* and *Auricularia polytrica* were observed and colony diameter of mycelia was measured daily using a clear (transpalent) ruler until the 7th day when three quarters of the plates were colonized completely by the mycelia.

3.7 Mineral content analysis

The dry fruit bodies of *Macrolepiota dolichaula*, *Pleurotus djamor* and *Auricularia polytrica* were analysed at Crop Nutrition Laboratory (Crop Nut) using Microwave digestion method. 200g of each species was obtained and was dissolved in concentrated nitric acid-Hydrochloric acid using microwave energy on the laboratory microwave unit. The constituents were placed in a fluorocarbon polymer (PFA or TFM) microwave vessel. The vessel was sealed and heated in the microwave unit for a specified period of time. After cooling, the vessel contents were diluted to volume and analyzed for Ca, Mg, K, Na, Mn, Fe, Cu, Zn, P mineral concentrations using Atomic Emission spectrometry (ICP-OES) method according to Fassel & Kniseley (1974).

3.8 Data analysis

3.8.1 Diversity data analysis

The frequency of occurrence of the functional groups of macrofungi was calculated as (total number of individual group /total number of all the groups x 100 (Wang & Jiang, 2015). The families were plotted against the total number of individual isolates (species) per a given family. The macrofungi species densities were calculated as total number of a species per unit area ($1m^2$) (Feest, 2006). Species richness was calculated as total number of species per 20x20m plot. Species Shannon–Wiener diversity index (H´) and Simpson index were calculated for each field plot using PAST programme (Hammer, *et al*., 2001). Simpson's diversity index (D) was calculated according to Megersa, *et al*., (2016) where $D=\Sigma Pi^2$ ……Pi = Ni / N, and Ni = Σ ni and Shannon-Wiener index as (H'= Σ [pi (log pi)], where; pi is the proportion of individuals found in species; ln is the natural logarithm) (Margalef, 2008). The frequency of occurrence (for each species) was calculated in accordance to (Wang, *et al*., 2015). Two-way ANOVA was performed to assess the effects of forest type and season on species richness, density, diversity measures, and mean diameter of macrofungi mycelia. Differences between treatment means were analysed by Turkey's *post hoc* test at $P < 0.05$ and standard deviation (SD) was used to separate the means. The effects of forest type and seasonality on macrofungi community composition was analysed by a multivariate redundancy analysis (RDA) using the Canoco 4.5 software (Braak & Smilauer, 1998). All data was tested for normality, and where necessary count data was logarithm (log+1) transformed to ensure conformity of the data with ANOVA assumptions.

3.8.2 Phylogenetic analysis

Both reverse and forward sequence data obtained from the nine mushroom samples were assembled and cleaned using DNA Baser v4.36.0 bioinformatics software. The homologues produced were queried using NCBI BLAST (Morgulis, *et al*., 2008; Zhang, 1997) in the non-redundant nucleotide database. Homologues of the queried sequences identified by BLAST as having the highest sequences identity/similarity were used to annotate closely related sequences for characterization. A Phylogenetic tree for the nine sequences reported in this study was generated by Mega7 (Kumar, *et al*., 2016) Bioinformatics Software. Mega7 used the Minimum Evolution statistical method and a 1000 bootstrap replication with a maximum sequence difference of 0.75. The phylogenetic tree was visualized and edited using Tree Graph2 (Stover & Muller, 2010).

3.8.3 Mycelial growth analysis

Two-way ANOVA was performed to assess the effects of macrofungi species and culture media on the growth of mycelia. Differences in means between the species and the media were analysed by Turkey's *post hoc* test at $P < 0.05$ using PAST programme.

CHAPTER FOUR

RESULTS

4.1 Macrofungi community within Kereita forest

A total of 28 families, 76 genera and 224 species distributed within the division Basidiomycota (223 genera within 27 families) and Ascomycota (1) species in the family Xylariaceae were encountered (Appendix 1). In the division Basidiomycota, the macrofungi species belonged to the class Agaricomycetes represented by 28 families and class Sordariomycetes represented by only 1 family (Xylariaceae). In the class Agaricomycetes, the order Agaricales (69%) represented the highest proportion of families followed by polyporales (14%). The family representation in other orders (Auriculariales, Haemenochaetales, Phallales and Xylariales) was at 3% each. Overall, the Agaricaceae family had the highest number of genera (44), followed by Mycenaceae (30), Polyporaceae (23), Marasmiaceae (19) and majority of the families (18) represented 1 genus each. Species belonging to the following families; Crepidotaceae, Physalacriaceae, Funariaceae, Gomphidiaceae, Meruliaceae, Niduliaceae, Pluteaceae, Typhulaceae and Xylariaceae were recorded only in the indigenous forest (Fig 5). The plantation also had species from 4 families and these are; Hydnangiaceae, Inocybaceae, Gomphidiaceae and Suillaceae not encountered in indigenous forest (Figure 5; Appendix 1). The rest of the species were found occurring in both forest types (Figure 5; Figure 7). 24% of the specimens were identified to species level, while 76 % were classified as a morphospecies belonging to some genus (Appendix 1; Appendix 2).

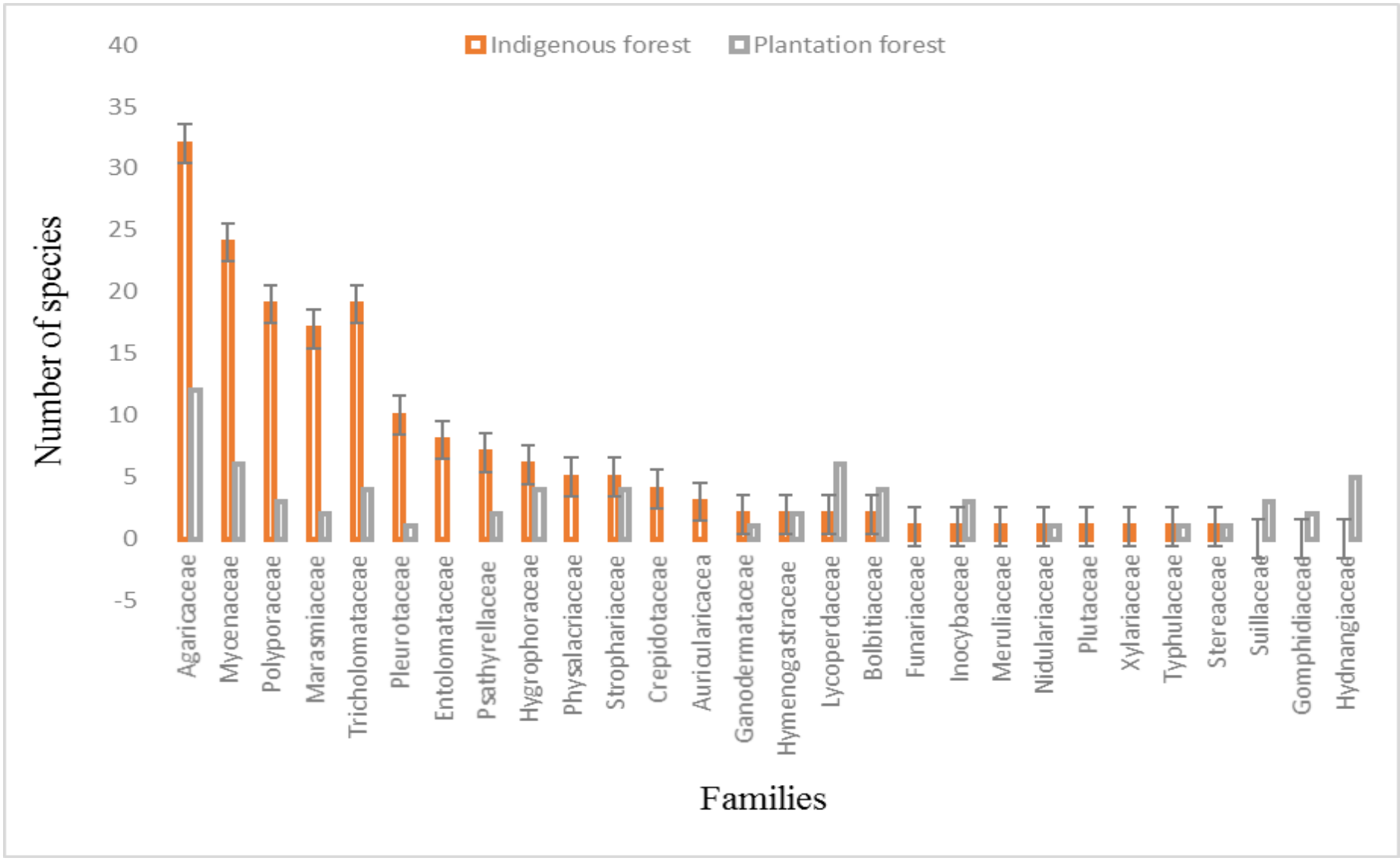

Figure 5: Species abundance of macrofungi in Kereita forest

Species accumulation curve showed that not all the species were sampled during the study and more can be recorded with additional sampling (Figure 6).

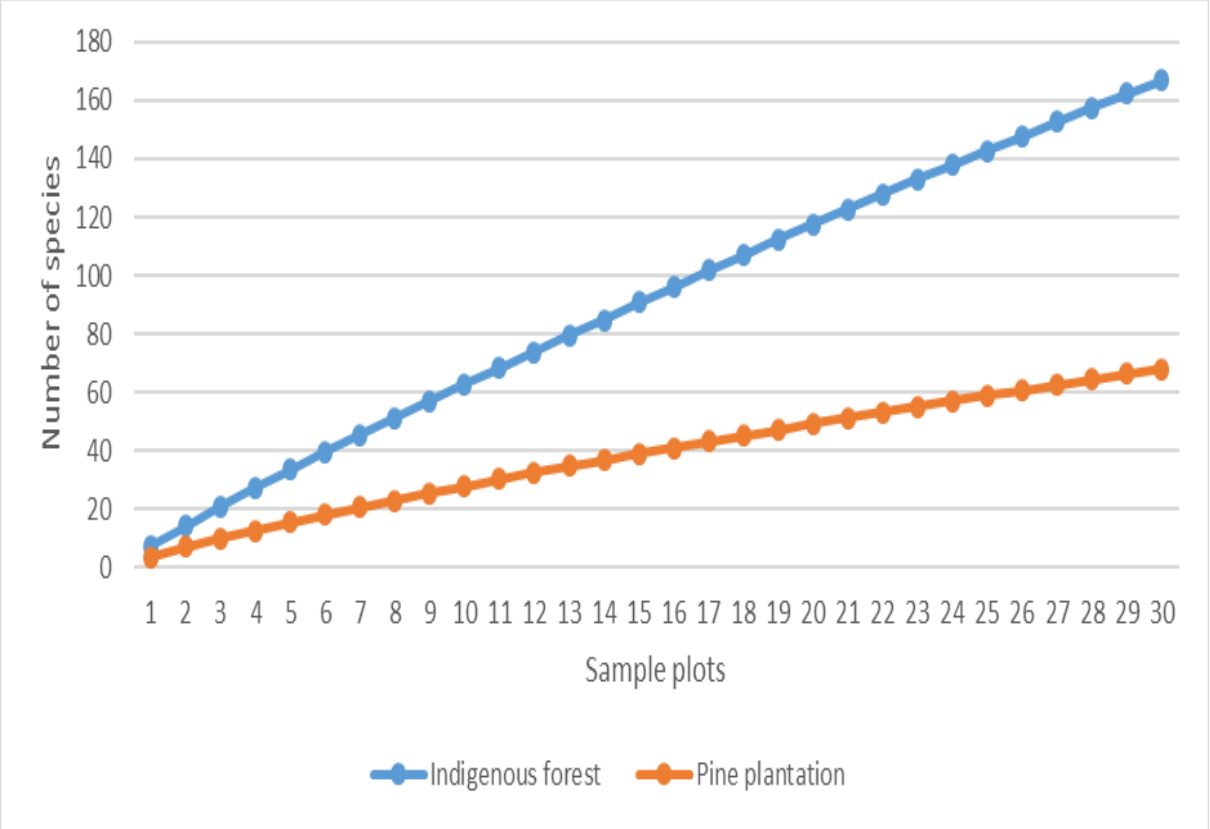

Figure 6: Number of species sampled in Kereita forest

4.2 Distribution of macrofungi by biotrophic groups

From the collected macrofungi, saprophytic group were 93% than the other groups during the wet and the dry seasons. The parasitic and ectomycorrhiza groups represented 3% and 5% respectively during the two seasons. Saprophytic species also dominated in the indigenous forest in both dry and wet season and were represented by wood rotters (50%), litter decomposers (29%) and soil (organic matter) colonizers (16%) (Fig 7). Pine plantation was dominated by both saprophytic and ectomycorrhiza species. Ectomycorrhiza species were only recorded in the Pine plantation forest and represented 6% of the total functional groups.

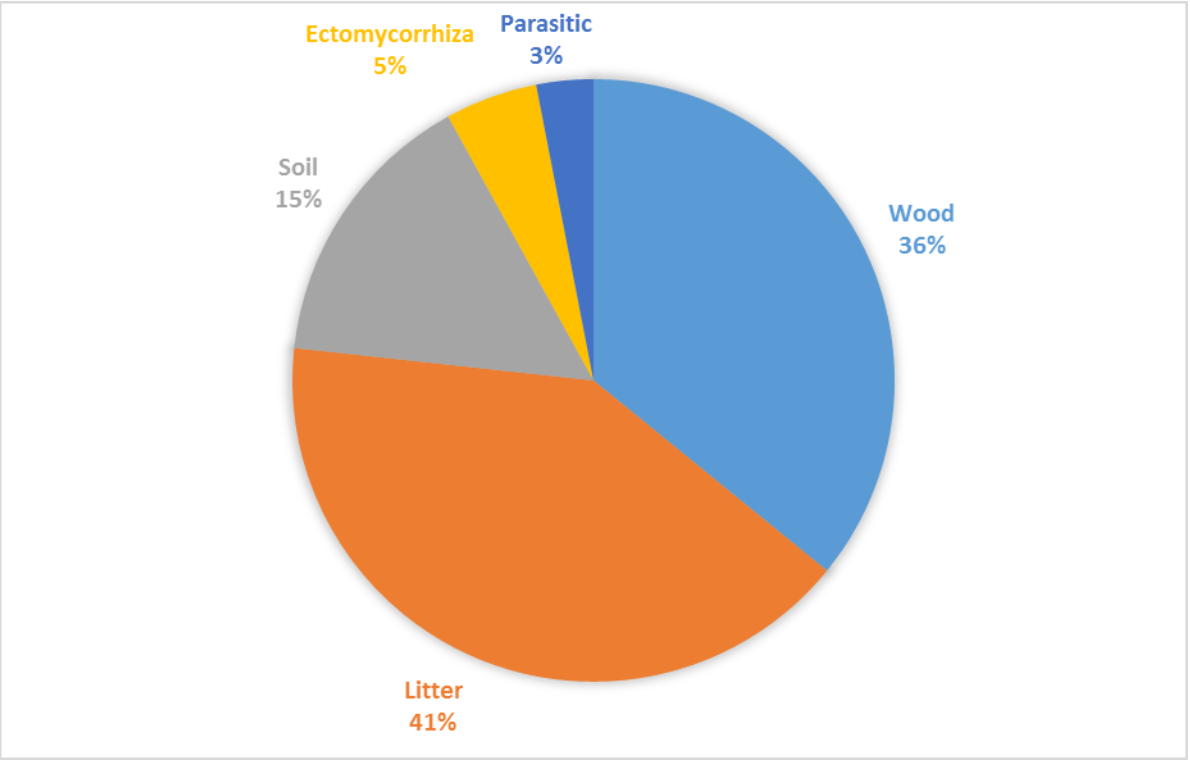

Figure 7: Distribution of macrofungi in Kereita forest

4.3 Effect of season and land use on macrofungi diversity

Seasonality and land use significantly affected macrofungi species richness (Season: F=13.03, p<0.05; land use F=7.32, p = 0.79) and species density (Season: F=50.89, p=<0.05; Forest type F=54.46, p<0.05). There were significant interaction between seasonality and forest types in species richness (F=3.94, p<0.05), density (F=36.14, p<0.05) and diversity (Shannon Weiner diversity Index) (F=0.14, p<0.05) but did not affect species diversity (Simpson diversity Index) (F=0.31, p=0.58) significantly. The indigenous forest had a significantly (p= <0.05) high density and species richness of fungi during the wet and dry seasons compared to the plantation forest (Table 3). Macrofungi density and species richness in the two forests were significantly (p<0.05) higher in wet season than in the dry season. There was no significant difference in species diversity during the wet and dry season in both forest types (Table 3).

Table 3: Effects of forest type and seasons on macrofungi diversity in Kereita

			Diversity indices and measures			
			Species richness (m)	Density (m^2.)	Shannon (H)	Simpson (I-D)
Interactions	A x B	Wet-Indigenous	10.13±1.41a	3.22±0.84a	0.84±0.14a	0.39±0.07a
		Dry-Indigenous	2.79±0.69b	0.19±0.09b	0.39±0.10a	0.211±0.06a
		Wet-pine	5.0±0.64a	0.15±0.05a	1.05±0.14a	0.53±0.07a
		Dry-Pine	2.0±0.26b	0.03±0.01a	0.51±0.11a	0.30±0.06a
	ANOVA	Forest type (A)	7.32(P<0.01)	54.46(p<0.01)	1.14(p=0.29)	2.25(p=0.1411)
		Season (B)	49.33(p<0.01)	50.89 (p<0.01)	13.03(p<0.01)	2.25(p=0.14)
		A x B	3.94(p<0.01)	36.14(p<0.01)	0.14(p<0.01)	0.31(p=0.58)

Key: Different letters within the same column show significant differences while same letters show no differences.

4.4 Macrofungi species composition

Macrofungi community composition in the Kereita forest was significantly affected by forest type (RDA, F = 5.47, P < 0.05) which explained 9% of the variability in the dataset. Land use type revelaed significant difference (p<0.05) in density of both saprophytic macrofungi genera such as *Armillaria*, *Pleurocybella*, *Cyathus* and *Galerina* (Figure 8; Figure 10) and parasitic species such as *Armillaria*, *Phellinus* and *Trametes* (Figure 8) and by more than 10%. The ectomycorrhiza species previously not in the indigenous forest especially species belonging to *Suillus* and *Laccaria* were introduced in pine plantation made up 14% macrofungi community in Kereita forest (Figure 9). The macrofungi species composition (community) was also significantly affected by seasonality (RDA, F = 3.97, P < 0.05) which explained 6% of the variability. The wet seasons was characterized by high number of fleshy wood rooting macrofungi species such *Pleurocybella*, *Cyathus*, *Hygrocybe*, A*rmillaria*, *Favolaschia*, *Myxomphalia*, *Micropsalliota occurring* in the indigenous forest only (Figure 8;

Figure 10). However, the polypores such as *Trametes*, *Microporus* and *Phellinus*, were present during the dry and the wet season in both land use types (Figure 11). The genus *Agaricus* appeared in both land use types during the dry and wet season. Therefore, seasonality and land use type was shown to have an effect on the community species composition of macrofungi in Kereita forest. The macrofungi density and species richness were significantly affected by season, forest type and their interaction ($p<0.05$). However, season and forest type had no significant effect on the two species diversity indices - Shannon and Simpson diversity Index ($P>0.05$). Macrofungi density and species richness were 2 times higher in indigenous forest compared to pine plantation. On seasonality, the increase was more during the wet season for both indigenous and pine plantations compared to those encountered during dry season (Figure 8). There was no significant difference in species diversity during the wet and dry season in both forest types.

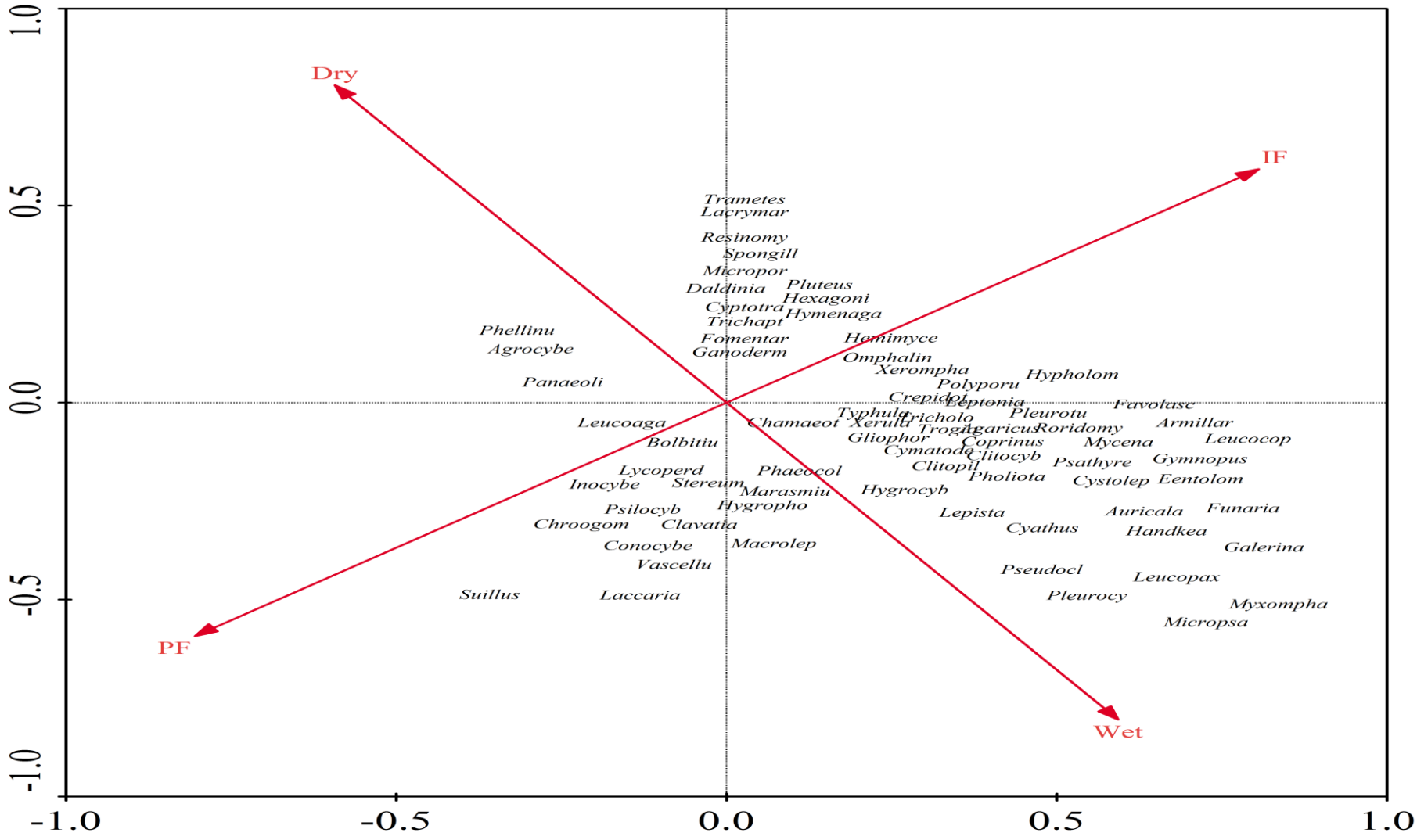

Figure 8: Redudancy analysis (RDA) on the species composition in Kereita forest block

Key:

Armillal - *Armillaria*
Auricala - *Auricularia*
Bolbitiu - *Bolbitus*
Chamaeot - *Chamaeta*
Chroogom - *Chroogomphus*
Clitopil - *Clitopilus*
Cymatode - *Cymatoderma*
Cyptotra - *Cyptotrama*
Macrolep - *Macrolepiota*
Micropsa - *Micropsaliota*
Phellinu - *Phellinus*
Pseudocl - *Pseudoclitocybe*
Roridomy - *Roridomycena*
Trichol - *Tricholoma*
Cytolep - *Cytolepiota*
Entolom - *Entoloma*
Favolasc - *Favolaschia*
Fomentar - *Fomentarius*
Ganoderm - *Ganoderma*
Gliophor - *Gliophorus*
Hexagoni - *Hexagonia*
Hygrocyb - *Hygrocybe*
Marasmiu - *Marasmius*
Omphalin - *Omphalina*
Pleurotu - *Pleurotus*
Psilocy - *Psilocybe*
Spongill - *Spongilipellis*
Vascellu - *Vascellum*
Hygropho - *Hygrophorus*
Hymenag - *Hymenaagaricus*
Hypholom - *Hypholoma*
Lacrymar - *Lacrymaria*
Leucoaga - *Leucoagaricus*
Leucocop - *Leucocoprinus*
Leucopax - *Leucopaxillus*
Leucoperd - *Leucoperdon*
Micropor - *Microporus*
Panaeoli - *Panaeolus*
Psathyre - *Psathyrella*
Resinomy - *Resinomyce*
Trichapt - *Trichaptam*
Xerompha - *Xeromphalina*

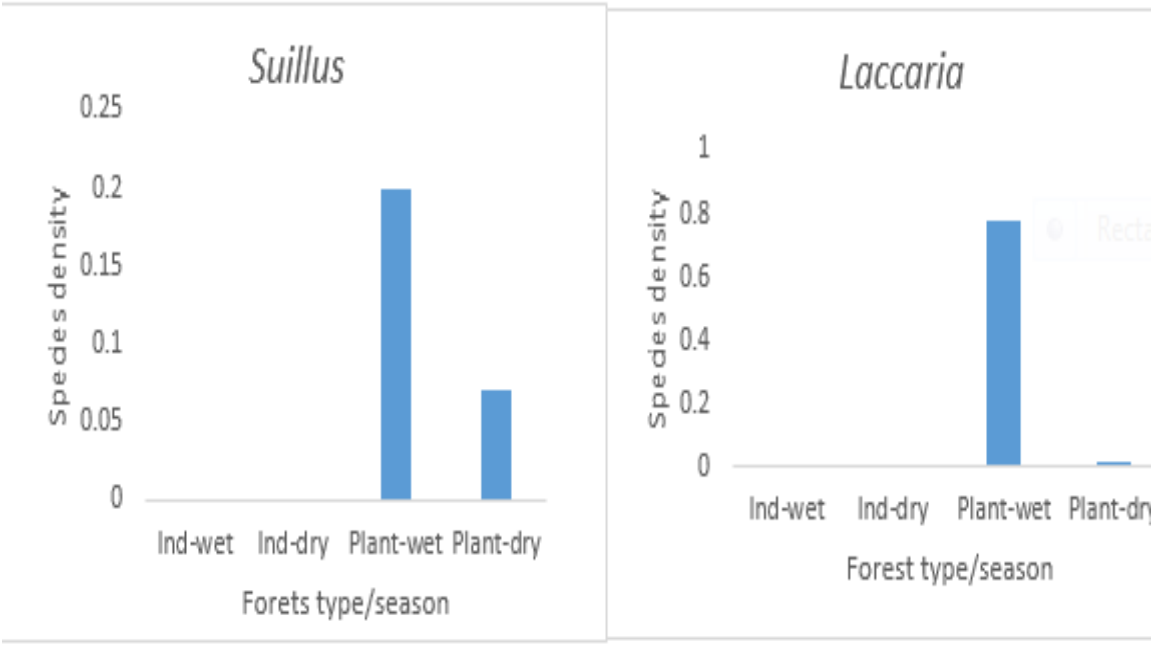

Figure 9: Ectomycorrhiza macrofungi genera occuring only in the plantation forest during the dry and wet season

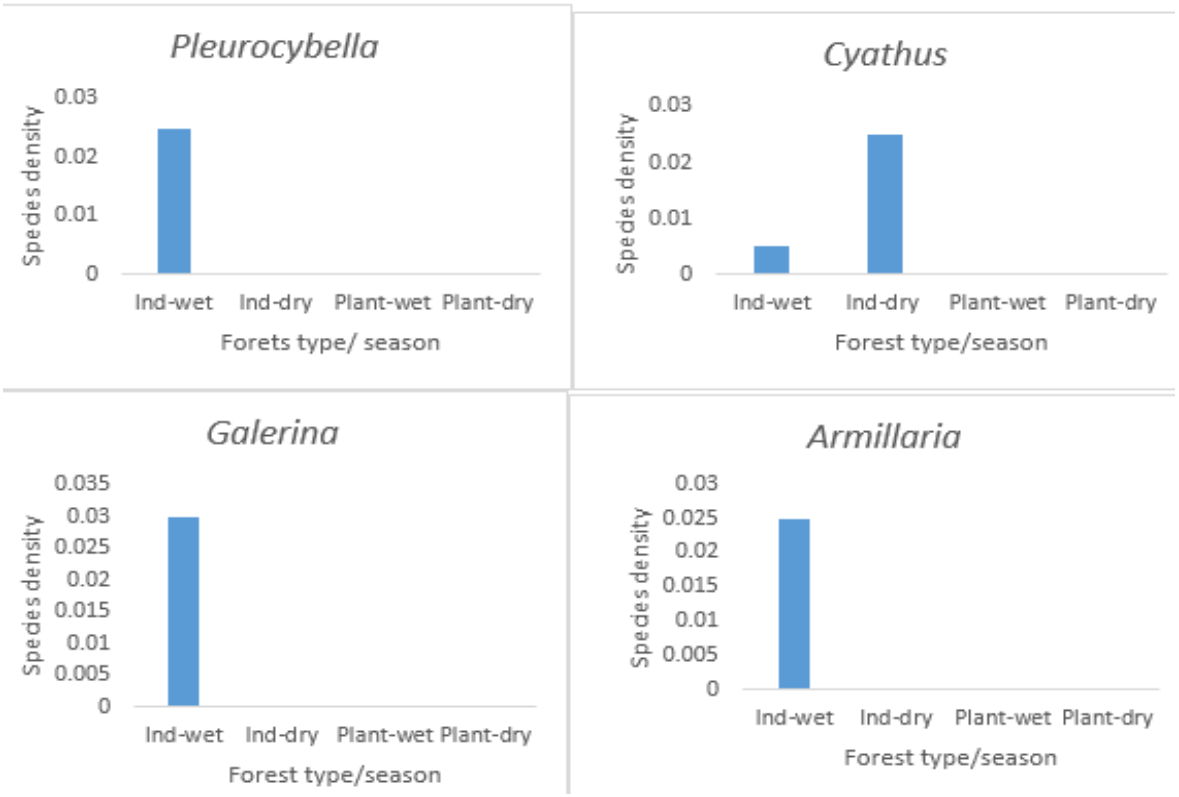

Figure 10: Fleshy wood rotting macrofungi genera in the indigenous forest during the dry and wet season

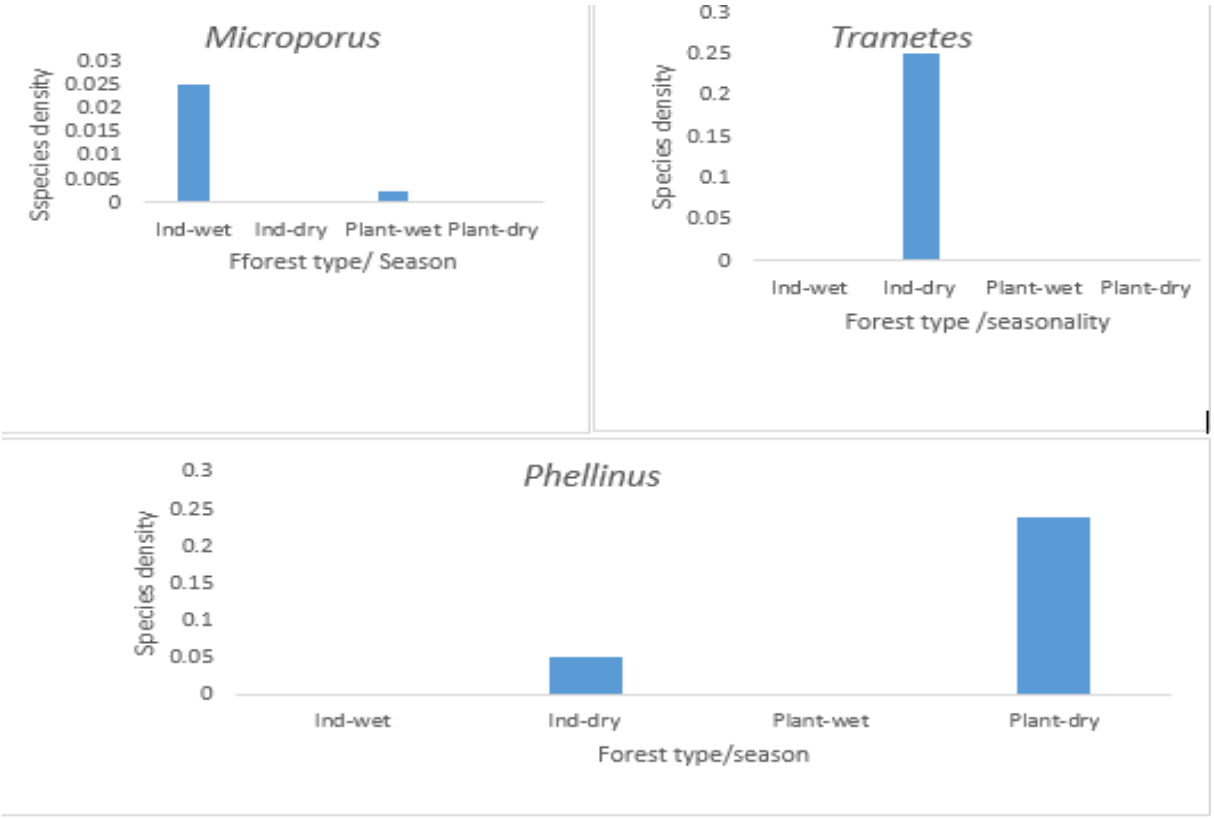

Figure 11: Polyporaceae (genera) in Kereita forest

4.5 Macrofungi species with potential for utilization

The indigenous forest had (70%) of the wild edible macrofungi while 30% occurred in the plantation forest (Appendix 3). From morphological and molecular characteristerization the genus *Pleurotus*, *Agaricus, Macrolepiota*, *Suillus*, *Auricularia*, *Stropharia*, *lycoperdon* and *Armillaria* were identified as edible. Other species notable for their medicinal value belonged to the families Ganodermataceae, Hymenochaetacea, Polyporaceae and Physalacriaceae (Fig 12)

Figure 12: Macrofungi species with potential for utilisation as food and medicine (Susan Njuguini, 2015)

Key: 1. *Agaricus avensis 2. Auricularia auricula* 3. *Suillus luteus 4. Ganoderma* sp 5. *Pleurotus* sp 6. *Agaricus silvaticus* 7. *Macrolepiota procera* 8. *Phellinus robustus 9. Agaricus avensis*

4.6 Molecular characterization of macrofungi

The Blast analysis summaries of different macrofungi species using Ribonucleic acid (5.8s rRNA) genes are shown in Table 4. The amplified ITS region for the 9 morphologically different species had length that ranged between 500-600bps with more than 94% identity and an impressively low E-value consistent with organisms that share the same species (Table 4). All the nine (9) samples were successfully identified based on the macrofungi sequence

similarity deposited at the Gene bank (Table 4; Figure13; Appendix 4). The sample KIC 001 and KPG 161 were identified as *Stropharia rugusoannulata* and *Macrolepiota dolichaula* both with 98% identity respectively. KPM 181and KIC 69 were affiliated to *Agaricus volvatulus* and *Agaricus inoxydabilis* with identity of 98% and 97% respectively. The *Agaricus* (KIC 60) was identified to genus level as *Agaricus /Hymenaagaric*us with 94 % identity. KPM 143 was identified as *Suillus lutea* with 100% identity. KIW 60 and KIL 109 were identified as *Auricularia polytrica* and *Pleurotus djamor* at 95% and 93% respectively while KIW 57 matched with *Fayodia leucophylla* at 94 % identity (Table 4)

Table 4: Sequence homology search for nine (9) macrofungi specimens

Sample No.	Specimen Accession	Description	E-Value	Ident	Accession
1	KIC 001	*Stropharia rugosoannulata*	0.0	98%	KC176328.1
10	KPM 143	*Suillus luteus*	0.0	100%	KX230614.1
11	KPG 161	*Macrolepiota dolichaula*	0.0	98%	KJ524564.1
12	KIW 57	*Fayodia Leucophylla*	0.0	94%	GU234142.1
13	KIL 109	*Pleurotus djamor*	1e-67	93%	KT273366.1
14	KIW 60	*Auricularia polytricha*	4e-60	95%	KM267729.1
5	KIC 60	*Agaricus /Hymenaagaricus*	0.0	94%	KU848188.1
7	KPM 181	*Agaricus Volvatulus*	0.0	98%	KU041660.1
9	KIC 69	*Agaricus Inoxydabilis*	0.0	97%	JF727841.1

The generated phylogenetic tree shared clades with the species consistent to those observed in the initial/primary sequence homology search with NCBI BLAST results and were compared with the morphological observations (Table 4; Appendix 4; Figure 13)

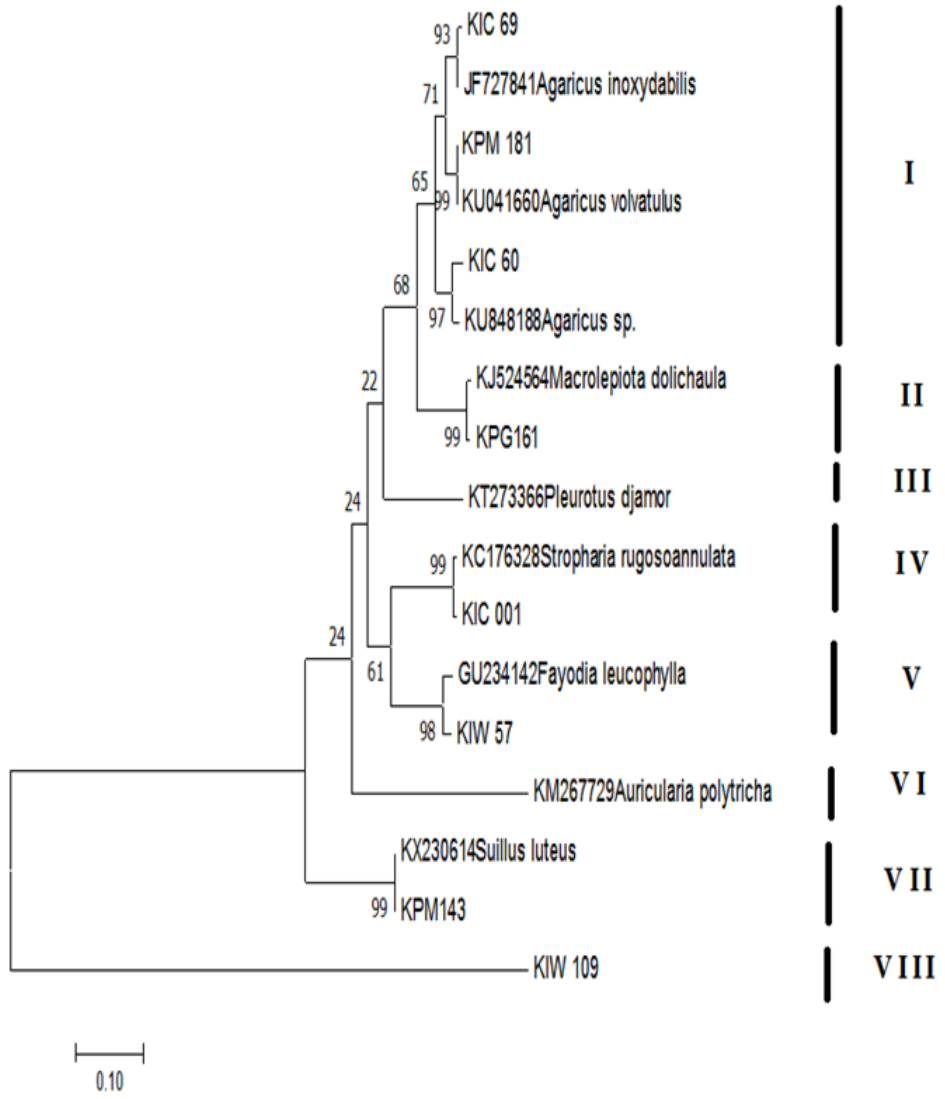

Figure 13: Phylogenetic tree displaying relationship of macrofungi specimens

4.7 Mineral content of wild edible macrofungi

Nine important mineral elements (P, K, Ca, Mg, Na, Fe, Mn, B, Cu, and Zn) were analyzed from the three mushroom varieties (*Macrolepiota dolichaula*, *Auricularia polytrica* and *Pleurotus djamor*). All the three mushroom species had all the nine elements but in different proportion (Table 5. Among the three species *Macrolepiota* has high P (850mg /100g), K

(2038 mg/100g), Cu (19 mg/100g) and Zn (10.2 mg/100g) but poor in Mn (2.1mg /100g) and Fe (12.6 mg/100g), Na (11.5 mg/100g) and Ca (69 mg/100g) compared to *Pleurotus djamor* and *Auricularia polytrica* (Table 5) *Pleurotus* and *Auricalaria* had similar levels of P, Ca, Fe, Mn, Cu and Zn but differed in accumulation of K and Na (Table 5). *Pleurotus* and *Auricalaria* had high levels of Ca (140-180mg/100g. The three species had high levels of K (2038mg/100g) compared to the other minerals (Table 5;). Among the four micronutrients (Fe, Mn, Cu and Zn), Fe was the richest mineral among the three species. *Macrolepiota* had the highest levels of Cu (19mg/100g) in comparison to the other two species. (Table 5). The Cu content of the reported species in literature is also twice higher that of *Macrolepiota*. *Auricularia* had very high levels of Na (110mg/100g) nine times higher than *Macrolepiota* and *Pleurotus* species.

Table 5: Mineral content analysis of selected wild edible species

Mineral content	*Macrolepiota dolichaula* mg/100g	*Pleurotus djamor* mg/100g	*Auricularia Polytrica* mg/100g
P	850	110	100
K	2038	570	900
Ca	69	140	180
Mg	120	140	180
Na	11.5	14.4	110
Fe	12.6	26.4	36
Mn	2.1	5.01	6.04
Cu	19	1.34	0.66
Zn	10.2	5.27	2.32

4.8 Mycelial growth of *Macrolepiota dolichaula, Auricularia polytrica* and *Pleurotus djamor*

Mycelia growth was significantly different among species (P<0.05) and among the different culture media (< 0.05) during the 7th day period (Table 6).

Table 6: Mycelial growth of the selected wild edible macrofungi

Species-Media	1	2	3	4	5	6	7
Mac-Malt	1.14±0.05	2.02±0.04	2.64±0.11	3.22±0.15	3.86±0.17	4.29±0.19	4.5±0.00
Aur -Malt	1.14±0.09	2.02±0.08	2.8±0.07	3.4±0.19	3.9±0.10	4.3±0.16	4.5±0.00
Pleu-Malt	0.4±0.09	1.06±0.08	1.78±0.07	2.26±0.15	2.76±0.22	3.68±0.26	4.06±0.15
Mac-PDYA	1.32±0.04	2.38±0.16	3.32±0.08	3.8±0.12	4±0.12	4.2±0.12	4.5±0.00
Aur-PDYA	1.34±0.05	2.34±0.05	3.22±0.84	3.92±0.08	4.14±0.19	4.5±0.00	4.5±0.00
Pleu-PDYA	0.52±0.04	0.82±0.44	1.14±0.09	1.42±0.04	1.74±0.09	2.18±0.08	2.66±0.11
Mac-PDA	0.94±0.13	1.96±0.09	0.15±0.15	3.18±0.11	3.6±0.07	4.4±0.07	4.5±0.00
Aur-PDA	1.08±0.04	1.78±0.44	3.02±0.11	3.4±0.07	3.72±0.11	4.1±0.07	4.46±0.05
Pleu-PDA	0.73±0.09	1.9±0.09	2.4±0.05	3.1±0.05	4.03±0.08	4.87±0.08	5.97±0.16
ANOVA							
Species:	403(P<0.01)	207.7(p<0.01)	1140 (p<0.01)	946.1(p<0.01)	593.1(p<0.01)	455.7(p<0.01)	712.2(p<0.01)
Media:	36.46(P<0.01)	6.722(P=0.003)	25.86(p<0.01)	14.56(p<0.01)	13.95p<0.01)	39.54(p<0.01)	105.8(p<0.01)
Species x Media	5.232(p=0.002)	10.77(p<0.01)	80.35(p<0.01)	60.23(p<0.01)	36.01(0.010)	54.44(p<0.01)	107.79 (p<0.01)

The mycelia growth of *Macrolepiota dolichaula* and *Auricularia Polytrica* was higher on the three culture media during the first four days compared to growth of *Pleurotus* on the same culture media (Figure 14). *Macrolepiota dolichaula* and *Auricularia Polytrica* reached full colonization of 4.5cm (diameter) by the 7th day on all the three media (Malt extract, PDA and PDYA media) while *Pleurotus* sp took longer and had an everage of 2.5, 3.5 and 4.0 cm in MEA, PDA and PDYA respectively by the 7th day (Figure 14). Among the culture media used, Malt extract was the best media followed by PDYA for culturing *Macrolepiota* and *Auricularia* species.

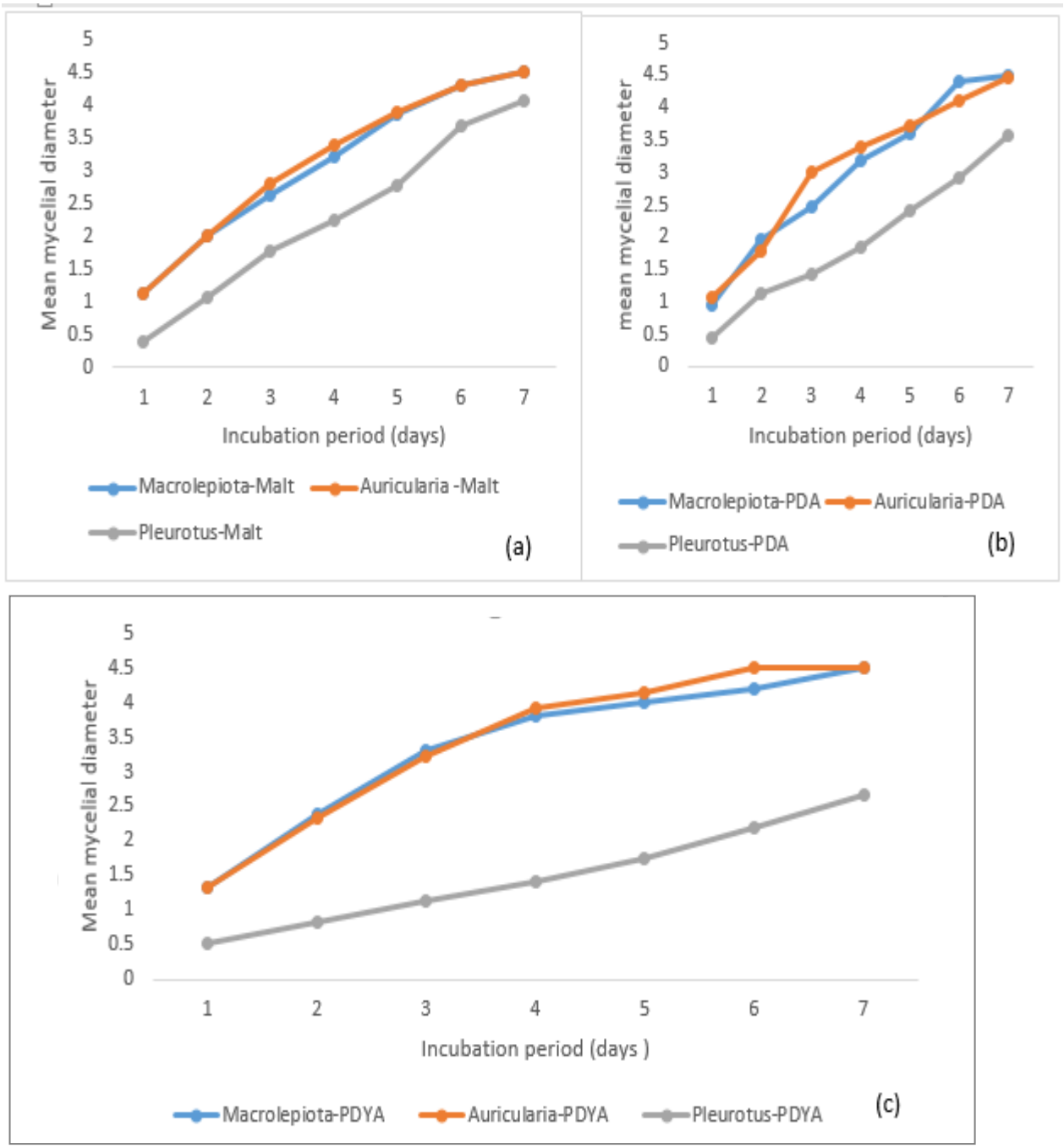

Figure 14: Mycelial growth of edible species on culture media

(a) Mean mycelial growth of edible macro fungi on Malt extract culture (b) Mean mycelial growth of edible macro fungi on potato dextrose agar culture media media (c) Mean mycelial growth of edible macro fungi on potato dextrose yeast agar culture media

CHAPTER FIVE

DISCUSSION, CONCLUSION & RECOMMENDATIONS

5.1 Discussion

The results from this study confirm the diversity of macrofungi assemblage in forested ecosystems in Kenya. The study has revealed diverse macrofungi community comprising of 224 species distributed in 28 families. Similar studies conducted in upland mountainous ecosystems reported 162 species (Kost, 2002) while the lowland region like Maasai Mara and Coast region reported less than 50 species (Tibuhwa, *et al*., 2011, Gateri, *et al*., 2014). This difference could be attributed to the unique habitats of the uplands (Aberdare forest) which might favor the diversified groups of macrofungi in Kereita forest. Upland ecosystems are known to harbor a rich diversity of vegetation sustained by rich and red volcanic soils which provides suitable conditions for the native forest (Muiruri, 1974). Again, the main ecosystem within the Abedares is the rain forest characteristic of dense vegetation cover for a wide range of biodiversity (Maina, *et al*., 2017). In this study, morphological methods used were mainly based on macro- and micro-morphological traits were used to identify macrofungi and only 24% of the macrofungi were identified to the species level. Although these methods have been used regularly, they are limited by presence of numerous convergent morphologies that limit adequate discrimination in several genus. (Martin, *et al*., 2004, Tang, *et al*., 2010). There is also possibility that several fungi species from this forest are new to science and molecular approaches are being followed to confirm this.

The species checklist from this study matches earlier reports showing diverse macrofungi diversity in Kenyan mountainous indigenous forested ecosystems (Kost, 2002). However, this study might have missed out several genera such as *Cerena, Cotylidia, Gryroon, Lopharia,*

Megasporospharia, Phaecogyroporus, Ripartitella, Schizopyrum, Scutellirinia among the species documented by Kost, 2002. This is possibly attributed to a short life of macrofungi and also the fact that different species are known to appear at different times during the year (Tibuhwa, *et al*., 2011). Since these results are based on study conducted only during the two seasons some of these species could have been missed during the sampling period. Therefore, to have a complete knowledge of macrofungi in a given habitat, continuous observation and sampling for many years has been suggested (Osemwegie, *et al*., 2010, Megersa, *et al*., 2016). This is reported as linear increase of species diversity with sampling effort especially in the indigenous forest indicating not all the species were sampled in the two forests during this study. This implies that more species can be recorded with additional sampling and detailed monitoring studies to reveal all macrofungi species are thus desirable.

90 % of the macrofungi recorded in Kereita forest during this study were saprophytic, mostly colonizing the litter-based, wood and soil organic substrates in both forest types. This could be attributed to ability to degrading many types of substrates present in indigenous forest (Lynch & Thorn, 2006). In this study, the genus *Agaricus* was distributed across the two forest type probably due to its saprophytic nature linked to organic matter colonization that is available everywhere. The Agaricaceae family is also not known to associate with a specific habitat. Species belonging to this family are able to establish and thrive anywhere provided the conditions are suitable therefore explaining the high number of genera (44) in Agaricaceae (Uzun, 2010). They were found growing in soil organic matter (*Agaricus*), forest litters (*Cytolepiota*), animal dung (*Coprinus*) in grassland patches under pine plantation where grazing was noted. The species were largely found growing on wild animal dung, which is thought to contribute in enriching organic matter substrate suitable for macrofungi diversity in this region (Karun & Sridhar, 2015). The high occurrence of Agaricaceae family

could further be explained by the fact that the Agaricaceae members have thick spores. The spores remain viable in the environment for a very long period of time especially when the conditions are not favourable for their sporulation (Priyamvada, *et al*., 2017). Other predominant families in this study were Tricholomataceae and Mycenaceae mostly predominant during the wet season. The mycenaceae family members are saprophytic species decomposing mainly litter based substrates. They are mainly favored by presence of dead twigs, leaf substrates while others occur on cowdung. The species were documented in both indigenous forest mainly in forest litter and in pine growing in cowdung. They are associated with small fruiting bodies that establishes at relatively shallow depth. This characteristic favours their appearance during the early rainy season and quick disappearance according to Enow, *et al*., (2013). Tricholomataceae is a large and diverse family with most of the members being wood degraders. The high number of species belonging to the Tricholomataceae in the indigenous forest during the wet season is linked to availability of diverse moist wood substrates. The wood based substrates have been shown elsewhere to support high mushroom diversity (Osemwegie, *et al*., 2010).

Ectomycorrhiza species only occured in the pine plantation and the common genera known to associate with pine trees such as *Suillus*, *Chroogomphus, Laccaria*, and *Inocybe* were documented (Karim & Kasovi, 2013). Other genera such as *Lactarius*, *Hebeloma* and *Rhizopogon* known to associate with pine trees were not documented (Kost, 2002). Such variations are expected since pine trees are exotic to Kenya and only ectomycorrhiza species introduced during the afforestation program may exist (Kost, 2002). Pine trees are among the major obligate hosts of ectomycorrhizal (ECM) fungi, explaining high diversity of this group in these forests. These species form symbiotic relationship with plant root where the plant provides fixed carbon to the fungus and in return, the fungus provides mineral nutrients,

water and protection from pathogens to the plant (Tapwal, *et al*., 2013). No ECM species were recorded in indigenous forests suggesting lack of mycorrhiza tree host species. Parasitic species belonging to the genus *Armillaria, Ganoderma* and *Phellinus* were recorded in the two land use types though they were few compared to other groups (Saprophytic and Ectomycorrhiza). The parasitic fungi in the forest ecosystem are a natural element if the pathogens are below a given population threshold. The fungus directly kills the trees opening the forest for the trees that demand light (Tapwal *et al*., 2013). The parasitic fungi occurring in these two land use types (*Ganoderma appalatum* and *Phellinus gilvus*) have medicinal value. These macrofungi can be harvested sustainably to contribute to the pharmaceutical industries (Tapwal, *et al*., 2013).

Indigenous forested ecosystems also harbored a wide range of macrofungi in terms of species density and richness compared to plantation forest (Claudia, *et al*., 2015, Pushpa & Purushothama, 2012). Saprophytic and parasitic species especially wood and litter decomposing species were more dominant in indigenous forest (*Armillaria*, *Pleurocybella*, *Cyathus* and *Galerina*, *Oudemansiella* and *Favolaschia*) while ectomycorrhiza species (*Suillus* and *Laccaria*) were found only in pine plantation (Figure 5-8).The results from this study is in line with several studies showing negative implication on the conversion of indigenous forest to single species tree plantation on macrofungi species composition (Paz, *et al*., 2015). Other findings have also shown high species density and richness in the natural forest compared to planted plantation forest (Osemwegie, *et al*., 2010, Claudia, *et al*., 2015). Pristine indigenous forests are associated with favorable macro and micro climate (humid conditions, temperature), reduced anthropogenic interferences, litter fall dynamics, readily available degradable wood substrates, high plant diversity and composition (Pushpa & Purushothama, 2012). Accumulation and availability of degradable substrates coupled by

presence of diverse tree species favors high turnover of litter decomposing and wood rotting macrofungi (Sefidi & Etemad, 2015, Yamatisha, *et al*., 2015). Litter decomposers are specialists in degrading the recalcitrant organic compounds in the litter materials to unleash nutrients and carbon to the soil (Wal, *et al*., 2013), while wood-degrading fungi decomposes wood type substrate to provide microhabitats important for soil dwelling fungi and other organisms *(*Rajala*, et al*., 2015*).*

70% of macrofungi species found in indigenous forest were not encountered in pine plantation. This suggests loss of macrofungi species that were previously associated with indigenous forest when the forest was converted to single species plantation forest.Conversion of indigenous forest to plantation forest causes drastic disturbance of natural ecosystem that destroys richer plant communities responsible for generating diversified microclimates and supporting different types of substrates such as diversified fine litter and dead wood in various sizes and stages of decomposition (Moore, *et al*., 2004, Waldrop, *et al*., 2006). Such changes alter the original environment creating drastic changes to degradable substrate from older and more diverse plant community in indigenous forest to woody and litter substrate dominated by a single tree species (Heilmann-Clausen & Christensen, 2003 & 2004, Norden, *et al*., 2004, Packham *et al*., 2002). Single species plantation forests have low plant diversity and high human disturbance linked to sivicultural practice such as thining and pruning of the trees (Baral, *et al*., 2015). Silvicultural practices are known to reduce the canopy cover to some extent causing the forest to be more open. As a result, high humidity and increased temperatures are experienced thus affecting the macrofungi fruit body formation (Baral, *et al*., 2015). The studied pine plantation forests was a single tree species forest making it less favorable habitats for diverse range of macrofungi species due to low woody and litter substrates, forest composition changes due to succession

and disturbance which ultimately affects macrofungi growth and development (Karim & Kasovi, 2013). In this study, pine plantations had very low woody and litter substrates, and were also highly grazed explaining the low species richness and density. Also only few species in the genera *Oudemansiella*, *Favolaschia*, *Campanella* and *Ripartitella* have the ability to utilise the wood substrates of pine plantation contributing significantly to the difference in species composition between the two land use types. Kasel *et al*. (2008) and Claudia *et al*. (2015) confirms that change in land use results to shift in species composition of macrofungi whereby plantation and indigenous forest support distinct groups.

Seasonality was a major factor explaining changes in macrofungi species community. Macrofungi species were more during wet season compared to the dry season in both forest types. Dominant species during wet season were fleshy macrofungi while non-fleshy fungi (polypore) were present in both seasons. This phenomenon could be well explained by adequate moisture levels in substrate and atmosphere alongside favorable temperature during the wet season (Priyamvada, *et al*., 2017). Climate is a critical factor in the fruiting, productivity and distribution of all fungi (Boddy, *et al*., 2014). Certain agaric species are also known to be associated with closed canopies of forests whereby fruiting may be sporadic and limited to the wet season (Karim & Kasovi, 2013). The high number of soil inhabiting fungi during the wet season is also linked to substantial amounts of decaying woody fragments which eventually turns to soil organic matter, and hence supports a wide range of soil resident fungi (Rajala, *et al*., 2015). The dry season is not favorable for the development of fleshy fruit bodies and instead both annual and perennial polypores are prevalent during this time (Enow, *et al*., 2013), (Yamatisha, *et al*., 2015). Woody perennial polypore survives both in the dry and moisture-rich periods. Their hard external upper fruiting body, deeply rooted vegetative mycelium and presence of long and narrow hymenial tubes help the fungus remain in a

relatively saturated state even in dry environmental conditions. They also have resistant spores survive for a very long time in the environment (Priyamvada, *et al*., 2017). Therefore, polypores are considered to experience minimal effect in regard to seasonality or annual variation. The present study coincides with the findings of Karim and Kasovi (2013) who studied the macrofungi of deciduous forest in Iran and explained that seasonality is critical in distribution of macrofungi. *Armillaria, Pleurocybella, Cyathus* and *Galerina* were common species with high density during the wet season in the indigenous forest.

The diversity indices did not reveal significant difference between the different land uses, although plantation forest had lower diversity. Plantation forest equally supports diverse community of macrofungi as the indigenous forest however, species composition might differ among forests (Tapwal, *et al*., 2013). Preference of macrofungi towards particular habitats may be driven mostly by ecological role of the species, as evidenced by the presence of ectomycorrhizal species in the forests (Pradhan, *et al*., 2013). The ectomycorrhizal species in the plantation were introduced during the afforestation when the exotic trees could not establish without the symbiotic macrofungi (Kost 2002). Only a few saprophytic species survived and it was due to their ability to utilize new sources of wood (Kost, 2002). This implies that conversion from indigenous forest to exotic plantation forest alters macrofungi species diversity and promotes a new community of macrofungi species (Claudia, *et al*., 2015).

The wild edible macrofungi recorded during this study do not only provide nutritious and healthy diet for humans but it is also a measure in the conservation of the macrofungi species existing in the natural habitat (Rizal, *et al*., 2014). Among the species are fleshy *Suillus* which is highly edible and priced as an important source of protein in other countries. These species

are exotic since they were introduced in Kikuyu Escarpment forest after the conversion of the indigenous forest to pine plantation forest (Kost, 2002). The pine tree species are ectomycorrhizal and dependent on these fungi for their establishment and survival. An inventory of macrofungi was carried out by Degreef, *et al.*, 2016 in the miombo woodland and also documented the occurrence of *Suillus* species as an important food source in Burundi and Rwanda.

Macrolepiota is a fleshy macrofungi in the family Agaricaceae, appreciated as food sources in China, Thailand and India and which is collected during their fructification period (Ge, *et al*., 2010). The species possess acceptable flavor and nutritional composition (Kumari & Atri, 2014). The species was widely collected in pine plantation forest in this study probably due to its habitat preference. *Macrolepiota dolichaula* is saprotrophic grasslands dweller that grows in clusters or individually (Rizal, *et al*., 2014). Shim, *et al*., (2005) and Rizal, *et al*., (2015) have proved the edibility and cultivability potential both indoor and outdoor. The other species that occurred in the pine plantation were *M. procera*, which is also highly edible and has been experimentally cultivated elsewhere (Shim, *et al*., 2005). This study confirms the occurrence of edible *Macrolepiota* species in Kenya. Mbaluto, (2015) carried out mycelial culture and spawn production of the *Macrolepiota* species from Aberdare forest. However, the utilization of *Macrolepiota* as a food source is yet to be implemented in Kenya.

The family Auriculariaceae serves both as food and medicine and its dominance in the indigenous forest could be as a result of abundant moist woody substrates. Onyango, *et al*., (2011) reported the occurrence of three varieties of *Auricularia auriculara* in the Kakamega forest in Kenya. The local populace surrounding the forest collects the *Auricularia* macrofungi as a delicacy. To conserve the germplasm of this species Onyango, *et al*., 2011

advocated its domestication. The *Auricularia* species possess antiparasitic, antibacterial, antiviral and antitumor polysaccharides making the species to be the fourth most cultivated species in the world among the macrofungi (Yan, *et al*., 2004). Several substrates and media have successfully been investigated for the cultivation of *Auricularia* species in Kenya (Onyango, *et al*., 2011).

The *Agaricus* species were recorded in both plantation and indigenous forest. Three species; *Agaricus agustus, Agaricus silvaticus* and *Agaricus volvatulus* encountered have potential to contribute to the mushroom industry due to the good flavour and taste associated with this genus. A few varieties*; Agaricus blanzei, A. blasiliensis* and *A. subrufescens* have been successfully domesticated in the temperate (Cotter, 2014). However, in Kenya, the exploitation of *Agaricus* species hasn't been implemented and only exotic *Agaricus* species are cultivated (Gateri, *et al*., 2004). Martínez Carrera, *et al*., (2001) demonstrated domestication of wild *Agaricus* species.

Armillaria mellea was collected predominantly in the indigenous forest. *Armillaria* species are good decomposers of wood based substrates especially tree stumps in native forests (Munyanziza, 1996). Although *Armillaria mellea* is an edible macrofungi in Greece and its nutritional composition and mineral content has been established (Ouzouni, *et al*., (2009), its utilisation in Kenya is not known.This macrofungi forms many fruit bodies which can be sustainably harvested for nutrition and some dried to be used during drought. Therefore studying the potential utilisation of this species is vital.

Ganoderma australe and *Ganoderma applantum* species occured in both land use types. The fungus kills old trees allowing the light requiring plants to emerge. Since *Ganoderma* has

hypoglycemic, antioxidant, and antihypertensive activity (Oyetayo, 2011) then the species can be domesticated for sustainable utilization in the pharmaceutical industries. The other wood decomposing macrofungi reported in this work are *Phellinus gilvus* and *Trametes versicolor* also known for their medicinal importance. In Kenya, the cultivated *Ganoderma* has its origin in China and therefore the indigenous species haven't been exploited for the same benefits as the imported varieties.

The members of the Agaricaceae family were the highest represented in both land use types though their utilization in Kenya is limited. The species belonging to *Lycoperdon*, *Coprinus* and *Stropharia* are litter decomposing fungi and have been documented as wild edible macrofungi (Boa 2004). Boa, (2004) has reported *Lycoperdon pyriforme* and *Coprinus Comatus* as edible species from Benin and Chile respectively and yet to be adopted as food sources in many parts of Africa. *Stropharia rugosoannulata* is an edible mushroom which can be cultivated for food whose novel lectin has been isolated and characterized (Zhang, *et al*., 2014).

In this study the identity of macrofungi species was established through ITS (5.8s rRNA) gene region. The molecular characterization of the common edible species revealed two species established as *Agaricus volvatulus* and *Macrolepiota dolichaula.* Both species belong to the family Agaricaceae explaining the reason the two organisms although in different genus shared clade 1 and Clade 11. *Agaricus* species are saprobic with both edible and non-edible species found growing gregariously in woods, forests, gardens, on roadside, fields, pasture-land, grassland, rubbish dumps, manure heaps and alluvial soils. More than 400 *Agaricus* species are described worldwide (Zhao, *et al*., 2010), with about half of these species (200 species) recorded from tropical ecosystems (Boa, 2004.) Amazingly only less

than 5% of these species are recorded in Kenya (Boa, 2004). This is the first record of *Agaricus volvatulus* and *Agaricus inoxydabilis* in forested ecosystems in Kenya. Although the species have wide occurrences in Africa and Asia (Chen *et al*., 2016; Boa 2004), complexity in taxonomy and delimitation of *Agaricus* species within the genus using morphological traits may have hindered its documentation in Kenya. The two species were recorded in Kereita forest under *Pinus patula* and *Cupressus lustanica* plantation growing gregariously on grass patches. They are edible (Rizal, *et al*., 2015; Boa, 2004) and have great potential in supporting mushroom industry in Kenya. The *Hymenagaricus* sp. requires further evaluation since the specimen was only identified at the genus level as evidenced by gene bank sequence Blast results. The results suggests that *Hymenagaricus* sp. could be a new species and still undescribed *Agaricus* / *hymenagaricus* from Kenya.

In this study, *Macrolepiota dolichaula* was found growing in soil under *Pinus patula* and *Cupressus lustinica* forest plantation. The species was macroscopically characterized by fleshy, big, pileus with squamules; white to cream lamellae; a prominent movable annulus and microscopically was characterized by clamp connections on the septa in lamellae and thick-walled white to cream basidiospores with metachromatic cresyl blue inner layer spore-wall (Singer, 1948). There are currently about 30 species recognized worldwide (Kirk, *et al*., 2008). The occurrence of *Macrolepiota dolichaula* was reported before in Aberdare forest (Mbaluto, 2015). *Macrolepiota dolichaula* is an edible species considered a delicacy in China. In Kenya, mycelia and grain cultures of *Macrolepiota dolichaula* have been established (Mbaluto, 2015), but cultivation of the species has not been realized.

This study also confirmed the identity of *Suillus lutea*. Morphologically the species is easily distinguishable by its partial veil that hangs on the stem. *Suillus lutea* is an ectomycorrhiza

species found growing in association with *Pinus patula* species. Pine trees are among the major obligate hosts of ectomycorrhizal fungi, explaining the presence of *Suillus* in the plantation forest. This finding is in agreement with the report by Kost, (2000) who recorded them in both Aberdares and Mount Kenya forest (Degreef , *et al*., 2016).

Morphological characterization of *Auricularia Polytrica* matched very well with molecular studies. Gene bank sequence results affiliated *Auricularia Polytrica* at 95% identity. Members of the family Auriculariaceae such as *Auricularia polytrica* and *Auricularia auricula* have been widely reported in Kenya (Onyango, *et al*., 2011). Mycelia and grain cultures have been produced and their growth and nutritional composition under different media have also been reported in Kenya *(*Onyango, *et al*., 2011).

From this study the identity of *Pleurotus djamor* was established using molecular characteristics. Although the genus *Pleurotus* mushrooms have worldwide distribution. They are recognized for their highly commercial edible species. Their morphological identification is complicated by numerous convergent morphologies such as shape, colour and size of hymenophore, length, thickness and colour of stipe, yield, and duration for maturation (Avin, *et al*., 2014). Phylogenetic analyses are powerful in establishing identity of *Pleurotus* species (Saha, *et al*., 2012). Homology search analysis against the GenBank database using 16 rDNA ITS sequences randomly selected from the one clades of AFLP dendrogram revealed identity of wild edible white ecological variety of *Pleurotus djamor*. The species is edible and has been cultivated worldwide and was collected previously in Kakamega, Arabuko Sokoke and Mount Kenya forest (Otieno, *et al*., 2015).

This study revealed for first time occurrence of *Stropharia rugussoannulata* in Kenya. The species was found growing on decomposing bark of fallen trees branches under *Pinus patula* plantation. The identity *Stropharia rugussoannulata* was revealed by 95% homology identity and morphological characteristics of species showed a high similarity to *Stropharia rugusoannulata* with cream cap besides the commonly known "wine cap" (Kuo, 2016). Although the cap of *S. rugosoannulata* is strikingly wine-red when fresh, the cap colour fades to cream-white when weathered. Unlike other *Stopharia* species, *Stopharia rugusoannulata* is edible and is easily cultivated in wood chips and straw (Adey, 1995). There are no records of their mycelia and grain culture as well as their cultivation or domestication potential in Kenya.

The morphological characterization of KIW 57 placed the macrofungi in the family Pleurotaceae yet the molecular identification grouped the sample in the Tricholomataceae family. The Phylogenetic analysis indicates that this specimen shares a significant evolutionally relationship with *Fayodia leucophylla* at 98% bootstrap support values. Following (Lange & Sivertsen, 1966) description of *Fayodia leucophylla*, disagrees with the morphological description of KIW 57 in this study which indicate that it bears a pileus that is fan and shell shaped with an eccentric stipe similar to those belonging to *Pleurotus* species. On the other hand, members of the group *Fayodia leucophylla* have been characterised by a cap with a deep depression, stipe and deccurent gills. Therefore, KIW 57 needs further molecular evaluation because its morphology is not in agreement with the molecular affiliated species.

Additionally, the mineral content revealed that wild edible mushrooms (*A. polytrica*, *M. dolichaula* and *P. djamor*) are an important source of essentials mineral elements and have

great potential in supporting human and nutrition health. All the three wild edible mushrooms had all essential minerals tested but in different proportions. Macrofungi species are known to biologically accumulate minerals from their growth substrates directly to the fruiting body (Mallikarjuna, *et al*., 2012), which prompted their use worldwide as sources of protein and minor elements such as Zn and Fe (Nakalembe, *et al*., 2015). Varying concentration of mineral elements in this study could as well be linked to various factors such as species of mushrooms and growth substratum (Rizal, *et al*., 2015). *Macrolepiota dolichaula* are organic matter dwelling species deriving its nutrient from well decomposed organic residues while *Auricularia polytrica* and *Pleurotus djamor* are wood rotting fungi that derive their nutrients from dead and decaying wood trees (Musieba, 2013; Gateri, *et al*., 2014).

High levels of various elements (P, K, Mg, Cu and Zn) in *Macrolepiota dolichaula* could be due to high concentration of such nutrients in well decomposed organic matter, making it a good accumulator of minerals (Rizal *et al*., 2015). In this study, results agree with mineral levels reported for *Macrolepiota* species by Rizal, *et al*., (2015). *Auricularia polytrica* and *Pleurotus djamor* which had similar levels of P, Ca, Fe, Mn, Cu and Zn but lower compared to their levels in *Macrolepiota dolichaula*. Levels of P, Ca, Fe, Mn, Cu and Zn in these species could be related to similar growth substrate and woody substrate (Rajaratnam, *et al*., 1998).

Levels of major minerals (P, K, Mg and Ca) in the three mushrooms species was in the range of what has been reported in literature. Adequate concentration of P, K minerals in the three species (10.2-2038mg/100g) agrees with the findings of Rizal *et al*., (2015). Musieba, (2013) reported that *Pleurotus citrinopileatus* have high levels of K concentrations of 2280 mg/100g among other minerals under investigation. This finding shows that the wood decomposing

fungi, such as *Macrolepiota* (up to 2038mg/100g) can also contain high levels K. K is an important macro nutrient that helps in maintenance of a healthy nervous system, body water balance and muscle movement (Nakalembe, *et al*., 2015). Macrofungi contains a rich source of this element and the stage of growth of the fruitbody is a determinant of the concentration of K (Wandati, 2014). Though the mineral content of K was higher compared to other minerals, it was still below the reported levels authenticating further analysis on the various stages of mushroom growth to confirm the levels of K in these mushroom species. The macrofungi exhibited rich sources of Ca mineral and its content in this study was above (170mg/100g) compared to those reported ranges of (11–16mg/100g) by Nakalembe, *et al*., (2015) and Chye, *et al*., (2008) (77–144.7 mg/100 g) . Other lower levels for Ca (0.28 mg/100 g) in particular have been reported by Mattilla, *et al*. 2001. Ca is an important source of healthy bones.

Mushrooms are also good sources of micro-nutrients (Cu, Fe, Mn and Zn). Micronutrients are essential metal required in regulation of metabolism, heartbeat, cellular pH, and bone density. Lack of micronutrients can lead to stunted growth in children and increased risk for various diseases in adulthood. Micronutrient concentrations in mushroom species usually range between (1-85mg/100g (Table 8). Our results show comparable levels of micronutrient with those reported in the literature. *Macrolepiota dolichaula* reported high levels of micronutrients than *Auricularia polytrica* and *Pleurotus djamor*. Zinc content in *Macrolepiota dolichaula* in this study (10mg/100g) is equivalent to the Zinc content of most meat types (10-50 mg/100g) (Pelkonen, *et al*., 2008). Therefore, this macrofungi can be an alternative source of Zinc when meat is not available due to the important role it plays in the body. Certain cellular functions such as DNA synthesis, wound healing, cellular metabolism are functions of Zinc (Nakalembe, *et al*., 2015). *Macrolepiota dolichaula* cultivation has great

potential in supporting mushroom industry in eradicating micronutrient deficiency experienced among young children in most areas in Kenya. *Macrolepiota dolichaula* are also medicinal and are used for treatment of indigestion and anaemia (Kumari, *et al*., 2014). Low level of zinc content in *Auricularia polytrica* and *Pleurotus* sp below (RDA 15.5 mg/day) is comparable to earlier reported values (Nakalembe, *et al*., 2015). Enriching growing substrate for *Auricularia polytrica* and *Pleurotus djamor* may be vital in increasing levels of these minerals during cultivation.

The values of Mg and Fe which were within the range of 120-180 mg/100g and Fe (12.6-36mg/100g) for the three species could be explained by the ability of the macrofungi to uptake Mg and Fe based minerals in the ecology such as soil which forms a good basis to determine the amount of minerals taken up by fungi from its substrate (Garcia, *et al*., 1998). The capacity of these macrofungi to accumulate minerals could be a potential bio-indicator of mineral concentration of their substratum. The Fe and Mg content in these mushrooms were slightly higher compared to those reported by Mallikarjuna *et al*., (2013), Fe (6.27 mg to 35.3 mg/100g) and (Mg 21.1mg –40.7 mg/100g) for *Lentinula edodes, Pleurotus florida, Lentinus cladopus* and *Pleurotus djamor.* The Mg content was below the recommended daily intake suggesting that the macrofungi requires combination with other foods for proper body functioning (Nakalembe, *et al*., 2015).

Cu is a micro element that is vital for human body functioning and health due to the role it plays in haemoglobin production. The micronutrient is found everywhere in the body mostly in the brain, heart, liver, and kidneys. The mineral also functions in energy production. The high levels of Cu in *Macrolepiota dolichaula* could be explained by its high ability to accumulate the minerals from its substrate (Rizal, *et al*., 2015) compared to the other two

macrofungi species. Therefore, *Macrolepiota dolichaula* can be a suitable species in mycoremediation to remove the Cu from the soils (Damodaran, *et al.*, 2013). The reported levels of copper in several macrofungi species are within the range of 0.1-9.5 mg/100g (Lalotra, *et al*., 2016; Mallikarjuna *et al*., 2012), authenticating safe levels of Cu content in the *Auricularia polytrica* and *Pleurotus* macrofungi species under the study. It also validates domestication of *Macrolepiota dolichaula* in substrate with low Cu concentration.

Mn acts as a co-factor of enzymes and an antioxidant in the human body. The content in this study is higher than the values (0.25 to 1.19 mg/100 g) reported by Nakalembe, *et al*., (2015) for the wild edible species. Despite these low levels compared to the recommended daily intake (12mg/g) (Nakalembe, *et al*., 2015), higher levels (14.3 mg/100g) have been reported elsewhere (Colak, *et al*., 2009).

Na regulates the electrolyte in the body and low levels of Na are commendable for the hypertensive patients and therefore qualifying *Macrolepiota dolichaula* and *Pleurotus djamor* species in this study as good source of the Na mineral. In contrast, lower levels of sodium contents have been reported elsewhere to be as low as 0.28 mg/100 g by Mattila, *et al*., (2001). Contrary to the reported levels of Na in several species *Auricularia* species in this study have been shown to have even higher levels and could be suitable species for the hypotensive patients who need to raise their blood pressure.

Three wild edible macrofungi species mycelia were successfully cultured in three culturing media (PDYA, PDA and MEA). Apart from nutrients, temperature and pH are important environment condition controlling growth of mushroom mycelia (Kalaw, *et al*., 2016). In this study, temperature and pH were maintained at constant level (25^{o}C and 7 respectively) hence

had minimal influence on mycelia growth. Among different growing agar media tested, results are in line with previous studies showing higher growth of mushroom mycelia under MEA in comparison to other media (Nasim, *et al*., 2001; Shim, *et al*., 2005; Kalaw, *et al*., 2016). Munj, *et al*., (1997) and Inqabal, *et al*., (2014) reported enhanced growth of *Ganoderma lucidum* and *Volvariella volvaceae* mycelia in MEA. Shim *et al*., (2005) also reported high growth rate of *Macrolepiota procera* in MEA compared to PDA. PDA is a basic and a commonly used medium in the laboratories. It's composed of potato extract, agar and dextrose. PDYA is composed of potato extract, agar dextrose and yeast. Although PDYA media is not commonly used to culture macrofungi, this study has found out that PDYA is also a suitable media and can be an alternative to malt extract agar for culturing *Macrolepiota dolichaula* and *Auricularia polytrica*.

The time taken for complete mycelial growth of *Pleurotus* on the three culture media compares with the findings of Nasim *et al*., 2010 who reported the number of days that *Pleurotus* mushrooms took (upto 10 days) for complete mycelial ramification. Though complete mycelia growth for *Macrolepiota dolichaula* in this study took a maximum of 7 days on PDA, PDYA and MALT extract, Mbaluto, (2015) reported complete mycelial colonization of *Macrolepiota* in 8 - 10 days on PDA, YEA or MEA.

5.2 Conclusion

- From this study, the indigenous and plantation land use types are a haven of diverse and distinct macrofungi communities. However indigenous forests (natural ecosystems) have a wide range of macrofungi assemblage, abundance and species richness compared to exotic plantation forest.

- Seasonality is a key factor in the occurrence of macrofungi and are dominant during the wet season.
- Use of both morphological and molecular methods is important for the identification of macrofungi. A single approach is inadequate since some macrofungi species especially young fruit bodies might have missing important morphological features.
- Among the three tested culture media, malt extract agar was the best media for culturing *Macrolepiota dolichaula* and *Auricularia polytrica* species followed by PDYA.
- The study forms a baseline on the diversity of macrofungi for further assessment of forested ecosystems.

5.3 Recommendations

- From this study it's recommended that a detailed monitoring study be carried out and additional sampling. This will enable the documentation of all fungi species which could have been missed during the two surveys and at the same time document the sprouting period of each fungal species after the on-set of rainy season.
- The rich biodiversity of wild edible macrofungi needs further research exploration to widen the culinary and medicinal utilization by the rural folk who depend on the mushrooms through conservation, cultivation and commercialization activities.
- More detailed taxonomic studies combining both morphological and molecular studies are required to confirm all the species to the lowest level and mostly species derived from mycelial cultures.

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Appendix 1: Checklist of macrofungi in Kereita Forest, Kikuyu Escarpment

Families	Species	Substrates	Wet	Dry	Wet	Dry
Mycenaceae	*Mycena inclinata*	*Wood*	+	_	_	_
Mycenaceae	*Mycena* sp 2	*Wood*	+	_	_	_
Agaricaceae	*Agaricus augustus*	*Soil*	+	_	_	_
Agaricaceae	*Agaricus inoxydabilis*	*Soil*	+	_	_	_
Agaricaceae	*Agaricus silvaticus*	*Soil*	+	_	_	_
Agaricaceae	*Agaricus* avensis	*Soil*	_	_	+	_
Agaricaceae	*Agaricus* sp 5	*Soil*	+	_	_	_
Agaricaceae	*Agaricus* sp 6	*Soil*	_	+	_	_
Agaricaceae	*Agaricus* sp 7	*Soil*	_	_	_	+
Agaricaceae	*Agaricus* sp 8	*Soil*	+	_	_	_
Strophariaceae	*Agrocybe* sp 1	*Litter*	_	_	_	+
Strophariaceae	*Agrocybe* sp 2	*Litter*	_	_	_	+
Physalacriaceae	*Armillaria mellea*	*Parasitic*	+	_	_	_
Physalacriaceae	*Armillaria* sp *1*	*Parasitic*	+	_	_	_
Physalacriaceae	*Armillaria* sp 2	*Parasitic*	+	_	_	_
Auriculariacea	*Auricalaria auricula*	*Wood*	+	_	_	_
Auriculariacea	*Auricalaria delicata*	*Wood*	+	_	_	_
Auriculariacea	*Auricalaria polytrica*	*Wood*	+	_	_	_
Auriculariacea	*Auricalaria* sp 1	*Wood*	+	_	_	+
Bolbitiaceae	*Bolbitius* sp 1	*Litter*	+	_	_	_
Bolbitiaceae	*Bolbitius* sp 2	*Litter*	+	_	_	_
Bolbitiaceae	*Bolbitius* sp 3	*Litter*	_	_	_	+
Bolbitiaceae	*Bolbitius* sp 4	*Litter*	_	_	_	+
Pluteaceae	*Chamaeota* sp	*Wood*	+	_	_	_
Gomphidiaceae	*Chroogomphus* sp *1*	*Ectomycorrhizal*	_	_	+	_
Gomphidiaceae	*Chroogomphus* sp *2*	*Ectomycorrhizal*	_	_	+	_
Gomphidiaceae	*Chroogomphus* sp *3*	*Ectomycorrhizal*	_	_	+	_
Agaricaceae	*Clavatia* sp 1	*Litter*	_	_	+	_
Tricholomataceae	*Clavatia* sp 2	*Litter*	_	_	+	_
Tricholomataceae	*Clavatia* sp 3	*Litter*	_	_	+	_
Tricholomataceae	*Clitocybe dilitata*	*Soil*	+	_	_	_
Tricholomataceae	*Clitocybe* sp 1	*Soil*	+	_	_	_
Tricholomataceae	*Clitocybe* sp 2	*Soil*	+	_	_	_
Tricholomataceae	*Clitocybe* sp 3	*Soil*	+	_	_	_
Tricholomataceae	*Clitopilus* sp 1	*Litter*	+	_	_	_
Tricholomataceae	*Clitopilus* sp 2	*Litter*	+	_	+	_
Bolbitiaceae	*Conocybe* sp 1	*Litter*	_	_	+	_
Bolbitiaceae	*Conocybe tenera*	*Litter*	_	_	+	_
Agaricaceae	*Coprinus comatus*	*Litter*	+	_	_	_
Agaricaceae	*Coprinus disseminatus*	*Litter*	+	_	_	_
Agaricaceae	*Coprinus jonesii*	*Litter*	_	_	+	_
Agaricaceae	*Coprinus* sp 1	*soil*	+	_	_	_
Agaricaceae	*Coprinus* sp 2	*Litter*	+	_	_	_
Agaricaceae	*Coprinus* sp 3	*Litter*	+	_	_	_

Agaricaceae	*Coprinus stercoreus*	*Litter*	+	_	_	_
Crepidotaceae	*Crepidotus applanatus*	*Wood*	_	+	_	_
Crepidotaceae	*Crepidotus* sp 1	*Wood*	+	_	_	_
Crepidotaceae	*Crepidotus* sp 2	*Wood*	+	_	_	_
Crepidotaceae	*Crepidotus* sp 3	*Wood*	+	_	_	_
Nidulariaceae	*Cyathus poeppigii*	*Wood*	+	_	_	_
Nidulariaceae	*Cyathus striatus*	*Wood*	+	_	+	_
Meruliaceae	*Cymatoderma elegance*	*Wood*	+	_	_	_
Physalacriaceae	*Cyptotrama* sp 1	*Wood*	+	_	_	_
Agaricaceae	*Cystolepiota* sp 1	*Soil*	+	_	_	_
Agaricaceae	*Cystolepiota* sp 2	*Soil*	+	_	_	_
Agaricaceae	*Cystolepiota* sp 3	*Soil*	+	_	_	_
Agaricaceae	*Cystolepiota* sp 4	*Soil*	+	_	_	_
Agaricaceae	*Cystolepiota* sp 5	*Soil*	+	_	_	_
Agaricaceae	*Cystolepiota* sp 6	*Soil*	+	_	+	_
Agaricaceae	*Cystolepiota* sp 9	*Soil*	+	_	_	_
Agaricaceae	*Cytolepiota* sp 7	*Soil*	+	_	_	_
Agaricaceae	*Cytolepiota* sp 8	*Soil*	+	_	_	_
Xylariaceae	*Daldinia concentrica*	*Wood*	_	+	_	_
Entolomataceae	*Entoloma* sp 1	*Soil*	+	_	_	_
Entolomataceae	*Entoloma* sp 2	*Litter*	+	_	_	_
Entolomataceae	*Entoloma* sp 3	*Litter*	+	_	+	_
Mycenaceae	*Favolaschia calocera*	*Wood*	+	+	_	_
Mycenaceae	*Favolaschia cyathea*	*Wood*	_	+	_	_
Tricholomataceae	*Fayodia leucophylla*	*Wood*	_	+	_	_
Polyporaceae	*Fomentarius fomes*	*Wood*	_	+	_	_
Funariaceae	*Funaria* sp	*Wood*	+	_	_	_
Hymenogastraceae	*Galerina* sp 1	*Wood*	+	_	_	_
Hymenogastraceae	*Galerina* sp 2	*Wood*	+	_	_	_
Hymenogastraceae	*Ganoderma applanatum*	*Parasitic*	_	+	_	_
Ganodermataceae	*Ganoderma australe*	*Parasitic*	_	_	+	_
Ganodermataceae	*Ganoderma* sp 1	*Parasitic*	_	+	_	_
Hygrophoraceae	*Gliophorus* sp 1	*Litter*	+	_	_	_
Hygrophoraceae	*Gliophorus* sp 2	*Litter*	+	_	_	_
Hygrophoraceae	*Gliophorus* sp 3	*Litter*	+	_	_	_
Marasmiaceae	*Gymnopus* sp 1	*Wood*	+	_	_	_
Marasmiaceae	*Gymnopus* sp 2	*Wood*	+	_	_	_
Marasmiaceae	*Gymnopus* sp 3	*Wood*	+	_	_	_
Marasmiaceae	*Gymnopus* sp 4	*Wood*	+	_	_	_
Marasmiaceae	Gymnopus sp 5	*Wood*	+	_	_	_
Marasmiaceae	Gymnopus sp 6	*Wood*	+	_	_	_
Marasmiaceae	*Gymnopus* sp 7	*Wood*	+	_	_	_
Marasmiaceae	*Gymnopus subpruinosus*	*Wood*	+	_	_	_
Agaricaceae	*Handkea* sp	*Soil*	+	_	_	_
Mycenaceae	*Hemimycena sp*	*Wood*	_	+	_	_
Polyporaceae	*Hexagonia* sp 1	*Wood*	_	+	_	_

Polyporaceae	*Hexagonia* sp 2	*Wood*	_	+	_	_
Polyporaceae	*Hexagonia tenuis*	*Wood*	_	+	_	_
Hygrophoraceae	*Hygrocybe conica*	*Soil*	_	_	+	_
Hygrophoraceae	*Hygrocybe persistens*	*Soil*	+	_	_	_
Hygrophoraceae	*Hygrophorus* sp 1	*Litter*	_	_	+	_
Hygrophoraceae	*Hygrophorus* sp 4	*Litter*	+	_	_	_
Hygrophoraceae	*Hygrophorus* sp 2	*Litter*	_	_	+	_
Hygrophoraceae	*Hygrophorus* sp 3	*Litter*	_	_	+	_
Hygrophoraceae	*Hygrophorus* sp 5	*Litter*	_	+	_	_
Agaricaceae	*Hymenagaricus* sp 1	*Litter*	+	_	_	_
Agaricaceae	*Hymenagaricus* sp 2	*Litter*	_	_	+	_
Agaricaceae	*Hymenagaricus* sp 3	*Litter*	+	_	_	_
Agaricaceae	*Hymenagaricus* sp 4	*Litter*	_	+	_	_
Strophariaceae	*Hypholoma fasciculata*	*Wood*	+	+	_	_
Inocybaceae	*Inocybe* sp 1	*Ectomycorrhizal*	+	_	_	_
Inocybaceae	Inocybe *sp* 3	*Ectomycorrhizal*	_	_	+	_
Inocybaceae	*Inocybe* sp 4	*Ectomycorrhizal*	_	_	_	+
Inocybaceae	*Inocybe* sp 2	*Ectomycorrhizal*	_	_	+	_
Hydnangiaceae	*Laccaria* sp 1	*Ectomycorrhizal*	_	_	+	_
Hydnangiaceae	*Laccaria* sp 3	*Ectomycorrhizal*	_	_	+	_
Hydnangiaceae	*Laccaria* sp 4	*Ectomycorrhizal*	_	_	+	_
Hydnangiaceae	*Laccaria* sp 2	*Ectomycorrhizal*	_	_	+	_
Hydnangiaceae	*Laccaria tortolis*	*Ectomycorrhizal*	_	_	+	+
Psathyrellaceae	*Lacrymaria velutina*	*Wood*	_	+	_	_
Agaricaceae	*Lepiota felina*	*Litter*	+	_	_	_
Agaricaceae	*Lepiota* sp 1	*Soil*	+	_	_	+
Tricholomataceae	*Lepista sordida*	*Litter*	+	_	_	_
Entolomataceae	*Leptonia* sp 1	*Litter*	+	_	_	_
Entolomataceae	*Leptonia* sp 2	*Litter*	+	+	_	_
Entolomataceae	*Leptonia* sp 3	*Litter*	+	_	_	_
Entolomataceae	*Leptonia* sp 4	*Litter*	+	+	_	_
Entolomataceae	*Leptonia* sp 5	*Litter*	+	_	_	_
Agaricaceae	*Leucoagaricus* sp 1	*Soil*	_	_	+	+
Agaricaceae	*Leucoagaricus* sp 2	*Soil*	_	+	_	_
Agaricaceae	*Leucocoprinus* sp 1	*Litter*	+	_	_	_
Agaricaceae	*Leucocoprinus* sp 2	*Litter*	+	_	_	_
Agaricaceae	*Leucopaxillus* sp	*Litter*	+	_	_	_
Lycoperdaceae	*Lycoperdon perlatum*	*Soil*	_	_	_	+
Lycoperdaceae	*Lycoperdon pyriforme*	*Soil*	_	_	_	+
Lycoperdaceae	*Lycoperdon* sp 1	*Soil*	_	_	+	_
Lycoperdaceae	*Lycoperdon* sp 4	*Soil*	_	_	+	_
Lycoperdaceae	*Lycoperdon* sp 5	*Soil*	+	_	_	_
Lycoperdaceae	*Lycoperdon* sp 6	*Soil*	_	_	_	+
Lycoperdaceae	*Lycoperdon* sp 2	*Soil*	+	_	_	_
Lycoperdaceae	*Lycoperdon* sp 3	*Soil*	_	_	+	_
Agaricaceae	*Macrolepiota dolichaula*	*Litter*	_	_	+	_

Agaricaceae	*Macrolepiota procera*	*Litter*	+	_	+	+
Agaricaceae	*Macrolepiota* sp *1*	*Litter*	+	_	_	_
Marasmiaceae	*Marasmius leucorotalis*	*Litter*	_	_	+	_
Marasmiaceae	*Marasmius* sp 1	*Litter*	_	_	+	_
Marasmiaceae	*Marasmius* sp 2	*Litter*	+	_	_	_
Marasmiaceae	*Marasmius* sp 3	*Litter*	+	_	_	_
Polyporaceae	Microporus sp	Wood	_	+	_	_
Polyporaceae	*Micropsalliota* sp 1	*litter*	+	_	_	_
Polyporaceae	*Micropsalliota* sp 2	*litter*	+	_	_	_
Mycenaceae	*Mycena* sp 1	*Litter*	+	_	_	_
Mycenaceae	*Mycena* sp 1	*Litter*	+	_	_	_
Mycenaceae	*Mycena* sp 4	*Litter*	+	_	_	_
Mycenaceae	*Mycena* sp 5	*Litter*	+	_	_	_
Mycenaceae	*Mycena* sp 8	*wood*	+	_	_	_
Mycenaceae	*Mycena* sp 9	*Litter*	+	_	_	_
Mycenaceae	*Mycena* sp 3	*Wood*	+	_	_	_
Mycenaceae	*Mycena* sp 6	*Litter*	+	_	_	_
Mycenaceae	*Mycena* sp 7	*Litter*	+	_	_	_
Tricholomataceae	*Myxomphalia* sp	*Litter*	+	_	_	_
Tricholomataceae	*Omphalia* sp	*Litter*	+	+	_	_
Tricholomataceae	*Omphalina epichysum*	*Litter*	+	_	_	_
Bolbitiaceae	*Panaeolina* sp 1	*litter*	_	_	_	+
Bolbitiaceae	*Panaeolina* sp 2	*litter*	_	_	_	+
Hymenogastraceae	Phaeocollybia sp	Ectomycorrhizal	_	_	_	_
Hymenochytaceae	*Phellinus* sp 1	*Parasitic*	_	+	_	_
Hymenochytaceae	*Phellinus gilvus*	*Parasitic*	_	+	_	_
Hymenochytaceae	*Phellinus robustus*	*wood*	_	_	_	+
Hymenochytaceae	*Phellinus* sp 4	*wood*	_	_	_	+
Polyporaceae	*Phellinus* sp 3	*wood*	_	_	_	+
Strophariaceae	*Pholiota* sp 1	*Wood*	+	_	_	_
Strophariaceae	*Pholiota* sp 2	*Wood*	+	_	_	_
Strophariaceae	*Pholiota squarrosus*	*Wood*	+	_	_	_
Pleurotaceae	*Pleurocybella porrigens*	*Wood*	+	_	_	_
Pleurotaceae	*Pleurotus djamor*	*Wood*	_	+	_	_
Pleurotaceae	*Pleurotus populinus*	*Wood*	+	_	_	_
Pleurotaceae	*Pleurotus* sp 1	*Wood*	+	_	_	_
Pleurotaceae	*Pleurotus* sp 2	*Wood*	+	_	_	_
Tricholomataceae	*Fayodia leucophylla*	*Wood*	+	_	_	_
Pleurotaceae	*Pleurotus* sp 3	*Wood*	+	_	+	_
Pleurotaceae	*Pleurotus* sp 4	*Wood*	+	_	_	_
Pleurotaceae	*Pleurotus* sp 5	*Wood*	+	_	_	_
Pleurotaceae	*Pleurotus* sp 6	*Wood*	+	_	_	_
Plutaceae	*Pluteus* sp	*Wood*	+	_	_	_
Polyporaceae	*Polyporus* sp 1	*Wood*	+	_	_	_
Polyporaceae	*Polyporus* sp 2	*Wood*	+	_	_	_
Polyporaceae	*Polyporus* sp 3	*Wood*	+	_	_	_

Polyporaceae	*Polyporus* sp 4	*Wood*	+	_	_	_
Polyporaceae	*Polyporus* sp 5	*Wood*	_	+	_	_
Psathyrellaceae	*Psathyrella longipes*	*Litter*	+	+	_	_
Psathyrellaceae	*Psathyrella* sp 1	*Wood*	+	_	_	_
Psathyrellaceae	*Psathyrella* sp 2	*Litter*	+	_	_	_
Psathyrellaceae	*Psathyrella* sp 3	*Litter*	+	_	_	_
Psathyrellaceae	*Psathyrella* sp 4	*Litter*	+	_	+	_
Psathyrellaceae	*Psathyrella* sp 5	*Litter*	+	_	+	_
Tricholomataceae	*Pseudoclitocybe*	*Ectomycorrhizal*	+	_	_	_
Hymenogastraceae	*Psilocybe* sp 1	*Wood*	_	_	+	_
Hymenogastraceae	*Psilocybe* sp 2	*Wood*	_	_	+	_
Marasmiaceae	*Resinomycena* sp 3	*Wood*	_	+	_	_
Mycenaceae	*Roridomyces* sp 1	*Wood*	+	_	_	_
Mycenaceae	*Roridomyces* sp 3	*Wood*	+	_	_	_
Mycenaceae	*Roridomyces* sp 4	*Wood*	+	_	_	_
Mycenaceae	*Roridomyces* sp 5	*Wood*	+	_	_	_
Mycenaceae	*Roridomyces* sp 6	*Wood*	+	_	_	_
Mycenaceae	*Roridomyces* sp 2	*Wood*	+	_	_	_
Strophariaceae	*Spongillipellis* sp 4	*Wood*	_	_	+	_
Polyporaceae	*Spongipellis* sp 1	*Wood*	_	+	_	_
Polyporaceae	*Spongipellis* sp 1	*Wood*	_	+	_	_
Polyporaceae	*Spongipellis* sp 3	*Wood*	_	+	_	_
Polyporaceae	*Stereum gausapatum*	*Wood*	+	_	_	_
Stereaceae	*Stereum ostrea*	*Wood*	_	_	+	_
Suillaceae	*stropharia rugosoannulata*	*Litter*	_	_	_	+
Strophariaceae	*Stropharia* sp 1	*Litter*	_	_	+	_
Strophariaceae	*Stropharia* sp 3	*Litter*	+	_	_	_
Strophariaceae	*Stropharia* sp 2	*Litter*	_	_	+	_
Tricholomataceae	*Suillus granulatus*	*Ectomycorrhizal*	_	_	_	+
Suillaceae	*Suillus lutea*	*Ectomycorrhizal*	_	_	_	+
Suillaceae	*Suillus* sp 1	*Ectomycorrhizal*	_	_	+	+
Polyporaceae	*Trametes* sp	*Wood*	_	+	_	_
Polyporaceae	*Trichaptum* sp	*Wood*	_	+	_	_
Tricholomataceae	*Tricholomopsis rutilans*	*Wood*	+	_	_	_
Marasmiaceae	*Tricholomopsis* sp *1*	*Wood*	+	_	_	_
Marasmiaceae	*Trogia* sp 1	*Wood*	+	_	_	_
Marasmiaceae	*Trogia* sp 3	*Wood*	+	_	_	_
Marasmiaceae	*Trogia* sp 2	*Wood*	+	_	_	_
Lycoperdaceae	*Typhula* sp	*Litter*	_	+	+	_
Physalacriaceae	*Vascellum pratense*	*Soil*	_	+	_	_
Marasmiaceae	*Xeromphalia* sp *1*	*Litter*	+	_	_	_
Marasmiaceae	*Xeromphalina* sp 2	*Litter*	_	+	_	_
Typhulaceae	*Xeromphalina* sp 3	*Litter*	_	+	_	_
Physalacriaceae	*Xerula radicata*	*Wood*	+	_	_	_

Appendix 2: Distribution of macrofungi in Kereita forest, Kikuyu Escarpment

		Indigenous Forest	Plantation Forest
Families	**Species**	**Abundance**	**Abundance**
Agaricaceae	*Agaricus* sp *1*	2	0
Agaricaceae	*Agaricus* sp *2*	0	13
Agaricaceae	*Agaricus* sp *3*	0	1
Agaricaceae	*Agaricus* sp *4*	5	0
Agaricaceae	*Agaricus* sp *5*	9	9
Agaricaceae	*Agaricus volvulatus*	0	1
Agaricaceae	*Agaricus inoxydabilis*	0	6
Agaricaceae	*Agaricus augustus*	1	0
Agaricaceae	*Agaricus silvaticus*	14	0
Strophariaceae	*Agrocybe* sp *1*	0	2
Strophariaceae	*Agrocybe* sp *2*	0	2
Auriculariaaece	*Auricalaria* sp *1*	200	0
Auriculariaaece	*Auricalaria polytrica*	20	0
Auriculariaaece	*Auricalaria auricula*	200	0
Auriculariaaece	*Auricalaria delicata*	1	0
Physalacriaceae	*Armillaria* sp *1*	40	0
Physalacriaceae	*Armillaria* sp *2*	20	0
Physalacriaceae	*Armillaria mellea*	4000	0
Bolbitaceae	*Bolbitius* sp *1*	2	0
Bolbitaceae	*Bolbitius* sp *2*	2	0
Bolbitaceae	*Bolbitius* sp *3*	0	4
Bolbitaceae	*Bolbitius* sp *4*	0	1
Pluteaceae	*Chamaeota* sp	2	0
Gomphidiaceae	*Chroogomphus* sp *1*	0	2
Gomphidiaceae	*Chroogomphus* sp *2*	0	12
Gomphidiaceae	*Chroogomphus* sp *3*	0	8
Agaricaceae	*Clavatia* sp *1*	0	1
Agaricaceae	*Clavatia* sp *2*	0	1
Agaricaceae	*Clavatia* sp *3*	0	14
Tricholomataceae	*Clitocybe* sp *1*	2	0
Tricholomataceae	*Clitocybe* sp *2*	3	0
Tricholomataceae	*Clitocybe* sp *3*	1	0
Tricholomataceae	*Clitocybe dilitata*	1000	0
Tricholomataceae	*Clitopilus* sp *1*	6	0
Tricholomataceae	*Clitopilus* sp *2*	0	3
Crepidotaceae	*Crepidotus* sp *1*	3	0
Crepidotaceae	*Crepidotus* sp *2*	400	0
Crepidotaceae	*Crepidotus* sp *3*	10	0
Crepidotaceae	*Crepidotus applanatus*	3	0
Bolbitiaceae	*Conocybe* sp *1*	0	2

Bolbitiaceae	*Conocybe tenera*	0	2
Agaricaceae	*Coprinus* sp *1*	4	0
Agaricaceae	*Coprinus* sp *2*	6	0
Agaricaceae	*Coprinus* sp *3*	1	0
Agaricaceae	*Coprinus comatus*	57	0
Agaricaceae	*Coprinus disseminatus*	30	0
Agaricaceae	*Coprinus jonesii*	0	1
Agaricaceae	*Coprinus stercoreus*	2	0
Nidulariaceae	*Cyathus striatus*	0	10
Nidulariaceae	*Cyathus poeppigii*	2	0
Meruliaceae	*Cymatoderma elegance*	7	0
Physalacriaceae	*Cyptotrama* sp 1	7	0
Agaricaicaceae	*Cystolepiota* sp 1	2	0
Agaricaicaceae	*Cystolepiota* sp 2	2	0
Agaricaicaceae	*Cystolepiota* sp 3	6	0
Agaricaicaceae	*Cystolepiota* sp 4	3	0
Agaricaicaceae	*Cystolepiota* sp 5	1	0
Agaricaicaceae	*Cystolepiota* sp 6	0	3
Agaricaicaceae	*Cytolepiota* sp 7	2	0
Agaricaicaceae	*Cytolepiota* sp 8	1	0
Agaricaicaceae	*Cystolepiota* sp 9	1	0
Plolyporaceae	*Daldinia concentrica*	4	0
Entolomataceae	*Entoloma* sp 1	25	0
Entolomataceae	*Entoloma* sp 2	53	0
Entolomataceae	*Entoloma* sp 3	30	0
Mycenaceae	*Favolaschia calocera*	5271	10
Mycenaceae	*Favolaschia cyathea*	10	0
Polyporaceae	*Fomentarius fomes*	1	0
Funariaceae	*Funaria* sp	1	0
Hymenogastraceae	*Galerina* sp 1	3	0
Hymenogastraceae	*Galerina* sp 2	9	0
Ganodermataceae	*Ganoderma* sp 1	6	0
Ganodermataceae	*Ganoderma appalnatum*	4	0
Ganodermataceae	*Ganoderma australe*	0	1
Hygrophoraceae	*Gliophorus* sp 1	1	0
Hygrophoraceae	*Gliophorus* sp 2	9	0
Hygrophoraceae	*Gliophorus* sp 3	3	0
Marasmiaceae	*Gymnopus* sp 1	3	0
Marasmiaceae	*Gymnopus* sp 2	2	0
Marasmiaceae	*Gymnopus* sp 3	20	0
Marasmiaceae	*Gymnopus* sp 4	2	0
Marasmiaceae	Gymnopus *sp 5*	5	0
Marasmiaceae	Gymnopus *sp 6*	1	0

Marasmiaceae	*Gymnopus* sp 7	40	0
Marasmiaceae	*Gymnopus subpruinosus*	45	0
Agaricaceae	*Handkea* sp	3	0
Mycenaceae	*Hemimycena* sp	1050	0
Polyporaceae	*Hexagonia* sp 1	6	0
Polyporaceae	*Hexagonia* sp 2	27	0
Polyporaceae	*Hexagonia tenuis*	200	0
Polyporaceae	*Hygrocybe persistens*	3	0
Hygrophoraceae	*Hygrocybe conica*	0	3
Hygrophoraceae	*Hygrophorus* sp 1	0	3
Hygrophoraceae	*Hygrophorus* sp 2	0	6
Hygrophoraceae	*Hygrophorus* sp 3	0	4
Hygrophoraceae	*Hygrophorus* sp 5	2	0
Hygrophoraceae	*Hygrophorus* sp 4	2	0
Agaricaceae	*Hymenagaricus* sp 1	7	0
Agaricaceae	*Hymenagaricus* sp 2	0	1
Agaricaceae	*Hymenagaricus* sp 3	30	0
Agaricaceae	*Hymenagaricus* sp 4	30	0
Strophariaceae	*Hypholoma fasciculata*	214	5
Inocybaceae	*Inocybe* sp 1	1	0
Inocybaceae	*Inocybe* sp 2	0	1
Inocybaceae	Inocybe *sp 3*	0	1
Inocybaceae	*Inocybe* sp 4	0	18
Hydnangiaceae	*Laccaria* sp 1	0	1
Hydnangiaceae	*Laccaria* sp 2	100	261
Hydnangiaceae	*Laccaria* sp 3	0	5
Hydnangiaceae	*Laccaria tortolis*	0	46
Hydnangiaceae	*Laccaria* sp 4	0	5
	Lacrymaria velutina	1	0
Agaricaceae	*Lepiota felina*	30	0
Agaricaceae	*Lepiota* sp 1	1	1
Entolomataceae	*Leptonia* sp 1	2	0
Entolomataceae	*Leptonia* sp 2	51	0
Entolomataceae	*Leptonia* sp 3	13	0
Entolomataceae	*Leptonia* sp 4	1	0
Entolomataceae	*Leptonia* sp 5	1	0
Tricholomataceae	*Lepista* sp	1	0
Agaricaceae	*Leucoagaricus* sp 1	0	4
Agaricaceae	*Leucoagaricus* sp 2	1	0
Agaricaceae	*Leucocoprinus* sp 1	7	0
Agaricaceae	*Leucocoprinus* sp 2	4	0
Tricholomataceae	*Leucopaxillus* sp	1	0
Lycoperdaceae	*Lycoperdon* sp 1	0	6

Lycoperdaceae	*Lycoperdon* sp 2	4	0
Lycoperdaceae	*Lycoperdon* sp 3	0	1
Lycoperdaceae	*Lycoperdon* sp 4	0	1
Lycoperdaceae	*Lycoperdon* sp 5	1	0
Lycoperdaceae	*Lycoperdon* sp 6	0	1
Lycoperdaceae	*Lycoperdonsp perlatum*	0	10
Lycoperdaceae	*Lycoperdon pyriforme*	0	1
Agaricaceae	*Macrolepiota* sp 1	5	0
Agaricaceae	*Macrolepiota* sp 2	0	1
Agaricaceae	*Macrolepiota procera*	1	8
Marasmiaceae	*Marasmius* sp 1	0	2
Marasmiaceae	*Marasmius* sp 2	5	0
Marasmiaceae	*Marasmius* sp 3	5	0
Marasmiaceae	*Marasmius leucorotalis*	0	128
Polyporaceae	Microporus sp	1	0
Agaricaceae	*Micropsalliota* sp 1	1	0
Agaricaceae	*Micropsalliota* sp 2	10	0
Mycenaceae	*Mycena* sp 1	3	0
Mycenaceae	*Mycena* sp 2	1	0
Mycenaceae	*Mycena* sp 3	2	0
Mycenaceae	*Mycena* sp 4	12	0
Mycenaceae	*Mycena* sp 5	2	0
Mycenaceae	*Mycena* sp 6	2	0
Mycenaceae	*Mycena* sp 7	1	0
Mycenaceae	*Mcena inclinata*	1	0
Mycenaceae	*Mycena* sp 8	24	0
Mycenaceae	*Mycena* sp 9	5	0
Mycenaceae	*Mycena* sp 10	1	1
Tricholomataceae	*Myxomphalia* sp	11	0
Tricholomataceae	*Omphalia* sp	10	0
Hymenogastraceae	*Omphalina epichysum*	1	0
Hymenogastraceae	Phaeocollybia sp	6	0
Bolbitiaceae	*Panaeolina* sp 1	0	3
Bolbitiaceae	*Panaeolina* sp 2	0	1
Hymenochytaceae	*Phellinus* sp 1	20	0
Hymenochaetaceae	*Phellinus* sp 2	0	94
Hymenochytaceae	*Phellinus* sp 3	0	2
Hymenochytaceae	*Phellinus* sp 4	0	1
Hymenochtaceae	*Phellinus glivus*	1	0
Strophariaceae	*Pholiota* sp 1	5	0
Strophariaceae	*Pholiota* sp 2	250	0
Strophariaceae	*Pholiota squarrosus*	13	0
Pleurotaceae	*Pleurocybella porrigens*	10	0

Pleurotaceae	*Pleurotus* sp 1	300	0
Pleurotaceae	*Pleurotus* sp 2	300	0
Pleurotaceae	*Pleurotus* sp 3	6	0
Pleurotaceae	*Pleurotus* sp 4	0	3
Pleurotaceae	*Pleurotus* sp 5	1	0
Pleurotaceae	*Pleurotus* sp 6	4	0
Pleurotaceae	*Pleurotus djamor*	10	0
Pleurotaceae	*Pleurotus populinus*	1	0
Pluteaceae	*Pluteus* sp	1	0
Polyporaceae	*Polyporus* sp 1	2	0
Polyporaceae	*Polyporus* sp 2	3	0
Polyporaceae	*Polyporus* sp 3	2	0
Polyporaceae	*Polyporus* sp 4	1	0
Polyporaceae	*Polyporus* sp 5	1	0
Psathyrellaceae	*Psathyrella* sp 1	1	0
Psathyrellaceae	*Psathyrella* sp 2	1	0
Psathyrellaceae	*Psathyrella* sp 3	2	0
Psathyrellaceae	*Psathyrella* sp 4	1	1
Psathyrellaceae	*Psathyrella* sp 5	15	1
Psathyrellaceae	*Psathyrella longipes*	2	0
Tricholomataceae	*Pseudoclitocybe*	100	0
Hymenogastraceae	*Psilocybe* sp 1	0	1
Hymenogastraceae	*Psilocybe* sp 2	0	1
Mycenaceae	*Resinomycena* sp 3	5	0
Mycenaceae	*Roridomyces* sp 1	15	0
Mycenaceae	*Roridomyces* sp 2	15	0
Mycenaceae	*Roridomyces* sp 3	5	0
Mycenaceae	*Roridomyces* sp 4	1	0
Mycenaceae	*Roridomyces* sp 5	150	0
Mycenaceae	*Roridomyces* sp 6	300	0
Stereaceae	*Stereum ostrea*	0	3
Stereaceae	*Stereum gausapatum*	11	0
Polyporaceae	*Trametes* sp	100	0
Polyporaceae	*Trichaptum* sp	170	0
Strophariaceae	*Stropharia* sp 1	0	2
Strophariaceae	*Stropharia* sp 2	0	1
Strophariaceae	*Stropharia* sp 3	0	2
Polyporaceae	*Spongipellis* sp 1	1	0
Polyporaceae	*Spongipellis* sp 1	20	0
Polyporaceae	*Spongipellis* sp 3	50	0
Polyporaceae	*Spongillipellis* sp 4	10	0
Suillaceae	*Suillus* sp 1	0	6
Suillaceae	*Suillus lutea*	0	88

Suillaceae	*Suillus granulatus*	0	15
Tricholomataceae	*Tricholomopsis rutilans*	2	0
Tricholomataceae	*Tricholomopsis* sp 1	1	0
Marasmiaceae	*Trogia* sp 1	5	0
Marasmiaceae	*Trogia* sp 2	50	0
Marasmiaceae	*Trogia* sp 3	7	0
Typhulaceae	*Typhula* sp	1	0
Agaricaceae	*Vascellum pratense*	4	30
Physalacriaceae	*Xerula radicata*	5	0
Marasmiaceae	*Xeromphalia* sp 1	5	0
Marasmiaceae	*Xeromphalina* sp 2	1	0
Marasmiaceae	*Xeromphalina* sp 3	2	0

Appendix 3: List of macrofungi occuring in the Kereita block during the dry and wet season with potential for utilisation as food and medicine

Species	Family	Habitat	State	Uses	Reference
Agaricus agustus	Agaricaceae	Indigenous forest	Fleshy	Food	(Cotter, 2014)
Agaricus silvicatus	Agaricaceae	Plantation forest	Fleshy	Medicine	(Munkhgerel, *et al.*, 2014)
Agaricus volvatulus	Agaricaceae	Plantation forest	Fleshy	Food	Degreef *et al*., 2016
Macrolepiota procera	Agaricaceae	Indigenous forest	Fleshy	Food	(Chim *et al*., 2005)
Macrolepiota dolichauli	Agaricaceae	Plantation forest	Fleshy	Food	Mbaluto, 2015
Suillus luteus	Suillaceae	Plantation forest	Fleshy	Food	(Degreef *et al*., 2016)
Suillus granulatus	Suillaceae	Plantation forest	Fleshy	Food	Degreef *et al*., 2016
Auricularia auricula	Auricalariaceae	Iindigenous forest	Fleshy	Food	(Onyango *et al*.,2011)
Auricularia delicate	Auricalariaceae	Indigeous forest	Fleshy	Food	(Onyango *et al*.,2011)
Auricularia polytrica	Auriculariaceae	Indigenous forest	Fleshy	Food	(Onyango *et al*., 2011)
Ganoderma austraule	Ganodermataceae	Indigenous forest	Non - fleshy	Medicine	(Cotter, 2014)
Ganoderma appalatum	Ganodermataceae	Indigenous forest	Non fleshy	Medicine	Tapwal *et al*., 2013
Pleurotus djamor	Pleurotaceae	Indigenous forest	Fleshy	Food	Tapwal *et al* ., 2103
Stropharia rugussoannulata	Strophariaceae	Plantation forest	Fleshy	Food	Kuo, 2016
Coprinus dessiminatus	Agaricaceae	Indigenous forest	Fleshy	Food	Tapwal *et al*., 2013
Lycoperdon pyriform	Lycoperdaceae	Indigenous	Fleshy	Food	Tapwal *et al*., 2013
Phellinus robustus	Hymenochaetaceae	Indigenous forest	Non-fleshy	Medicine	(Bisko *et al*., 2007)
Phellinus gilvus	Hymenochaetaceae	Indigenous forest	Non-fleshy	Medicine	Tapwal *et al*.,2013
Trametes versicolor	Polyporaceae	Indigenous forest	Non fleshy	Medicine	(Cotter, 2014)
Armillaria mellea	Physalacriaceae	Indigenous forest	Fleshy	Food	(Cotter, 2014)

Appendix 4: Internal Transcribed Sequences of selected macrofungi from Kereita forest, Kikuyu Escarpment.

Sample KIC 001/ Gene bank Accession Number KC176328.1

AAATTGTCCAAATTAACAGACGATTAGAAGCAGTGCTWWAAACGGTAAACA
GTCCACGGCGTAGATAATTATCACACCAATAGACTGGTTTACACAAGGCAAC
CAGCTAATGTATTTCAGGAGAGCTGATTTCAAAAGAGAAACCGGCAAACTCC
CACATCCAAGCCATTTATCAACCAAAAAGCTGATAAAGGTTGAGAATTTAAT
GACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTC
AAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCG
CTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATA
TAGTTTATAAGACATAAGTCTAATAATGACATTCTGTTACATTCTTATGGTGT
ATATGAAACATAGGCTTGAAGACATTCAAGGAAAGCCGTTTAAAGCAATTCC
TCACGACCGAGTTGCCTCGGAAAACTGTTTTCAAGTCTACAAAAGGTGCACA
GGTGGAAATATAAAGATGACAAGGCGTGCACATGTCTCCGAAAAGACCAGC
AACAACCAAGCCAGGTTTATTCAATAATGATCCTTCCGCAGGTTCACCTACGG
AAGGATCATTATTGAATAAACCTGGCTTGGTTGTTGCTGGTCTTTTCGGAGAC
ATGTGCACGCCTTGTCATCTTTATATTTCCACCTGTGCACCTTTTGTAGACWT
GAAAACAG

Sample KIC69 /Gene bank Accession Number JF727841.1
TGAGACGATTAGAAGCTGAACAACAGAGAGSAATCCCCTCGCTAGTGTAGAT
AATTTATCACACTTGTGGCAGATCGCAAACGGTTCCGCTAATGCATTTCAGAG
GAGCTGACCTCAAGAAGTGGCCAGCAAGCCTCCACAGTCCAAGCTCTCCTTT
ACAACAAAGTACAAGAGAGTTGAGAATTTAATGACACTCAAACAGGCATGCT
CCTCGGAATACCAAGGAGCGCAASATGCGTTCAAAGATTCGATGATTCMCTG
AATYCTGMAATTCACATTACTTATCGCATTTCGCTGCGKTCTTCATCGATGCG
AGAGCCAAGAGATCCGTTGCTGAAAGTTGTATTATAATTTTCATAGGCCATCA
AAGCCCATGTAAAGACATTCAATAACATTCTATAGGGTATAATGATTGACAT
AGACCCTGATAGGAAAAAGATTCCATGGCCAAGTMRAGGACAGCRATGCTTT
CGCACTGSAGGTSCTCACATSCAGCAGCAGCTGAGAGGCTGACCACTTCTTCC
ATAACCCAACRGAGACTACAATAGGTGCACAGGTGGATGAAAATGAAGTCC
AGGCAGGCGTGCACATGCCCCGAAGAGCCAGCTACAACCCATCTAGAAAAC
ATAATTCAATAATGATCCTTCCGCAGGTTCACCTACGGAAGGATCATTATTGA
RTTATGTTTTCTAGATGGGTTGTAGCTGGCTCTTCGGGGCATGTGCACGCCTG
CCTGGACTTCATTTTCATCCACCTGTGCACTATTGTAGTCTCTGTTGGGTTATG
GAAGAAGTGGTCAGCCTCTCAGCTGCTGCTGGATGTGAGGACCTGCAGTGCG
AAAGCATTGCTGTCCTTTACTTGGCCATGGAATCTTTTTCCTATCAGGGTCTAT
GTCAAYCATTATACCCTATAGAATGTTATTGAATG

Sample KPM181/Genebank Accession Number KU041660.1
TGAAGACGATTAKAAGCTGAACACMGAGAGMAAWCCCCTCGCCAGWGTAK
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CTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATG
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CGGAAGGATCATTATTGAATTATGTTTTCTAGATGGGTTGTAGCTGGCTCCTG
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Sample KPM 143 Gene Bank Accession NumberKX230614.1
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Sequence 5 (KIC 60)/ Gene Bank Accession Number KU848188.1
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Sample KPG 161/Gene Bank Accession Number KJ524564.1

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Sample KIW 57/ Gene Bank Accession Number GU234142.1

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Sample KIW 109 Gene Bank Accession Number KT273366.1

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TTTGAACGCACCTTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGTG
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Sample KIW 60/ Gene Bank Accession Number KM267729.1

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Appendix 5: Morphological characterization of macrofungi isolates collected in Kereita forest

Specimen Accession Genus Number		Cap	Gills	Stipe	Forest type	Spore print	Spore
K1C 001 ***Stropharia***		5-7cm diameter , convex dry and wrinkles with age, margin entire	Attached, initially white and turn black with maturity, crowded	Whitish, upto 5cm, Veil present, attached firmly as a permanent membranous ring at the upper region of the stipe.	Indigenous	Purplish black	Smooth, ellipsoid with a germ pore
KPM 143 ***Suillus***		3-10cm diameter, convex in young specimens, broadly convex to flat in older, orange-brown (sienna to cinammon), wax & shiny texture.	Yellow – pale ochreceous, angular pores	Light yellow with a partial veil hanging on the stem from the margin, Granular small dots on the stem, base not swollen	Plantation	Brown	Smooth and sub-fusoid.
KPG 161 ***Macrolepiota***		10-20 cm diameter, young ones button like latter expanding to a flattened shape, prominent umbo , covered by white to brown shaggy scales which are more pronounced at the umbo	Free and white, closely spaced	Upto 24 cm, Cream, ring large and movable on the stem, base of the stipe approximately 3 cm	Plantation	White	Dextrinoid
KIW 57	***Pleurotus***	White cream, thin flesh, fan shaped in overlapping groups with smooth inrolled margin and covered with very conspicuous hairs (looks like oyster)	Decurrent, crowded with alternating lengths and white	Absent	Indigenous	Cream	
KIL 109	***Pleurotus***	3-5 diameter , cream – light brown, fan shaped, margin wavy in older specimens and smooth slightly inrolled in the	Decurrent, closely spaced	Short		White	

	young specimens					
KIW 060 ***Auricularia***	Flat, wavy , jelly ,rubberly like cap	-	Absent	Indigenous	White	
KIC 60 ***Agaricus/ Hymenagaricus***	Convex (umbrella) shaped, purple with dark purple scales (3-5cm diameter), dark purple area near the crown that breaks into pruple scales, violet areas between scales with white to violet peeling, white dust (partial veil) which falls off.	Brown gills. Crowded, free (not attched to stipe)	Purplish date stipe with white dust which rubs off to give brown purple stipe, club shape base (5-7cm long).Young ones covered with a veil present and no ring observed in the older specimens	Indigenous	Grey brown	
KIC 69 ***Agaricus***	White with brown scales, convex, brown centre which breaks to hairy like scales, white areas between scales, smooth and entire	Chocolate brown, free (not attached), crowded light	brown stipe with white dust which rubs off to give light brown stipe	Indigenous	Black - Grey	
KPM 181 ***Agaricus***	Cap 1-10 cm diameter, with brown and white scale , cap with velvety texture, umbrella shaped with a smooth entire margin	Free and closely spaced, with alternating lengths, Cream – grey	Smooth from the cap to the ring region, slightly rough and dirty cream below the ring	Plantation	Grey–Black	Small brown and ellipsoidal