Analytical and Mycochemical Studies on Selected Wild Growing Mushrooms of the Genus *Boletus, Russula, Lactarius* and *Termitomyces* Distributed in KwaZulu-Natal, South Africa.

By

MUVHANGO RASALANAVHO

2021

PREFACE

Analytical and Mycochemical Studies on Selected Wild Growing Mushrooms of the *Boletus, Russula, Lactarius* and *Termitomyces* Families Distributed in KwaZulu-Natal, South Africa.

MUVHANGO RASALANAVHO

2021

A thesis submitted to the school of Chemistry and Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, for the degree of Doctor of Philosophy.

This thesis has been prepared according to **Format 4** as outlined in the guidelines from the College of Agriculture, Engineering and Science which states:

This is a thesis in which chapters are written as a set of discrete research papers, with an overall introduction and final discussion where one (or all) of the chapters have already been published. Typically, these chapters will have been published in internationally recognized, peer- reviewed journals.

As the candidate's supervisor and co-supervisor, we have approved this thesis for submission.

Supervisor:

Signed: [redacted] Name: Prof. SB Jonnalagadda Date: 5/1/2021

Co-supervisor:

Signed [redacted] Name: Prof R Moodley Date: 5/1/2021

ABSTRACT

Mushrooms are a highly biodiverse group of organisms and play various roles in nature, the economy, environmental science, food science and health. They are also involved in soil mineral weathering, organic substrate decomposition and element recycling. For many centuries, edible mushrooms have been collected from forests or cultivated and consumed due to their nutritional value, medicinal utility and unique flavour. In some instances, certain inedible mushrooms are collected and used during religious ceremonies and for medicinal purposes.

Unlike green plants, mushrooms can take up large quantities of heavy and radioactive metals such as Pb, Cd, ^{134}Cs and ^{137}Cs that can pose a health risk to humans. Cases of mushroom poisoning including fatalities have often occurred after consumption of wild growing mushrooms and this problem is increasing worldwide. Research studies devoted to mushrooms revealed a lack of focus on wild growing mushrooms from South Africa as their attention is on cultivated mushrooms.

The aim of this study was to evaluate the nutritional value of six species of wild growing mushrooms, namely *Amanita pantherine, Boletus edulis*, *Boletus mirabilis, Lactarius deliciosus, Russula sardonia* (all species alien to South Africa) and *Termitomyces sagittiformis* (indigenous to South Africa), and to determine the impact of soil parameters on uptake. A mycochemical analysis was also performed on the species indigenous to South Africa to assess for medicinal benefit.

Our study revealed that absorption and accumulation of metals from the soil substrate depends on the species of the mushroom as well as the soil quality and its environment. We found that mushrooms under this study bioaccumulate minerals as follows: *Amanita pantherina*

(K>Na>Zn>Cu>Mg); *Boletus edulis* (K>Cu>Zn>Se>Na>Mg); *Boletus mirabilis* (K>Cu>Zn>Na>Mg); *Lactarius deliciosus* (K>Zn>Mg); *Russula sardonia* (K>Na>Zn>Cu>Mg) and *Termitomyces sagittiformis* (K>Cu>Si>Zn>Na). Of the six species studied, *B. edulis* was found to be rich in selenium and could contribute positively towards its recommended dietary allowance (RDA). The content of toxic metals, namely; As, Cd, and Pb, analysed in these mushroom species were found to be below the maximum permissible level for cultivated mushrooms. The moisture content of each part (pileus and stipe) of the mushrooms was within the range 90% - 95%. The dried pileus and stipe of the edible fruiting bodies of *B. edulis, B. mirabilis, L. deliciosus,* and *T. sagittiformis* contained nutrients in the following range: proteins (25% - 55%), carbohydrates (34% - 69%), ash (3% - 10%), lipid (0.8% - 5.3%) and energy (365 – 410 kcal/100 g). Correlation coefficients (r) revealed that soil parameters influenced accumulation of metals by edible mushrooms more than inedible mushrooms.

Antioxidant activities on methanolic extracts from *T. sagittiformis* were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP). The results showed the mushroom to have moderate antioxidant properties, which could be attributed to ergosterol, glycosphingolipid, oleic acid, uracil and mannitol that were the results of the mycochemical analysis. A research base is essential to position mushrooms as a superfood. This study provides the scientific basis for the health benefits of wild growing mushrooms in South Africa.

SUMMARY OF ISOLATED COMPOUNDS

Compounds A1 – A6 isolated from *Termitomyces sagittiformis*

A1 – Linoleic acid

A2 – Ergosterol

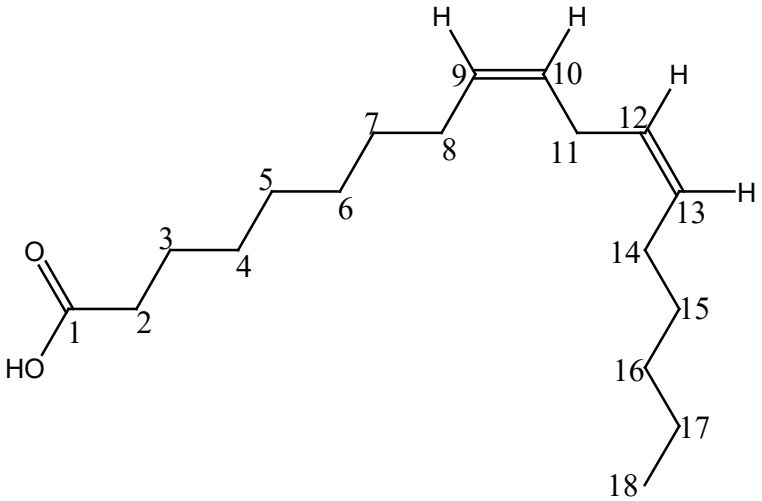
A3 – Glycosphingolipid

A4 – Mannitol

A5 – Oleic acid

A6 – Uracil

A1



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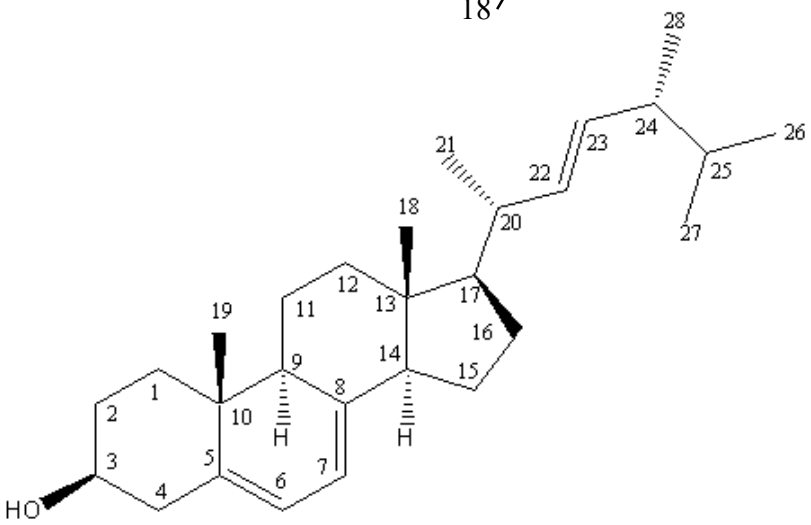

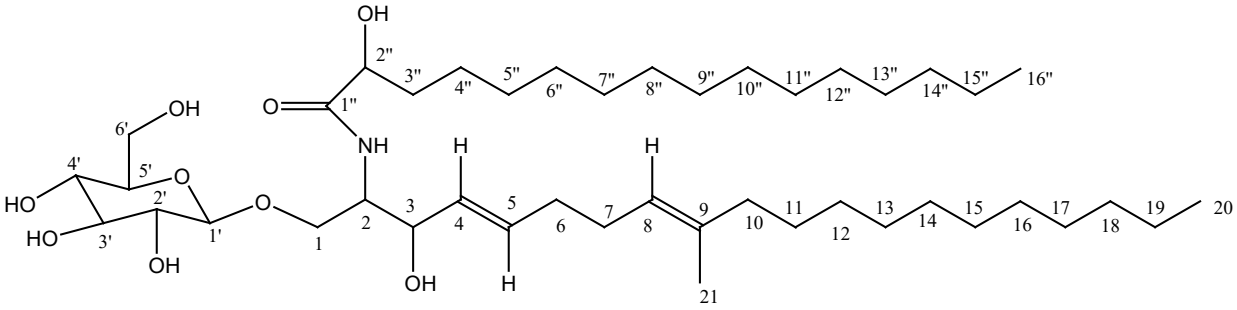

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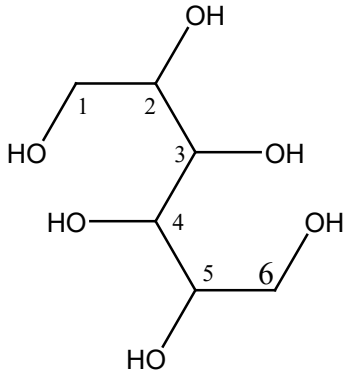

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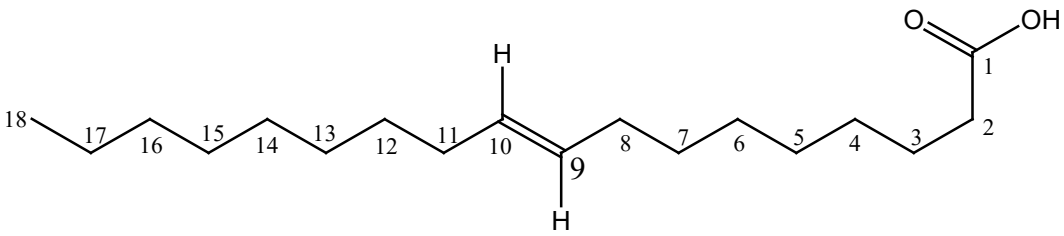

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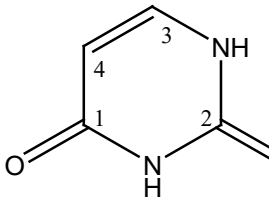


A6

ABBREVIATIONS

^{13}C NMR - Carbon-13 nuclear magnetic resonance spectroscopy

^{1}H NMR - Proton nuclear magnetic resonance spectroscopy

AIs - adequate intakes

ANOVA - Analysis of variance

BAF - Bioaccumulation factors

BCF - Bioconcentration factors

BHT - Butylated hydroxytoluene

CC - Column chromatography

COSY - Correlated spectroscopy

CRM - Certified reference material

d - Doublet

dd - Doublet of doublets

DEPT - Distortionless enhancement by polarization transfer

DPPH - 2, 2-Diphenyl-1-picrylhydrazyl

ECM - Ectomycorrhizal

FCR - Folin-Ciocalteu's phenol reagent

FRAP - Ferric reducing antioxidant potential

GC-MS - Gas chromatography-mass spectrometry

HDPE - High density polyethylene

HMBC - Heteronuclear multiple bond coherence

HSD - Honestly significant difference

HSQC - Heteronuclear single quantum coherence

ICP-OES - Inductively coupled plasma-optical emission spectrometry

IR - Infrared spectroscopy

LDPE - Low density polyethylene

m - Multiplet

MDGs - Millennium Development Goals

NCC - Na^+ - Cl^- cotransporter

NDP - National Development Plan

RDA - Recommended dietary allowance

RSA - Radical scavenging activity

PCA - Principal component analysis

PCBs - Polychlorinated biphenyls

PREM - The South African National Collection of Fungi

PTFE - Polytetrafluoroethylene

SD - Standard deviation

SLs - Sphingolipids

SOM - Soil organic matter

TLC - Thin-layer chromatography

TOF MS - Time-of-flight mass spectrometry

TPC - Total phenolic content

VAM – Vesicular-arbuscular mycorrhizal

UL - Tolerable upper intake limit

UV - Ultraviolet spectroscopy

DECLARATION 1: PLAGIARISM

I, Muvhango Rasalanavho, declare that:

(i) the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

(ii) this thesis has not been submitted in full or in part for any degree or examination to any other university;

(iii) this thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

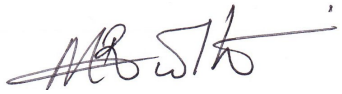
a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this thesis is primarily a collection of material, prepared by myself, published as journal articles and oral presentations at conferences. In some cases, additional material has been included;

(vii) this thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signature:  Date: 5th January 2021

Muvhango Rasalanavho

DECLARATION 2: PUBLICATIONS

Details of contributions to publications that form part and include research presented in this thesis: The research presented in the following three papers was conceptualized, planned, executed and written-up by the candidate, Muvhango Rasalanavho, who in each case is reflected as the first author. Roshila Moodley (Co-supervisor) and Sreekantha B. Jonnalagadda (Supervisor) performed the expected supervisory duties which included facilitating the performance of the research, engaging in regular discussion and deep conceptual debate with the candidate about the work and assisting in the editing of the final manuscripts.

Publication 1

Title: Elemental distribution including toxic elements in edible and inedible wild growing mushrooms from South Africa.

Authors: Muvhango Rasalanavho, Roshila Moodley, Sreekantha B. Jonnalagadda
Journal: Environmental Science and Pollution Research, 2019, 26(8), pp.7913-7925.

Publication 2

Title: Bioaccumulation of minerals and nutritional contents of five species of wild growing mushrooms from South Africa.

Authors: Muvhango Rasalanavho, Roshila Moodley, Sreekantha B. Jonnalagadda
Journal: Food Chemistry, 2020, pp.126596.

CONFERENCE

Oral Presentation

6th International Conference on Food Chemistry & Technology (FCT- 2020) – A Virtual Conference, Figure A4.2.

10th International Conference of African Materials Research Society (AMRS 2019), Figure A4.1.

In all of the publications I have collected samples, performed all the experimental work and written the manuscripts.
The co-authors were involved in discussion of the results and were responsible for verifying the scientific content and accuracy of the results as well as editing the manuscripts.

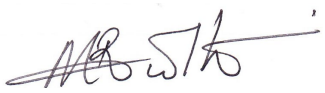
Signature.  Name: Mr. Muvhango Rasalanavho Date: 5th Jan 2021

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Issues relating to food security and environmental impact have received a lot of attention from the scientific community including the United Nations that established the Millennium Development Goals (MDGs) with food security as the first goal. The MDGs resulted in the adoption of 17 Sustainable Development Goals (SDGs) in 2015. These goals include, no poverty, no hunger, good health, quality education, gender equality, clean water and sanitation, clean energy, good jobs and economic growth, innovation and infrastructure, reduced inequalities, sustainable cities and communities, responsible consumption, protect the planet, life below water, life on land, peace and justice and partnerships for the goals (United Nations, 2015). In South Africa, to fast-track the attainment of these goal, the government implemented Vision 2030 or the National Development Plan (NDP) (Hendriks, 2013). Social grants were introduced to help mitigate the challenges of food security; however, this response fell short of its goal (Neves *et al*., 2009). The lack of nutritious foods in many underdeveloped and developing countries poses a serious threat to food security. Food security is maintained when all people at all times have access to sufficient, safe, nutritious food to keep a healthy and active life (Shaw, 2007). In many underdeveloped countries, people scavenge for food from their immediate environment due to food shortages.

Since ancient times, mushrooms have been used for medicinal value, as food and during religious ceremonies in South America, China and Europe (Miles & Chang, 2004; Miles & ShuTing, 1997). The medicinal value of mushrooms has been validated by studies that have shown them to possess anti-tumour, immunomodulating, antioxidant, cardiovascular, anti-

hypercholesterolemic, anti-viral, anti-bacterial, anti-parasitic, antifungal, detoxifying and anti-diabetic properties and can be used in the treatment of cancers (Levin & Branch, 1985; Liu et al., 2012; Miles & ShuTing, 1997; Sharma *et al*., 2018).

Because of the high level of food security worldwide, the quality and safety of food products have received significant attention. One of the fastest-growing industries in the past five decades is that of mushroom production and distribution (Eiker, 1990; Hall *et al*., 2003). At the same time, studies have also revealed mushrooms to be more vulnerable to toxins and toxic metals from the environment than vascular plants (Hall et al., 2003; Kabata-Pendias, 2011; Petkovšek & Pokorny, 2013). This poses a serious health risk due to increased industrialization in both developed and developing countries.

It is estimated that there are more than 74 000 species of mushrooms worldwide with 10% being considered poisonous (Miles & Chang, 2004; Miles & ShuTing, 1997). Though a large amount of knowledge on mushrooms is available in other countries, there is very little knowledge on edible and non-edible wild-growing mushrooms in South Africa. Knowledge on cultivated mushrooms is available, but these are highly priced and people from rural communities in the country and those living below the poverty line cannot afford them (Eicker, 1987; Jonnalagadda *et al*., 2006; Labuschagne *et al*., 2000). These people depend on wild-growing mushrooms as a food supplement and for dietary diversity.

1.1 Aims

The focus of this study was to determine the elemental uptake and distribution in six species of wild growing mushrooms, namely *Amanita pantherina* (inedible); *Boletus edulis* (edible); *Boletus mirabilis* (edible); *Lactarius deliciosus* (edible); *Russula sardonia* (inedible) and

Termitomyces sagittiformis (edible) as well as the isolation and characterisation of mychochemical compounds from the indigenous species, *Termitomyces sagittiformis* (edible).

1.2 Objectives

This research project had the following objectives:

- To determine the proximate chemical composition (protein, ash, carbohydrate and fat content) of the five-mushroom species.
- To determine and compare elemental distributions in the five mushroom species and evaluate their contribution to recommended dietary allowances (RDAs).
- To determine the bioaccumulation of metals as a function of geographical location and evaluate the impact of soil quality on uptake.
- To determine the antioxidant and antibacterial activities of the crude extract of *Termitomyces sagittiformis* and to extract, isolate and identify the secondary metabolites.

1.3 Outline of the thesis structure

Chapters are written in manuscript format, containing a mini abstract, introduction, materials and methods, results and discussion, conclusions and references.

Chapter 2 discusses the fungi, different families, mushroom and secondary metabolite families isolated from edible and nonedible species.

Chapter 3 focuses on the comparison of elemental distribution in 30 species of edible and inedible mushrooms and as well as determining the importance of edible species towards Recommended Dietary Allowances.

Chapter 4, looks at the elemental bioaccumulation and nutritional value of five species, namely *Amanita pantherina, Boletus edulis, Boletus mirabilis, Lactarius deliciosus* and *Russula sardonia,* wild growing mushrooms.

Chapter 5 is devoted to the mushroom species *Termitomyces sagittiformis* with regard to extraction, antioxidant activities, phenolic content as well as isolation and structural elucidation of secondary metabolites.

Chapter 6, integrates the work and provides conclusions and documentation of the contributions of this research. Future research possibilities are also highlighted.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The branch of science that studies fungi is called Mycology; hence people who conduct such studies are called mycologists. The mycology community believes that between 2.2 and 3.8 million fungal species exist on the earth's surface. Although the number is growing yearly, at around 2 000 species per year, so far only 144 000 species have received particular attention and several studies are being conducted on them (Willis, 2018).

The early civilizations of the Greeks, Egyptians, Romans, Chinese, and Mexicans appreciated mushrooms as a delicacy and often used them in religious ceremonies (Miles & Chang, 2004). *Auricularia auricular* was the first species to be cultivated in China as far back as around A.D. 600; hence China is regarded as the pioneer when it comes to growing mushroom for food, medicine and an incredibly rich natural diversity of fungi (Boa & Boa, 2004; Levin & Branch, 1985). Today a variety of mushroom species are cultivated worldwide, with *Agaricus bisporus* (the champignon or the button) being the species produced in large quantities. This mushroom was firstly cultivated in France around 1600 on a composted substrate (Miles & Chang, 2004).

Most fugal species grow naturally in the environment and these are collectively referred to as "wild growing mushrooms". Some of these mushrooms are edible and medicinal, while others are inedible and medicinal or toxic. This project is based on mushroom species that grow naturally in the wild during rainy seasons.

2.2 What are fungi?

Fungi are eukaryotic and filamentous groups of organisms containing an interconnected network, or mycelium, of minute, protoplasm-filled tubes called hyphae (Levin, 2013). They play a crucial role in the ecosystem through the decomposition of dead organic matter (lignocellulosic material), nutrients cycling and nutrient transporter (Mueller, 2011; Peralta et al., 2017; Walker & McGinnis, 2014).

Besides their essential role in nature, they also cause significant challenges like plant and animal diseases. Research studies are conducted with the sole purpose to mitigate the challenges fungi pose to agricultural products. For this reason, countries are very vigilant on the type of food visitors can bring to their shores as well as the movement of stock within as they can introduce unknown crop pathogens that can devastate the agricultural sector.

Fungi cannot manufacture their own food but are dependent on the host species for their nutrition (Blackwell, 2011; Hall *et al*., 2003; Willis, 2018). Unlike plants, they lack chlorophyll and vascular tissue and live by decomposing and absorbing organic matter from a dead or living source, hence are heterotrophic (Levin, 2013; Van Der Westhizen & Eicker, 1994; Walker & McGinnis, 2014). They are eukaryotes, making them distinguishable from bacteria (Levin, 2013; Smith & Moss, 1985). DNA evidence and the presence of chitin in their cell wall, makes them more closely associated with the animal than the plant kingdom (Levin, 2013; Walker & McGinnis, 2014).

Molecular evidence suggests a shared common ancestry between fungi and animals, as shown in Figure 2.1 (Blackwell, 2011; Boa & Boa, 2004; Money, 2017; Willis, 2018). Their mode of nutrition is, however, different from that of animals (absorptive rather than digestive); they first digest and then ingest (Miles & Chang, 2004). They are capable of getting their food from both

dead (saprophytes) and living (parasitic or mutualistic symbionts) organisms. Like animals, they store their food as glycogen, i.e. different from plant species which store food as starch (Levin, 2013; Watkinson *et al*., 2015). The focus of this study is on the fungal species that fall under the phyla, Basidiomycota, collectively referred to as mushrooms.

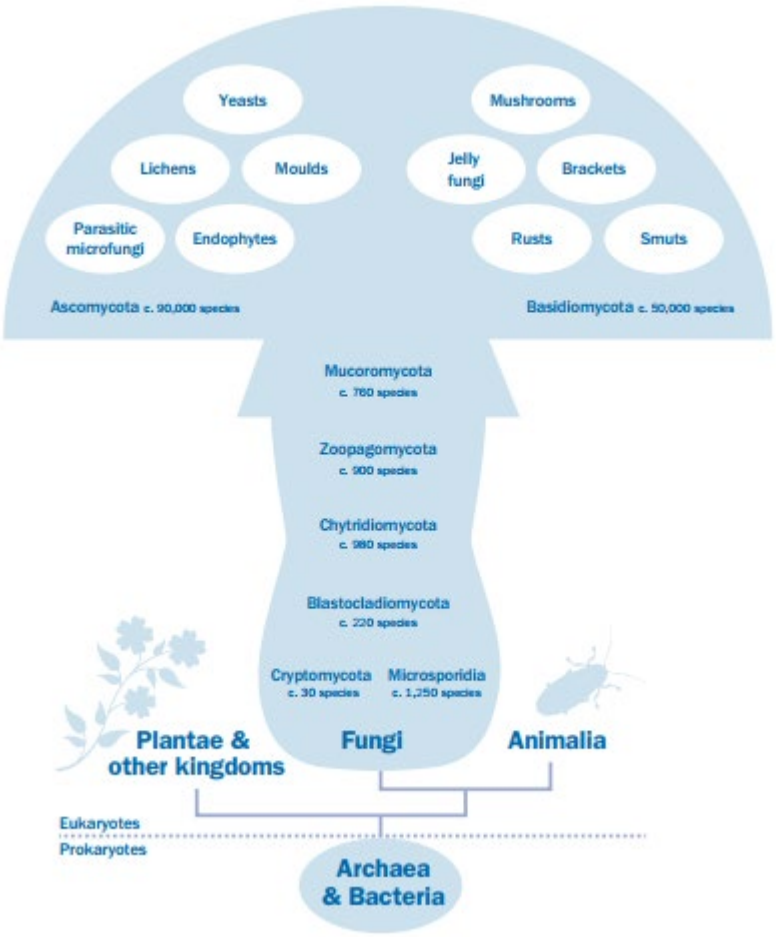


Figure 2. 1 The phylogenetic tree of the kingdom Fungi, fungal phyla (Willis, 2018).

2.3 Basidiomycota

Basidiomycota, a diverse phylum of fungi with an estimated 50 000 species, is the second-largest phyla after Ascomycota with an estimated 90 000 species (Willis, 2018). Species of the

phylum Basidiomycota are large filamentous fungi that reproduce sexually with a club-shaped spore-bearing organ (basidium), as shown in Figure 2.2, which grows from gills or tubes on the under-surface of the cap (Levin & Branch, 1985). Each basidium has two or four spores which are released and dispersed by the wind for them to germinate when conditions are favourable.

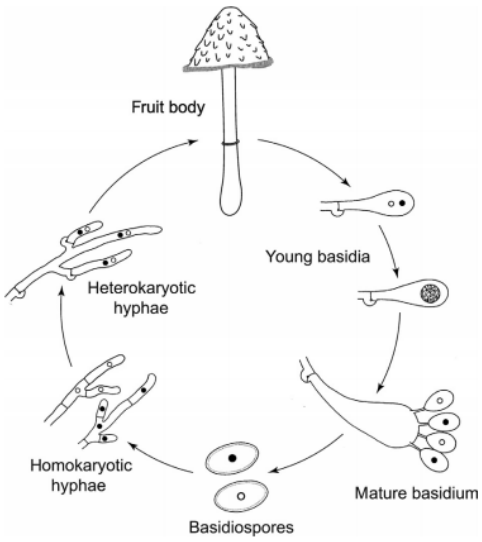


Figure 2. 2 Basidiomycota life cycle, example using *Coprinus comatus*, the lawyer's wig, showing four basidium on its gill (Watkinson et al., 2015).

2.4 Mushrooms

Mushrooms refer to the large fleshy fungi (fruiting body) with stalks (stipe) and caps (pileus), shown in Figure 2.3, which are edible or nonedible, poisonous or toxic, medicinal or/and gourmet. They are broadly defined as follows "a mushroom is a macro fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand" (Miles & Chang, 2004). Main features used to identify mushroom species is based on its morphology including the characteristic of annulus, pileus, spore print, partial or universal veil, vulva and lamellae

(Koppel, 1993; Lamaison & Polese, 2005). Commercialised edible species are derived from 18 orders of fungi and collectively making up at least 350 species, which are eaten worldwide (Willis, 2018). These species are either cultivated or collected from the wild environments. Owing to their mode of nutrition and the nature of their relationship with the host species, mushrooms are placed into three major groups, namely mycorrhizae, saprophytes and parasitic.

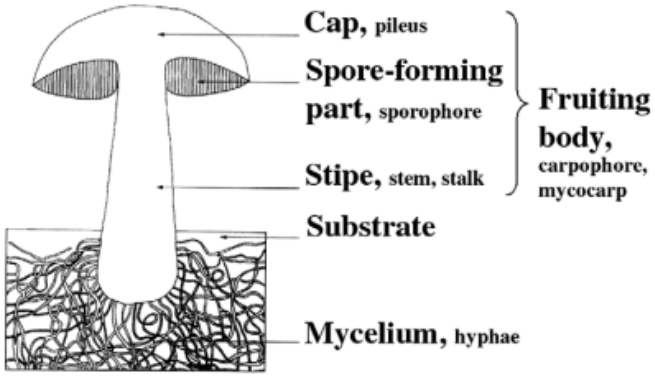


Figure 2. 3 A sketch of a mushroom and used mycological terms (Kalač, 2009).

2.4.1 Mycorrhizae mushrooms

The word mycorrhizae is derived from *myco* which means "fungus" and *rhiza* which means "root". Mycorrhizae are divided into two groups, namely vesicular-arbuscular mycorrhizal (VAM) or endomycorrhizal and ectomycorrhizal (ECM) (Hall *et al*., 2003). The VAM fungi are mostly found forming colonies with roots of most agricultural food and bioenergy crops, and their mycelium growing intercellularly in the cortex and forming intracellular vesicles and branched. The ECM only colonize plant species extracellularly throughout the plant's root system, such as pine roots (Spiteller, 2015; Watkinson *et al*., 2015). These mushrooms form symbiotic relationship with more than 90% of plant species and both fungi and the plant benefit.

Because the hyphae cover a large area, the fungi are able to absorb and provide the host plant with much needed nutrients such as phosphate, nitrogen and potassium as well as abiotic (drought, salinity, heavy metal) and biotic (root pathogens) stress resistance (Blackwell, 2011; Bücking et al., 2012; Hall *et al*., 2003; Levin, 2013; Miles & Chang, 2004; Watkinson *et al*., 2015). In return the fungi receive carbohydrates from the root plant. Therefore, both ecologically and economically benefits are met.

Well-known examples include the truffles (for example, *Tuber*), matsutake (*Tricholoma matsutake*), porcini (*Boletus edulis*), chanterelle (*Cantharellus cibarius*), and the saffron milk cap (*Lactarius deliciosus*) (Hall *et al*., 2003). The host plant for ectomycorrhizal fungi include birches (Betulaceae), oaks and beeches (*Fagaceae*), eucalypts (*Myrtaceae*), pines and spruces (*Pinaceae*), poplars and willows (*Salicaceae*), rock roses (*Cistaceae*), and dipterocarps (*Dipterocarpaceae*) (Hall *et al*., 2003). Others form symbiotic relationship with termites as they emerge from termite hills, e.g. *Termitomyces* species and *Podaxis pistillaris.*

2.4.2 Saprophyte mushrooms

This type of mushroom obtains the nutrients from dead organic material, e.g. dead tree trunk, animal dung, agricultural crop residue (Van Der Westhizen & Eicker, 1994). They therefore fulfil the important ecological role in nature of returning minerals and humus back to the soil (Hall *et al*., 2003; Willis, 2018). Saprophytes can grow on selected habitat or substrate, namely, fresh wood residue, slightly composted lignocellulose, well composted material or an animal dung, soil and humus, dead grass and straw as well as dead wood of specific tree species (Levin & Branch, 1985; Miles & Chang, 2004).

Based on their substrate of choice and the target part of the plant (i.e. cellulose, hemicellulose and lignin), they are classified as either brown or white rot (Miles & Chang, 2004). Some well-known families of saprophytes mushroom include, *Lentinula, Pleurotus, Flammulina, Auricularia, Pholiota, Tremella, Agrocybe, Ganoderma, Volvariella, Stropharia, Coprinus, Lepiota, Leptista, Morchella, Gyromitra, Cora galapagoensis, Gymnosporangium, Pseudofibroporia* and *Pleurotus* (Miles & Chang, 2004; Willis, 2018).

2.4.3 Parasitic mushrooms or pathogens

This type of mushroom obtains the nutrients from living plants and animals, causing harm to the host. They can damage crops and cause severe damage to the host species, limit growth and fruiting ability (Callan, 2003). The parasitic fungi benefit from the host by getting nutrients without providing anything, often causing severe damage to the host in the process. Some examples of parasitic mushrooms include the honey mushroom (*Armillaria mellea*) and some bracket and oyster mushrooms (*Pleurotus eryngii* and *Pleurotus ostreatus*) (Hall *et al*., 2003).

2.5 Mushrooms in this study

The current study looked at six species of Basidiomycetes fungi which includes: *Amanita pantherine* (inedible); *Boletus edulis* (edible); *Boletus mirabilis* (edible); *Lactarius deliciosus* (edible); *Russula sardonia* (inedible); and *Termitomyces sagittiformis* (edible).

2.5.1 The genus *Amanita*

The genus *Amanita* contains most species that contributes to cases of severe poisoning, causing degeneration of the cells of the internal organs; directly affecting the nervous system and gastro-intestinal upsets (Levin & Branch, 1985). The species *A. phalloides* (death cap), *A. pantherine* (panther), *A. muscaria* (fly agaric), *A. virosa* (destroying angel), *A. verna* (the fool's mushroom), *A. foetidissima* (stinker) are the most dangerous species known. *Amanita phalloides* is the species that contributes to most fatalities after ingestion and the deadly poison is associated with three groups of toxins, namely amatoxins (bicyclic octapeptides), phallotoxins (bicyclic heptapeptides), and virotoxins (monocyclic peptides) (Bresinsky & Besl, 1990; Garcia *et al*., 2015; Vetter, 1998; Wong & Ng, 2006). Amatoxins inhibit RNA polymerases (especially polymerase II) while phallotoxins and virotoxins stimulate the polymerization of G-actin and stabilize the F-actin filaments (Garcia *et al*., 2015). Active toxins present in the panther cap (*A. pantherina*) are ibotenic acid and muscimol (Satora *et al*., 2006).

2.5.1.1 *Amanita pantherina*

Amanita pantherina, also known as panther cap, warted agaric and tiersampioen in Afrikaans, falls under the class, Agaricomycetes, family, Amanitaceae and genus, Amanita. This mushroom species is widely distributed in pine, gum and oak forests. This mushroom is poisonous and cause nausea, vomiting, loss of consciousness and even death.

Cap: It is about 5 – 13 cm in diameter, fleshy, hemispherical and at maturity it flattened with margins turning upwards, even and striate, see Figure 2. 4 (b). Surface smooth, sticky and pale greyish brown at first and later smoky brown, yellowish brown or brownish olive and covered

with large, white, floccose warts (remains of universal veil), often in concentric rings, see Figure 2. 4 (a). The cap is very fragile and feels/looks very damp.

Stipe: The stipe/stem is central, widening towards the basal base bulb (volva), white, brittle, solid at first, hollow at maturity and about 7 – 13 cm long x 1 – 2 cm wide, see Figure 2. 4 (d). It has a tattered yellowish white ring/skirt, with double margins, persistent and membranous without striae or grooves and attached high up on stipe, see Figure 2. 4 (b & d). Basal bulb has one or two white belt-like rings above it and closely wrapped by the volva which forms a distinct free rim, see Figure 2. 4 (a).

Lamellae: The spore bearing organs are called gills and are underneath the cap, free, crowded thin and white in colour, see Figure 2.4 (d).

Flesh: The flesh is thin, firm, white, unchanging with odour and a taste of a raw potato.

Spore print: The spore print is white while the spores are hyaline, broadly ovoid, smooth, thin-walled, non-amyloid and 9 – 10.5 x 6.2 – 8 µm (Branch, 2001; Lamaison & Polese, 2005, Levin & Branch, 1985; Van Der Westhizen & Eicker, 1994).

A.  **B.**  **C.**  **D.** 

Figure 2. 4 Sectional views of the mushroom species, *A. pantherina:* A. Juvenile mushroom showing whites warts, reminiscence of the universal veil, B. Fully grown fruiting body, C. Cap of fully-grown mushroom showing warts and brown centre and D. Matured mushroom showing white gills, volval ring (skirt) on the fragile stipe and basal bulb.

A literature review shows substantial amounts of work done internationally on the elemental concentrations of this species (Table 2.1). These studies include those conducted in Finland, France, Japan and Poland. A study carried out in South Africa was only devoted to toxins in the species (Hallen *et al*., 2002). Other studies conducted in Central Bohemia only looked at specific elements such as gold (Au) which was found to be 27.1 μg kg^{-1} (Borovička *et al*., 2010).

2.5.2 The genus *Boletus*

The genus *Boletus* contains many species which are edible and very nutritional. The most sought-after species is the mushroom hunter's delight, the *Boletus edulis*, (porcini or king bolete or cèpe), which is expensive at international supermarkets and restaurants (Joubert, 2012). In South Africa, this species is collected from the vast pine plantations [Figure 2.5 and Figure A1.1 (c)], situated in Mpumalanga, KwaZulu-Natal and Eastern Cape Provinces. Being an alien mushroom introduced in South Africa through pine plantations, majority of South Africans do not consume these mushrooms, as a result, *Boletus edulis* mushrooms harvested in this country are generally exported to international markets.

2.5.2.1 *Boletus edulis*

Boletus edulis is an ectomycorrhizal fungus, meaning that the mycelia of the fungus infest the roots of the host plant, forming a symbiotic relationship with it, especially pine and spruces at higher altitudes; oak and chestnut at lower altitudes. It is also called eetbare boleet or sep in Afrikaans and other names used includes Penny Bun, porcini or king bolete or cèpe. *B. edulis*

is edible, dubbed the mushroom hunter's delight, and harvested commercially for export (Figure 2. 5). In addition, it is used as a medicinal mushroom for healing dog bites and preventing freckles (Levin & Branch, 1985).

Cap: It is about 5 – 25 cm in diameter, hemispherical in juvenile, with a smooth thick, fleshy and rounded surface which is dry but tends to be greasy at maturity. It is mostly fawn to a dull light brown or buffy brown to reddish or chestnut-brown in colour, hemispherical at first expanding to shallow convex; margin smooth and projecting slightly beyond the tubes.

Stipe: The pale stipe/stem is central, swollen, barrel-shaped or club-shaped or more or less cylindrical and about 7 – 19 cm long x 1.5 – 5.5 cm at apex, swelling towards the base. The base is smooth, hard, firm, solid, becoming spongy with age. It has a network of raised white threads over its upper part. There is no visible ring on the stem.

Tubes: The spore bearing organs are tubes underneath the cap instead of gills, very close but free, depressed around stipe and long, 2.5 cm deep, creamy turning yellow on maturity and eventually becoming olive- coloured.

Flesh: The flesh is white, solid and firm with no distinct smell and does not discolour on bruising or when cut.

Spore print: The spore print is olive brown while the spores are pale olive-brown, fusiform, smooth and 15 – 17 x 4 – 5 µm (Branch, 2001; Lamaison & Polese, 2005, Levin & Branch, 1985; Van Der Westhizen & Eicker, 1994).

Several studies conducted Internationally (China, Croatia, Finland, France, Italy, Mexico, Norway, Poland, Portugal, Spain, Sweden, Turkey and USA) on *B. edulis* with respect to elemental concentration shows that the species is an excellent accumulator of Ca, Fe, K, Mg, Mn, Se and Zn, as shown in Table 2.1.

Other research studies conducted on *B. edulis* samples collected from Italy and Poland showed that it can be used as a bioindicator for pollution with radioactive elements. In other studies, ^{137}Cs & ^{40}K, mercury, selenium and ^{90}Sr, were exclusively determined from samples collected at several places in Mexico, Poland and the north of Portugal, wherein species was found to contain an average of 2.0 – 5722 Bq kg^{-1} ^{137}Cs, 51 – 1500 Bq kg^{-1} ^{134}Cs, 77 – 912 Bq kg^{-1} ^{40}K, 1.2 – 7.6 mg kg^{-1} Hg, 14.9 mg kg^{-1} Se and 0.33 Bq kg^{-1} ^{90}Sr dry biomass (Cocchi et al., 2017; Costa-Silva *et al.*, 2011; Falandysz *et al*., 2007; Falandysz *et al*., 2015; Gaso *et al*., 2000; Saniewski *et al*., 2016, Szántó *et al*, 2007).

Figure 2. 5 Collection of *Boletus edulis* species destined for European markets: A. Different Grades of *Boletus edulis,* juvenile is Grade 1 while matured can be Grade 2 (tubes underneath the cap whitish in colour) or Grade 3 (tubes underneath the cap greenish in colour) B. Seasonal workers collecting the mushroom *B. edulis* into crates.

Some edible *Boletus* species found in South Africa include *Boletus aestvalis* (Oak Bolete), *Boletus mirabilis* (Admirable bolete), *Chalciporus piperatus* (Peppery Bolete), *Gyroporus castaneus* (Chestnut Bolete), *Leccinum duriusculum* (Poplar Bolete), *Phlebopus sudanicus* (Bushveld Bolete), *Suillus bellini* (Pine Bolete), *Suillus bovinus* (Cow Bolete), *Suillus granulatus* (Granular Stalk Bolete), *Suillus luteus* (Slippery jack), *Xerocomus badius* (*Boletus* badius) and *Fistulina Africana*. (Levin & Branch, 1985; Van Der Westhizen & Eicker, 1994).

2.5.2.2 *Boletus mirabilis*

Unlike *B. edulis*, *Boletus mirabilis* is a saprophyte fungus found on decaying stumps of pine trees. This means it decomposes the dead stump. It is also known as the admirable bolete, the bragger's bolete, and the velvet top.

A.  **B.**  **C.** 

Figure 2. 6 *Boletus mirabilis* species: A. growing on a decaying stump. B. velvet dark reddish-brown cap showing crowded whitish tubes on the underneath the cap B. stipe, thick and reddish-brown with long narrow reticulations.

Cap: It is about 5 – 15 cm in diameter, smooth thick, fleshy, dry and rounded velvet surface. It is dark reddish-brown to a chestnut chocolate brown sheen colour, as shown in Figure 2.6 A.

Stipe: The stipe/stem is central, yellowish with brown reticulations and about 5 – 12 cm long x 1.2 – 1.9 cm at apex, thinner towards the base as shown in Figure 2.6 (B) and (C). There is no visible ring on the stem.

Tubes: The spore bearing organs are tubes underneath the cap, 2.5 cm long, crowded, almost lime green when cut horizontally.

Flesh: The flesh is white, solid and firm with no distinct smell.

Spore print: The spore print is olive-brown while the spores are spindle-shaped to roughly elliptical. Smooth and 18 – 22 x 7 – 9 μm (Levin & Branch, 1985).

No data on the elemental concentrations in *B. mirabilis* was available, as such, this is the first report on the species.

Table 2. 1 A review of elemental analysis carried out on the species chosen for this research project

Country	Species	Analyte Concentration in mg kg^{-1}																	Ref.
		Ag	Al	As	Ca	Cd	Co	Cr	Cu	Fe	Hg	K	Mg	Mn	Na	Ni	Pb	Se	Zn	
China	BE				111-468	7.7-85.4	1.1-7.2	18-161	24-53	412-1550			421-655	15-37	60-2720	14-42			73-129	A
	LD			0.8	247	1.9		4	1.3	197			1244	23.1			0.9		52.3	B
Croatia	BE					1.9		1.9			2.4					2.4	1.3			C
Finland	BE	16		0.1	44	3.4	0.08	0.01	42.2	27.5		23840	715	6.9	519	1.8	0.3	18.5	91.6	D
France	AP	8.1	33.1		1650	10.3	2.7	1.5	38.5	136	64.9		573	16.9		8.9	33.2	38.8	116	E
	BE	2.7	68.7		1310	1.4	1.9	1.3	14.9	163	40.6		752	59.1		5.4	21.2	31.2	55.4	
	AP	8.1	61.2		1750	11.4	1.8	1.3	45.4	183	53.4		654	21.6		6.6	27.6	30	128	F
	BE	4.3	36.4		1930	1.9	2	2.1	14.2	113	46.6		827	71.1		7.2	27.1	27.4	77.2	
Greece	LD					0.2			6.9	29.8				5.7		0.2	0.1		81.1	G
Italy	BE			0.1		4					2.7						1.2	30.8		H
Japan	AP		556		254							26800	721		197					I
Mexico	BE		1200	3.8	260	0.7	0.6		54.1	340		14440	300	20			2.9	1.3	172.7	J
	RS			3		1.8	1.4		143.6								3.3	1.3	143.1	
Norway	BE					2.4-120			20-290		0.4-15								120-450	K
Poland	AP		416			19.4				105				15.2			2.3		175	L
	BE				273				31	85.2		24000	700	16	351				155	M
	LD				400				19.3	190		29000	1100	41	64				160	
	BE				200	14			36	47		32000	850	8.6	190				210	N
	BE	7.6	43		100	18	0.22	0.3	57	61	2.3	20000	890	21	400		2	32	210	O
	BE				92	5.5			54	110				14					210	P
	BE		79		94	5.5			47	79	4.9	29000	960	17	220				190	Q
	BE				39				62	50		34000	1100		180				290	R

Country	Species	Analyte Concentration in mg kg^{-1}																	
		Ag	Al	As	Ca	Cd	Co	Cr	Cu	Fe	Hg	K	Mg	Mn	Na	Ni	Pb	Se	Zn	Ref.
	BE					4.3	1.1		10.3		1.7					1.8	1		51.6	S
	LD					2.4	0.4		14		1					0.9	1.3		88.7	
	LD				500	3.6		2.6	17.7			38800	800			1	6.4		124.2	T
	RS				300	13.8		3.5	35.8			37800	700			1.2	7.8		115.5	
	BE		13		55	3.3			25			25000	800		190					U
Portugal	BE				350				44	67		38860		14			2.7		172	V
	LD				250				11	222		26000		24			2.2		87	
Serbia	LD					0.5	0.1	1.1	15.5	217				6		1.6	0.4		123.6	W
Spain	BE								52										63.7	X
	LD								18.6										152	
	BE					1.2														Y
	LD					0.4														
Sweden	BE	1.1			200	2.4			34	63		28000	820	5.4	190		6.7		80	Z
Turkey	BE					1.1			20.1	764				96.3				9.9	158	AA
	LD					0.5		4.2	10.6	146				16.8		2.5	0.4		68.9	AB
	LD	0.02	13.6	0.1	124	0.03	0.04	0.4	0.02	7.6		75.6	13.2	0.36	4	0.2	0.02	0.1	0.56	AC
	LD					0.4	2.3		47	493				12		3.8	3		88	AD
USA	BE						3.8		104	5841						54.9		16.3	186	AE

Where: AP – *Amanita pantherina*, BE – *Boletus edulis*, LD – *Lactarius deliciosus*, RS – *Russula sardonia*.

A (Su *et al*., 2018); B (Xu *et al*., 2019); C (Širić *et al*., 2016); D (Nikkarinen & Mertanen, 2004); E (Michelot *et al.*, 1998); F (Siobud-Dorocant *et al.*, 1999); G (Aloupi *et al.*, 2012); H (Cocchi *et al.*, 2017); I (Yoshida & Muramatsu, 1998); J (Gaso *et al*., 2000); K (Collin-Hansen *et al.*, 2005); L (Rudawska & Leski, 2005); M (Brzezicha-Cirocka *et al.*, 2019); N (Brzezicha-Cirocka *et al.*, 2016); O (Falandysz et al., 2008); P (Falandysz *et al*., 2011); Q (Frankowska *et al.*, 2010); R (Falandysz *et al*., 2001); S (Mleczek *et al*., 2013); T (Pająk *et al*., 2020); U (Zhang *et al*., 2010); V (Carvalho *et al.*, 2005); W (Kosanić *et al.*, 2016); X (Alonso *et al.*, 2003); Y (Melgar *et al.*, 2016); Z (Mędyk *et al.*, 2017); AA (Tuzen *et al.*, 2007); AB (Yamaç *et al.*, 2007); AC (Konuk *et al.*, 2007); AD (Yilmaz *et al.*, 2002); AE (Wuilloud *et al.*, 2004).

2.5.3 The genus *Lactarius*

The genus *Lactarius* contains species that are edible and have neither a ring nor volva (Levin & Branch, 1985). Two species that are found in South African pine plantations are the edible ectomycorrhizal fungi *L. deliciosus* (The Saffron milk cap or Pine ring) and *L. hepaticus* (Plowright apud Boudier) (Lamaison & Polese, 2005; Van Der Westhizen & Eicker, 1994).

2.5.3.1 *Lactarius deliciosus*

Lactarius deliciosus, shown in Figure 2.6 is also called Pine ring or Saffron Milk Cap, and Orange Melkswam in Afrikaans. It grows on humus under pine trees and found in large groups.

Cap: It is 3 – 15 cm in diameter, convex at first, then flattening and opening into a wide funnel. The cuticle has concentric rings of darker patches or greyish against the bright orange background, as shown on Figure 2.7 (a). Turns green with age or bruising. The margins are overhanging but flattens out with age.

Stipe: The stipe/stem is 3 – 9 cm long x 1.5 – 4 cm, central, generally short and sturdy, attenuating at the base. Carrot-coloured, flecked with green. It becomes hollow and soft from top to bottom. The upper part is covered with whitish colour and at the bottom it is pitted (like scars) with bright orange crevices against and orange background, as seen on Figure 2.7 (b). If cut horizontally, it shows a distinct orange outer ring. It has no ring or skirt on the stipe.

Lamellae: The spore bearing organs called gills are underneath the cap, orange in colour, crowded and fragile, decurrent, unequal in length and forked at the base. With maturity, they gradually show patches of green, as shown in Figure 2.7 (c).

Flesh: The flesh ranges from white to pale orange and is firm and brittle. When broken or squeezed it reveals a bright orange milk which darkens on contact with air.

Spore print: The spore print is white to pale ochre, see Figure 2.7 (d) while the spores are hyaline, ellipsoidal, covered by a network of thin ridges, 7 – 9 x 6 – 7 µm (Branch, 2001; Lamaison & Polese, 2005, Levin & Branch, 1985; Van Der Westhizen & Eicker, 1994).

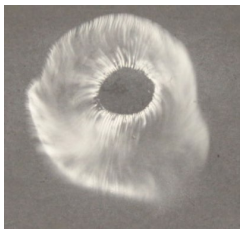
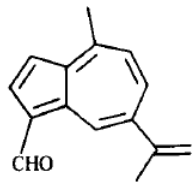
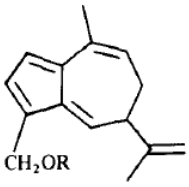
A.  **B.**  **C.**  **D.** 

Figure 2. 7 Different views of the mushroom species, *L. deliciosus*, A. Cap showing concentric zones and depressed centre, B. stipe with spotted orange, C. Decurrent, forked lamellae (gills) and D. Spore print

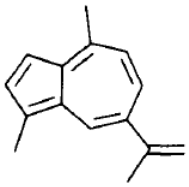
Elemental concentration studies conducted on *L. deliciosus* in China, Greece, Poland, Portugal, Serbia, Spain and Turkey have shown this mushroom species to be an excellent accumulator of Ca, Fe, K, Mg and Zn, as presented in Table 2.1. Species of the genus *Lactarius* contain only one sesquiterpenes if undamaged, while injured fruiting bodies contains five sesquiterpenes (lactaroviolin, lactarazulene, lactarofulvene, delicial and deterrol) shown in Figure 2.8. (Bergendorff & Sterner, 1988). Besides these compounds, a polysaccharide, LDG-A, with suspected antitumor activities has been isolated from *L. deliciosus* and its proposed structure is given in Figure 2.9 (Ding *et al.*, 2012).



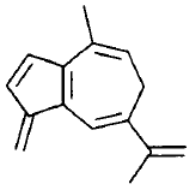
Lactaroviolin



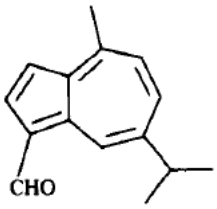
Alcohol, R = H
Acid ester, R = $CO(CH_2)_{16}Me$



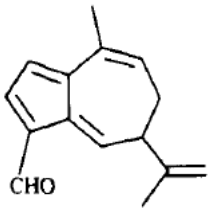
Lactarazulene



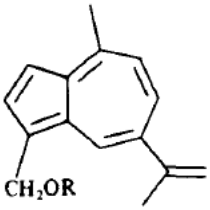
Lactarofulvene



Aldehyde



Delicial



Deterrol, R = H
Acid ester, R = $CO(CH_2)_{16}Me$

Figure 2. 8 Sersquiterpenes isolated from the injured species of *L. deliciosus* and *L. deterrimus*

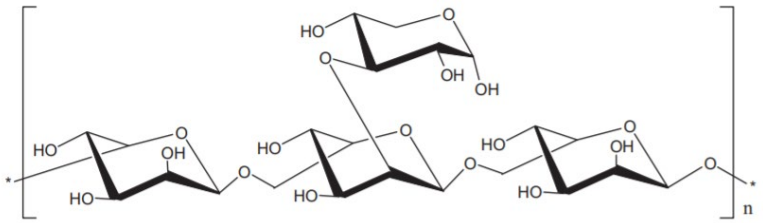


Figure 2. 9 The polysaccharide, LDG-A isolated from *L. deliciosus*

2.5.4 The genus *Russula*

Similar to *Lactarius*, the genus *Rassula* neither has a ring nor volva and both belong to the same order (Russulales) and family (Russulaceae) (Bresinsky & Besl, 1990). They have a peppery effect that causes flow of tears when being cleaned as well as stuffing the nostrils. Most species are considered inedible because of the pungent and bitter taste, but some people do consume them after parboiling. *Russula* species are not indigenous to South Africa as they only appeared after the planting of pine trees. Their cap of this genus can be purple, lilac or green in colour. Species found includes *R. caerulea, R. capensis, R. sardonia* (purple-stemmed), and *R. sororia* (Levin & Branch, 1985; Van Der Westhizen & Eicker, 1994).

2.5.4.1 *Russula sardonia*

Rassula sardonia (called Perssteelrooimus in Afrikaans) has a purplish colour of the stipe and by the deep greyish-purplish colours of the cap, see Figure 2.10. When tested fresh, it brings out a hot, acrid taste to the tongue. When cleaning this species, it produces a pungent smell that creates stuffy noise and its peppery feature results in tears in the eyes while cleaning with a brush. It forms mycorrhizae with pine trees and found in large groups.

Cap: It is 4 – 12 cm in diameter, fleshy, thick, at first convex with margin wavy, faintly striate and somewhat incurved in the centre, later flat or somewhat depressed with margin upturned and splitting at maturity. Surface smooth, matt, dry or slightly sticky when wet, deep rose-pink, greyish magenta to greyish ruby or blackish purple. Colour often fades as a result of rain.

Stipe: The stipe/stem is 3 – 9 cm long x 1 – 2 cm, central, expanding at the apex and occasionally towards the rounded base, crisp, apple like texture flattened and elliptical in cross section. It has no ring or skirt on the stipe.

Lamellae: The spore bearing organs called gills are underneath the cap, close, adenaxed, crowded and lightly decurrent, forked near the stipe, brittle, edges thin, whitish to pale yellow and golden with maturity. With maturity, they gradually show patches of green.

Flesh: The flesh is thick, firm, creamy white with a faint greenish tinge, unchanging, with a faintly fruity odour and hot acrid taste.

Spore print: The spore print is pale cream while the spores are pale ochreas cream, ovoid, covered with warts and fine network of lines, amyloid, 6.5 – 8.5 x 6 – 7.5 µm (Levin & Branch, 1985; Van Der Westhizen & Eicker, 1994).

A.  **B.** 

Figure 2. 10 Different views of the mushroom species, *R. sardonia*, A. Cap showing concentric zones and depressed centre, B. stipe with spotted orange and Decurrent, forked lamellae (gills).

As seen from Table 2.1, only two studies (from Mexico and Poland) were conducted on *R. sardonia* mushroom species.

2.5.5 The genus *Termitomyces*

The genus *Termitomyces*, first described in 1942 by Roger Heim, is an agaric mushroom cultivated by termites, hence they form symbiotic or mutualistic relationship with termites (Pegler & Vanhaecke, 1994; Van Der Westhizen & Eicker, 1994; Van der Westhuizen & Eicker, 1990; Wei *et al,*, 2009). Termites manage them in their nest as "fungus gardens" whose responsibility is to degrade lignin and cellulose of plant material for termites to use as food, as well as providing much needed enzymes and vitamins. Species of *Termitomyces* are edible and sought-after in Africa, Asia and China. Morphologically, they are very different from each other with *T. titanicus* having the largest pileus, measuring more than 1 m in diameter.

Some of the well-known species include, *T. clypeatus, T. microcarpus, T. sagittifomis, T. schimperi, T. striatus, T. umkowaani, T. reticulatus* (all seven species are found in South Africa), *T. titanicus* (from Zambia), *T. singidensis* (from Tanzania), *T. bulborhizus*, *T. entolomoides, T. tylerianus, T. heimii, T. mammiformis, T. aurantiacus, T. eurrhizus, T. globulus, T. badius, T. cylindricus, T. fuliginosus, T. le-testui, T. medius, T. radicatus, T. spiniformis, T. striatus, T. indicus, T. albiceps, T. perforans, T. lanatus* (from China and India) just to name the few (Borkar *et al.*, 2015; Karun & Sridhar, 2013; Pegler & Vanhaecke, 1994; Tibuhwa, 2012b; Van Der Westhizen & Eicker, 1994; Van der Westhuizen & Eicker, 1990; Wei et al., 2009).

2.5.5.1 *Termitomyces sagittiformis*

Termitomyces sagittiformis, also known as “Inkowankowane” in the local isiZulu language. This mushroom species is found scattered in groupes on soil over nest of the wood-destroying termite *Odontotermes latericius,* under Allamanda plants, as shown in Figure 2.11 (e). It has a smooth, somewhat shiny greyish-brown cap, acutely pointed perforatorium centre as shown in Figure 2.11(b).

Cap: The pileus part of the mushroom is 2.5 – 6.5 cm in diameter, conical at first and acutely pointed perforation as shown in Figure 2.11 (a) uneven to almost lobate margins, incurved and at maturity it cracks or split radically and concentrically, as in Figure 2.11 (b).

Stipe: The stalk is central, 6 – 12 mm x 10 – 39 mm above soil level, narrowing downwards into a long pseudorrhiza, no annulus or ring as shown in Figure 2.11 (d)

Lamellae (Gills): Free or slightly adnexed, crowded, thin edges and cream white becoming buffy pink, becoming irregularly crenulate to lacinate, as shown in Figure 2.11 (c).

Flesh: Creamy white, unchanging, firm, thin with a faint mushroom odour.

Spore print: ochraceous pink to buffy pink.

Spores: hyaline, ellipsoidal, smooth, apiculate, thin-walled, non-amyloid 7.4 -10.1 x 5.8 - 6.4 µm (Van Der Westhizen & Eicker, 1994; Van der Westhuizen & Eicker, 1990; Vrinda & Pradeep, 2009).

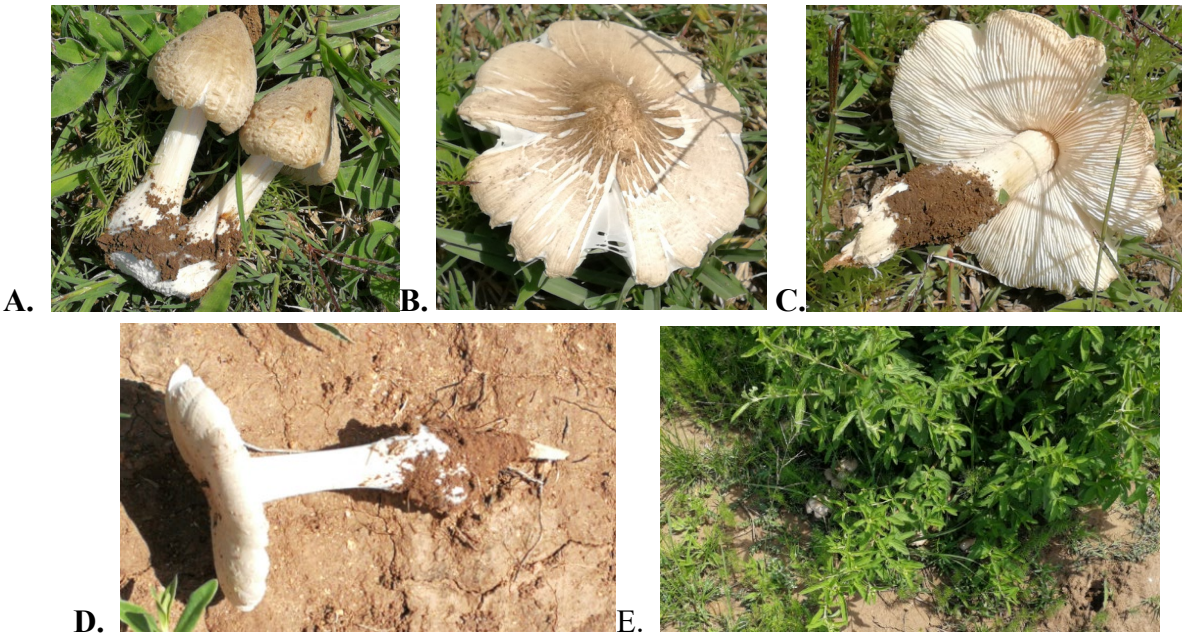


Figure 2. 11 Different views of the mushroom species, *T. sagittiformis*, A. juvenile mushroom, showing both the conical cap and stipe B. fully grown mushroom showing a smooth cracked cap with the centre of the cap acutely pointed and brownish, C. lamellae (gills) crowded, free or slightly adnexed, full and intermediate lengths, thin and creamy white. D. Matured cap showing uneven margins incurving and a stipe which is bulbous at the base and then narrowing downwards. E. *T. sagittiformis* under Allamanda plants.

As seen from Table 2.1, no elemental nor biological activities of the extracts from *T. sagittiformis* has so far been conducted. This study represents the analysis carried out on this species. Research studies conducted on certain *Termitomyces* species have shown certain ethnomedicinally beneficial for the treatment of illnesses (Hsieh & Ju, 2018). The medicinal benefits associated with species of *Termitomyces* are owed to several secondary metabolites isolated from certain known species, some of which are shown in Table 2.2 and hence their consumption provides healthy dietary supplements and treatment of certain illnesses.

Table 2. 2 Heath benefits associated with certain species of the genus *Termitomyces*

Species	Health Benefit	References
T. albuminosus	Improving brain and stomach functions and curing hemorrhoids	(Qi *et al*., 2000; Ying, 1987)
T. aurantiacus	Alleviate gastrointestinal problems, such as abdominal pain, constipation, stomach ache and ulcers.	(Tibuhwa, 2012a)
T. clypeatus	Antiproliferative activity against growth cells of a human liver cancer cell line	(Majumder *et al*., 2016)
T. eurrhizus	Curing of rheumatic disorder and diarrhea, lowering high blood pressure, alleviate gastrointestinal problems, such as abdominal pain, constipation, stomach ache and ulcers.	(Sachan *et al.*, 2013; Tibuhwa, 2012a)
T. heimii	Treatment for fever, cold, and fungal infections as well as blood tonics during wound healing and blood coagulation	(Venkatachalapathi & Paulsamy, 2016)
T. letestui	Alleviate gastrointestinal problems, such as abdominal pain, constipation, stomach ache and ulcers.	(Tibuhwa, 2012a)
T. microcarpus	Treatment for fever, cold, and fungal infections (gonorrhoea), boosting immune system for people with long illness, possessed cytotoxic activities against most tumor	(Venkatachalapathi & Paulsamy, 2016)
T. robustus	exhibited immunomodulatory properties in significantly activating macrophages, splenocytes and thymocytes. Can inhibit growths of human pathogenic microbes	
T. striatus	Enhancing the immune system.	
T. titanicus	Alleviate gastrointestinal problems, such as abdominal pain, constipation, stomach ache and ulcers	(Tibuhwa, 2012a)

2.6 Mushrooms as nutraceuticals

Nutraceuticals are foods that provide medical or health benefits, including the prevention and treatment of disease. Human beings have used mushrooms as a source of food and medicine for many centuries and some species played a role in traditional ceremonies.

2.6.1 Nutritional value of mushrooms

Edible mushrooms, either cultivated or collected from the wild during rainy seasons are known to contain about 90% moisture and appreciable amounts of proteins, dietary fibre, carbohydrates, vitamins, ash and much lower amount of lipid. The crude protein found in mushrooms rank below that of meat but it is higher than what is found in most other foodstuff and moreover contains all nine essential amino acids required by humans (Doelle *et al.*, 2009). Substantial amounts of research have been conducted to determine the nutritional value of edible cultivated or collected species of mushrooms (Alam *et al*., 2008; Ayodele & Okhuoya, 2009; Heleno *et al.*, 2009; Heleno *et al*., 2015; Kalač, 2009; Wang *et al*., 2014). The most nutritive mushrooms rank alongside meat and milk in potential nutritive value and considerably higher than most legumes and vegetables (Miles & Chang, 2004).

They also absorb minerals from the soil solution, accumulated in various parts of the mushroom as well as exchanged between vascular plants, especially in symbiotic mycorrhizae (Whiteside *et al*., 2019). These amounts are dependent on the species of mushroom as well as the environmental conditions, which include mushrooms' substratum, atmospheric conditions, age and part of the fructification (Alam *et al*., 2008). It is now generally understood that mushrooms translocate nutrients from where there is an abundance of resources to areas where there is scarcity of a particular nutrient (Whiteside *et al*., 2019). The major mineral elements

are potassium (K), followed by phosphorus (P), sodium (Na), calcium (Ca), and magnesium (Mg), while the minor mineral elements are copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), and cadmium (Cd) (Miles & Chang, 2004). The nutritional content is then calculated based on analytical results and compared with dietary reference intakes (DRIs), which are shown in Table 2.3 and 2.4 (Trumbo *et al.*, 2001).

Table 2. 3 Dietary Reference Intakes (DRIs): Recommended intakes for Individual elements

Dietary Reference Intakes (DRIs): Recommended Intakes for Individuals, Elements

Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Calcium (mg/d)	Chromium (µg/d)	Copper (µg/d)	Fluoride (mg/d)	Iodine (µg/d)	Iron (mg/d)	Magnesium (mg/d)	Manganese (mg/d)	Molybdenum (µg/d)	Phosphorus (mg/d)	Selenium (µg/d)	Zinc (mg/d)	Potassium (g/d)	Sodium (g/d)	Chloride (g/d)
Infants															
0–6 mo	210*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	0.4*	0.12*	0.18*
7–12 mo	270*	5.5*	220*	0.5*	130*	**11**	75*	0.6*	3*	275*	20*	**3**	0.7*	0.37*	0.57*
Children															
1–3 y	500*	11*	**340**	0.7*	**90**	**7**	**80**	1.2*	**17**	**460**	**20**	**3**	3.0*	1.0*	1.5*
4–8 y	800*	15*	**440**	1*	**90**	**10**	**130**	1.5*	**22**	**500**	**30**	**5**	3.8*	1.2*	1.9*
Males															
9–13 y	1,300*	25*	**700**	2*	**120**	**8**	**240**	1.9*	**34**	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 y	1,300*	35*	**890**	3*	**150**	**11**	**410**	2.2*	**43**	**1,250**	**55**	**11**	4.7*	1.5*	2.3*
19–30 y	1,000*	35*	**900**	4*	**150**	**8**	**400**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
31–50 y	1,000*	35*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
51–70 y	1,200*	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.3*	2.0*
> 70 y	1,200*	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.2*	1.8*
Females															
9–13 y	1,300*	21*	**700**	2*	**120**	**8**	**240**	1.6*	**34**	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 y	1,300*	24*	**890**	3*	**150**	**15**	**360**	1.6*	**43**	**1,250**	**55**	**9**	4.7*	1.5*	2.3*
19–30 y	1,000*	25*	**900**	3*	**150**	**18**	**310**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
31–50 y	1,000*	25*	**900**	3*	**150**	**18**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
51–70 y	1,200*	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.3*	2.0*
> 70 y	1,200*	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.2*	1.8*
Pregnancy															
14–18 y	1,300*	29*	**1,000**	3*	**220**	**27**	**400**	2.0*	**50**	**1,250**	**60**	**12**	4.7*	1.5*	2.3*
19–30 y	1,000*	30*	**1,000**	3*	**220**	**27**	**350**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
31–50 y	1,000*	30*	**1,000**	3*	**220**	**27**	**360**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
Lactation															
14–18 y	1,300*	44*	**1,300**	3*	**290**	**10**	**360**	2.6*	**50**	**1,250**	**70**	**13**	5.1*	1.5*	2.3*
19–30 y	1,000*	45*	**1,300**	3*	**290**	**9**	**310**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*
31–50 y	1,000*	45*	**1,300**	3*	**290**	**9**	**320**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*

NOTE: This table presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all (97 to 98 percent) individuals in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover needs of all individuals in the group, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); and *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2004). These reports may be accessed via http://www.nap.edu.

Table 2. 4 Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels (UL[a]), elements

Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Arsenic[b]	Boron (mg/d)	Calcium (g/d)	Chromium	Copper (µg/d)	Fluoride (mg/d)	Iodine (µg/d)	Iron (mg/d)	Magnesium (mg/d)[c]	Manganese (mg/d)	Molybdenum (µg/d)	Nickel (mg/d)	Phosphorus (g/d)	Potassium	Selenium (µg/d)	Silicon[d]	Sulfate	Vanadium (mg/d)[e]	Zinc (mg/d)	Sodium (g/d)	Chloride (g/d)
Infants																					
0–6 mo	ND[f]	ND	ND	ND	ND	0.7	ND	40	ND	ND	ND	ND	ND	ND	45	ND	ND	ND	4	ND	ND
7–12 mo	ND	ND	ND	ND	ND	0.9	ND	40	ND	ND	ND	ND	ND	ND	60	ND	ND	ND	5	ND	ND
Children																					
1–3 y	ND	3	2.5	ND	1,000	1.3	200	40	65	2	300	0.2	3	ND	90	ND	ND	ND	7	1.5	2.3
4–8 y	ND	6	2.5	ND	3,000	2.2	300	40	110	3	600	0.3	3	ND	150	ND	ND	ND	12	1.9	2.9
Males, Females																					
9–13 y	ND	11	2.5	ND	5,000	10	600	40	350	6	1,100	0.6	4	ND	280	ND	ND	ND	23	2.2	3.4
14–18 y	ND	17	2.5	ND	8,000	10	900	45	350	9	1,700	1.0	4	ND	400	ND	ND	ND	34	2.3	3.6
19–70 y	ND	20	2.5	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	ND	400	ND	ND	1.8	40	2.3	3.6
>70 y	ND	20	2.5	ND	10,000	10	1,100	45	350	11	2,000	1.0	3	ND	400	ND	ND	1.8	40	2.3	3.6
Pregnancy																					
14–18 y	ND	17	2.5	ND	8,000	10	900	45	350	9	1,700	1.0	3.5	ND	400	ND	ND	ND	34	2.3	3.6
19–50 y	ND	20	2.5	ND	10,000	10	1,100	45	350	11	2,000	1.0	3.5	ND	400	ND	ND	ND	40	2.3	3.6
Lactation																					
14–18 y	ND	17	2.5	ND	8,000	10	900	45	350	9	1,700	1.0	4	ND	400	ND	ND	ND	34	2.3	3.6
19–50 y	ND	20	2.5	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	ND	400	ND	ND	ND	40	2.3	3.6

[a] UL = The maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to lack of suitable data, ULs could not be established for arsenic, chromium, silicon, potassium, and sulfate. In the absence of ULs, extra caution may be warranted in consuming levels above recommended intakes.

[b] Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements.

[c] The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.

[d] Although silicon has not been shown to cause adverse effects in humans, there is no justification for adding silicon to supplements.

[e] Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding vanadium to food and vanadium supplements should be used with caution. The UL is based on adverse effects in laboratory animals and this data could be used to set a UL for adults but not children and adolescents.

[f] ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); and *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2004). These reports may be accessed via http://www.nap.edu.

2.6.2 Medicinal value of mushrooms

Like vascular plants, insects and other forms of life, mushrooms produce secondary metabolites believed to be used as a form of communication amongst fungi or between fungi and other species, like in mycorrhizae (Section 2.4.1) and chemical defence for keeping preys afar. After the discovery of Penicillin by Fleming in 1928 from *Penicillium rubens,* chemists are constantly looking for and interested in the isolation and structure elucidation of bioactive secondary metabolites from fungi that are useful to the livelihood of humans (Spiteller, 2015).

Major medicinal properties attributed to mushrooms include anticancer, antibiotic, antiviral activities, boosting the immune system, controlling blood lipids, antitumor function, pro-sexual, anti-inflammatory, anti-oxidant/anti-aging, anti-leukemic, anti-proliferative, anti-metastatic, immunomodulatory, anti-microbial, anti-bacterial, anti-viral, anti-fungal, anti-protozoal, insecticidal, larvicidal, anti-fibrotic, steroidogenic, hypoglacaemic, hypolipidaemic, antiangiogenetic, anti-diabetic, anti-HIV, anti-malarial, anti-fatigue, neuroprotective, liver-protective, reno-protective as well as pneumo-protective and blood lipid lowering effects (Das *et al*., 2010; Rathore *et al.*, 2017; Wang et al., 2014). Secondary metabolites and bioactive molecules include polysaccharides, terpernoids, steroids, nucleobases and glycoproteins. Some mushrooms are used as raw material for producing therapeutic and medicinal agents in many countries (Kadnikova *et al.*, 2015)

2.6.2.1 Polysaccharides

Almost all Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium and culture broth (Miles & Chang, 2004; Wasser, 2002).

Polysaccharides found in mushrooms are a source of pharmacologically active compounds for many ailments such as antitumor and immune-stimulating properties. Though most of them belongs to the β-glucans group (example: krestin (PSK) from *C. versicolor,* lentinan from *L. adodes* and schizophyllan from *S. commune*, they have different chemical structures based on the β-(1-3) linkages and β-(1-6) branch points that are needed for their antitumor action, as shown in Figure 2.12 (Mizuno *et al*., 1999; Rathore *et al*., 2017; Wasser, 2002).

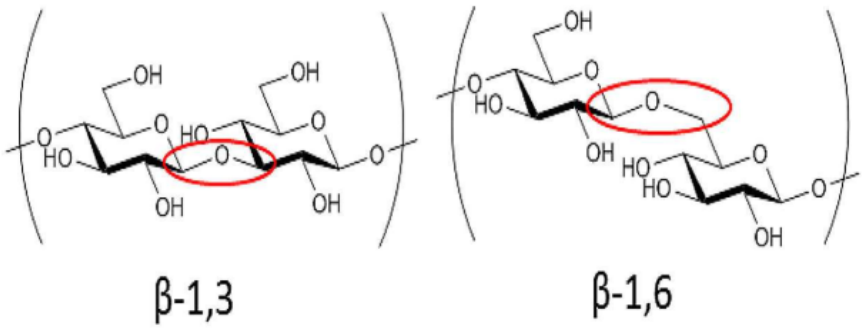


Figure 2. 12 Structure of β-glucan linkages (Rathore *et al*., 2017).

The activity of polysaccharides can be improved by altering them through well-known procedures like Smith degradation (oxydo-reducto-hydrolysis), formolysis, and Carboxymethylation (Wasser, 2002). A number of polysaccharides, some shown in Table 2.5 have been isolated from mushroom species and these includes plearan from *Pleurotus* species, lentinan and erothionine in *L. edodes,* ganoderan from *Ganoderma lucidiun*, agaritine from *Agaricus* and calocyban from *Calocybe indica* (Rathore *et al*., 2017).

2.6.2.2 Terpenoids

Terpenoids are one of the major secondary metabolites found in plant species and certain species of mushrooms. Terpenoids are classified based on the number and structural organization of carbons formed by the linear arrangement of isoprene units followed by cyclization and rearrangements of the carbon skeleton (Ludwiczuk et al., 2017). The "building block" of terpenoids, is 2-methylbuta-1,3-diene (C_5H_8) called isoprene. Terpenoids are divided into families based on the number of isoprene units, namely, terpenoids, monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), sesterterpenoids (C_{25}), triterpenoids (C_{30}), and tetraterpenoids (C_{40}) (Yaoita, Kikuchi, & Machida, 2014). The biosynthesis of varieties of these terpenoids follows the mevalonate (MVA) pathway or the plasticidal 2-C-methyl-D-erythriol 4-phosphate (MEP) pathway as shown in.

Pharmacological studies have shown that terpenoids possess anti-inflammatory, antitumor, antibacterial, antioxidant, hepatoprotective activities properties (Yao *et al*., 2016). A number of terpenoids arising from mushroom samples are being isolated and characterised from a number of edible and non-edible species. Some of the isolated terpenoids are listed in Table 2.6.

Table 2. 5 Common bioactive polysaccharides isolated from mushrooms having antitumor and anticancer effects, adopted from (Miles & Chang, 2004)

Species	Tumor or Cancer Treated	Polysaccharide origin[a]	Polysaccharide structure
Coriolus versicolor	Cancer of digestive organ, lung and breast	CM	(1→4), (1→3) or (1→4), (1→6)-β-D-glucan
Lentinula edodes	Cancer of stomach	FB	(1→3)-β-D-glucan
Schizophyllum commune	Cervical cancer	CB	(1→3), (1→6)-β-D-glucan
Ganoderma lucidum	Antitumor	FB, CM	
Agaricus blazei	Antitumor	FB, CM	(1→6)-β-D-glucan
Grifola frondosa	Antitumor	FB	(1→6), (1→3)-β-D-glucan
	Antitumor	CM	(1→3), (1→6)-β-D-glucan

[a] CM, culture mycelium; FB, fruiting body; CB, culture broth.

[b] (1→3)-β-D-Glucans including glucurono-β-D-glucan, arabinoxylo-β-D-flucan, xylo-β-D-glucan, mano-β-D-glucan, and xylomanno-β-D-glucan, as well as their protein complexes.

Table 2. 6 Some known isolated and characterised terpenoids as well as the corresponding mushroom species extracted

Mushroom species	Type of terpenoids	Isolated compound	References
Russula sanguinea	Lactarane-Type Sesquiterpenoids	Sangusulactones A – C	(Yaoita *et al*., 2012)
Lactarius subvellereus		Subvellerolactones B, D & E	(Kim *et al*., 2010a)
Lactarius repraesentaneus		Repraesentins A – C	(Hirota *et al*., 2003)
Lactarius piperatus	Marasmane-Type Sesquiterpenoids	Lactapiperanols A – D	(Yaoita *et al*., 1999)
Lactarius pubescens		Pubescenone	(Yaoita *et al*., 2014)
Russula foetens		8a,13-dihydroxymarasm-5-oic acid g-lactone 13-hydroxymarasm-7(8)-en-5-methoxy g-acetal	(Kim *et al*., 2010b; Wang *et al*., 2005)
Russula lepida	Nardosinane-Type Sesquiterpenoids Aristolane-Type Sesquiterpenoids	Rulepidanol Rulepidasiene Rulepidadiene, rulepidadiol, rulepidatriol, lepidamine, (1R,2S)-1,2-dihydroxyaristolone, (2S,11S)-2,12-dihydroxyaristolone, (1S & 1R,2S,11S)-1,2,12-trihydroxyaristolone	(Clericuzio *et al*., 2012; Jian-Wen *et al*., 2002; Tan *et al*., 2003; Vidari *et al*., 1998)
Lactarius repraesentaneus	Russulane-Type Norsesquiterpenoids	Repraesentin A – C,	(Hirota *et al*., 2003)
Rusula japonica		Russujaponols A – F	(Yoshikawa *et al*., 2006)
Russula delica		Russulanorol	(Yaoita *et al.*, 2003)
Naematoloma sublateritium	Lanostane-Type Triterpenoids	Sublateriols A – C	(Yaoita *et al*., 2001).
Tyromyces fissilis		Tyromycic acids B – G	(Quang *et al*., 2004)
Ganoderma lucidum		7-oxoganoderic acid, 15-hydroxyganoderic acid	(Cör *et al*., 2018; Jong & Birmingham, 1992; Komoda *et al*., 1985)
Hebeloma versipelle		24(E)-3b-hydroxylanosta-8,24-dien-26-al-21-oic acid	(Shao *et al*., 2005)
Ga. orbiforme		Ganorbiformins A – G	(Hapuarachchi *et al*., 2017; Isaka *et a*l., 2013)
Fomitopsis spraguei		Fomitopsins A – C	(Quang *et al*., 2005)
Elfvingia applanata		Elvingic acids A – H	(Yaoita *et al*., 2014)
Leucopaxillus gentianeus	Cucurbitane-Type Triterpenoids	Cucurbitacin B, leucopaxillones A – B	(Clericuzio *et al*., 2006)
Russula lepida		(24E)-3,4-secocucurbita-4,24-diene-3,26-dioic acid (24E)-3,4-secocucurbita-4,24-diene-3,26,29-trioic acid (24E)-3,4-secocucurbita-4,24-diene-3-hydroxy-26-carboxylic acid	(Jian-Wen *et al*., 2000)

2.6.2.3 Steroids

Steroids are a type of hormones that are important endocrine chemical messengers, nonpolar in nature and as such are classified as lipids produced by living organisms (Lucki & Sewer, 2008). Their polarity makes it easy for these compounds to cross through the cell membranes (Bruice, 1995). Steroids' backbone contains tetracyclic ring system numbered as A, B, C, D, with A, B and C being six membered ring and D a five membered ring as shown in Figure 2. 13

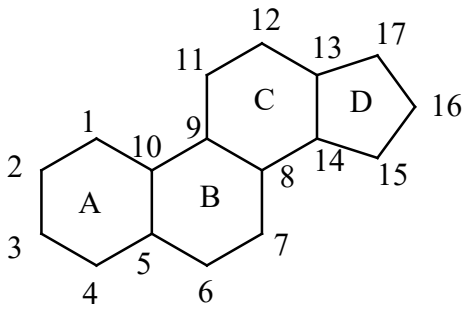


Figure 2. 13 The steroid system ring and the numbering of carbon atoms

Most fungal species, except those of the family Pythiacea are capable of synthesizing sterols. The most common sterol found in Basidiomycetes is ergosterol, which is considered the predominant sterol in fungus (Weete, 2012). This is different from the predominant sterol found in plants or animals, sitosterol and cholesterol respectively, as shown in Figure 2. 14. Ergosterol was first extracted from ergot in 1889 by Tanret, while its structure was determined in 1933 (Weete, 2012). This sterol is used as a precursor of vitamin D (Parks & Adams, 1978).

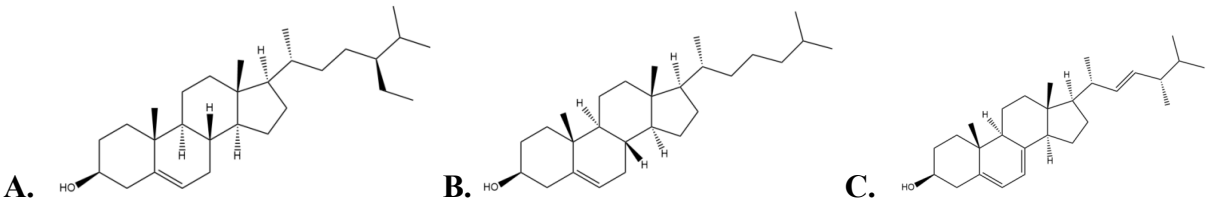


Figure 2. 14 Most prominent sterols in: (A) plants – sitosterol; (B) animals – cholesterol and (C) fungi – ergosterol.

2.6.2.4 Sphingolipids

Sphingolipids (SLs) represent a class of lipids that form an integral part of eukaryotic cell membranes (Heung *et al.*, 2006). SLs share the common structural feature that all are comprised of backbones called "long-chain-" or "sphingoid" bases, which are represented by sphingosine, (2S,3R,4E)-2-aminooctadec-4-ene-1,3-diol (also referred to as (E)-sphing-4-enine), amide-linked with a long- or very-long-chain fatty acid to form ceramides and a substituent group at C1 to form more complex sphingolipids such as sphingomyelin (SM), glucosylceramide (GlcCer), galactosylceramides (GalCer) and complex glycosphingolipids (GSLs) (Merrill Jr, 2011). They are formed through the de nova pathway which start with the condensation of serine and palmitoyl-CoA catalyzed by serine palmitoyl transferase (SPT) to generate 3-ketodihydrosphingosine followed by rapid reduction of the intermediate ketone to produce sphinganine (Bartke & Hannun, 2009; Guimarães *et al.*, 2014). The SL scheme is represented in Figure 2. 15.

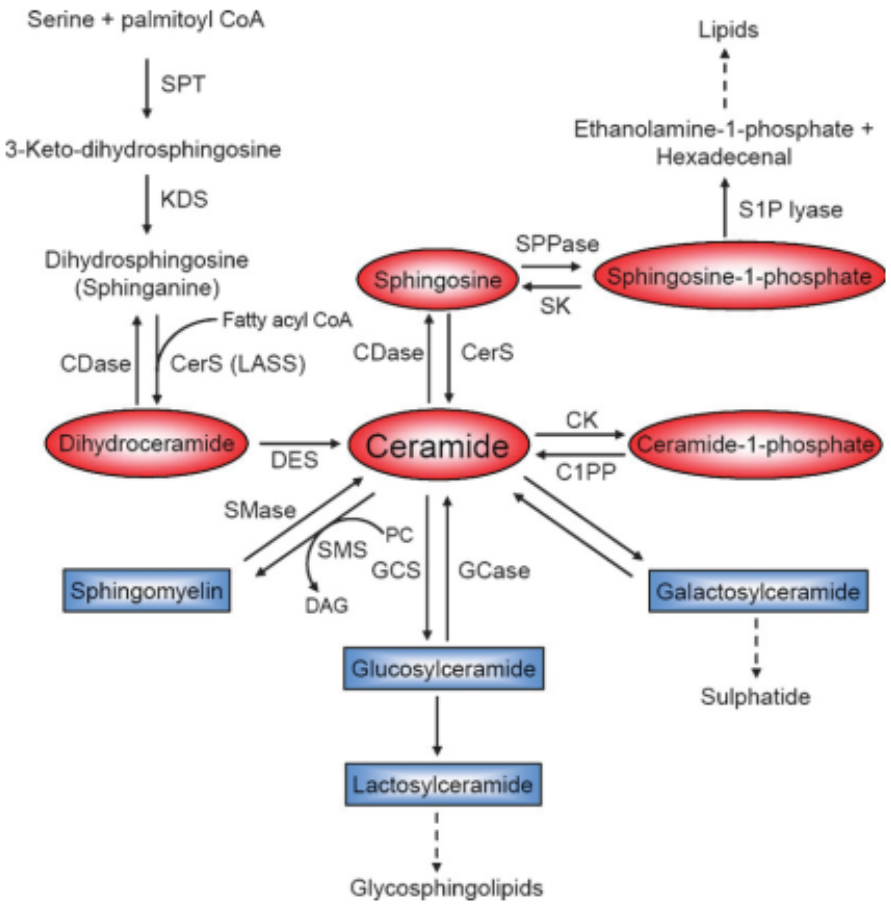


Figure 2. 15 Scheme of SL metabolism.

Bioactive SL metabolites are highlighted in red or oval shaped circle. SPT, serine palmitoyltransferase; KDS, 3-keto-dihydrosphingosine reductase; DES, dihydroceramide desaturase; SPPase, Sph phosphate phosphatase; CK, Cer kinase; C1PP, C1P phosphatase; SMS, SM synthase; PC, phosphatidylcholine; DAG, diacylglycerol; GCS, glucosylceramide synthase; GCase, glucosyl CDase (Bartke & Hannun, 2009).

These compounds play important roles that include regulation of signal transduction pathways; oncogenic transformation; embryonic development; control of cell division; cell adhesion and motility; and control of cell phenotype through direction of protein sorting to the mediation of cell-to-cell interactions and recognition (Bartke & Hannun, 2009; Guimarães *et al.*, 2014). This means they regulate pathobiological processes, such as cancer, cardiovascular and neurodegenerative disorders, and inflammation or infectious diseases (Heung *et al*., 2006). The SL family, includes ceramide (Cer), sphingosine (Sph), Sph-1-phosphate (S1P), and Cer-1-phosphate (C1P). Studies on SLs derived from fungal species have shown them to be associated with the heat stress response (Jenkins *et al*., 1997), endocytosis (Munn & Riezman, 1994), apoptosis (Cheng *et al.*, 2003), and fungal pathogenesis. As mentioned already the basic structure of a sphingolipid, shown in Figure 2.16, consists of a long-chain sphingoid base backbone linked to a fatty acid via an amide bond with the 2-amino group and to a polar head group at the C-1 position via an ester bond (Heung *et al*., 2006; Macher & Sweeley, 1978).

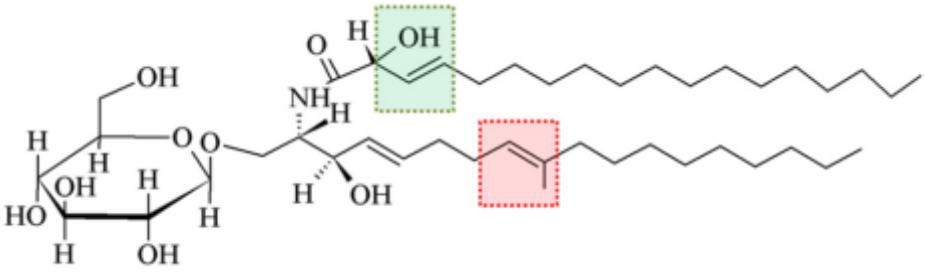
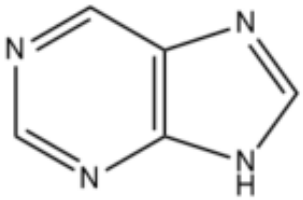


Figure 2. 16 A typical glucosylceramide from fungi, showing the sphenoid base (red box); fatty acid moiety (green box) and glucose or galactose sugar attached to C1 (Bartke & Hannun, 2009).

2.6.2.5 Nucleobases

There are five naturally occurring nucleobases that form part of the nucleic acid of the RNA and DNA. These includes cytosine, guanine, adenine, uracil and thymine. The first four are found in the RNA strand while uracil is replaced by thymine in the DNA (Emel'yanenko *et al.*, 2015). These nucleobases are grouped under two major groups, namely purines (two ring structures) and pyrimidine (one ring structure), shown in Figure 2.17.

The derivatives of purines are adenine and guanine while pyrimidines have three derivatives, cytosine, thymine and uracil.

A. 

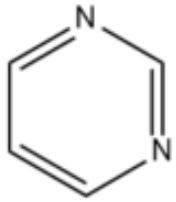
B. 

Figure 2. 17 A typical structure of purine (A) and pyrimidine (B).

2.7 Mycotoxins and Mycotoxicoses

Mycotoxins refer to compounds produced by fungi, isolated examples include: amatoxins, orellanine, gyromitrin, muscarine, isoxazoles, psilocybin, thermolabile and coprine, toxic to animals (Hallen *et al.*, 2002; Smith & Moss, 1985). Fungi such as mushrooms produce these secondary metabolites for yet unknown ecological role. It is however speculated that these compounds are used by fungi as a form of chemical communication amongst fungi species, between fungi and plants as well as between fungi and animals, and as chemical defence (as a warning to its prey or competitors) (Spiteller, 2015). Once such species are ingested, either raw or cooked they cause mycotoxicoses.

Globally it is believed that fatality rate due to the consumption of poisonous mushrooms is at least one hundred persons per year, but this can be an underestimated number as cases in underdeveloped countries are not well reported or not fully investigated (Stöver *et al*., 2019). In China alone, it is known that food poisoning cases are mostly due to ingestion of toxic mushrooms, with Amanita species accounting to 70.49 % of all recorded fatalities (Chen *et al.*, 2014). South Africa has had its own spate of mushroom poisoning after wild growing mushrooms were collected and consumed in 2012.

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CHAPTER 3
Elemental distribution including toxic elements in edible and inedible wild growing mushrooms from South Africa

3.1 Abstract

Macro-elements (Ca, Fe, K, Mg and Na) and trace elements including some toxic (As, Be, Cd, Co, Cu, Mn, Ni, Pb, Se and Zn) were determined in edible and inedible wild growing mushrooms (*Amanita rubescence, Auricularia polytricha, Boletus edulis, Boletus mirabilis, Clavulina cristata, Helvella crispa, Lacterious delicious, Pleurotus ostreatus, Suillus luteus, Termitomyces microcarpus, Termitomyces reticulatus, Termitomyces clypeatus, Termitomyces umkowaanii, Amanita foetidissima, Amanita muscaria, Amanita pantherina, Aseroe rubra, Chlorophyllum molybdites, Ganoderma lucidum, Gymnopilus junonius, Hypholoma fasciculare, Lentinus villosus, Lepista caffrorum, Pycnoporus sanguineus, Panaeolus papilionaceus, Pisolithus tinctorius, Podaxis pistillaris, Russula sardonia, Scleroderma citrinum, Scleroderma michiganense).* Analyses of samples were carried out using inductively coupled plasma-optical emission spectrometry. The elemental content in both edible and inedible mushrooms, in decreasing order, was found to be K >> Na > Ca > Mg > Fe > Mn > Zn > Cu > Se > Co > Ni > Be > Pb ≥ Cd > As. Our study revealed that accumulation of metals from the soil is independent of whether mushrooms are edible or inedible as uptake is dependent on the soil quality and its environment. Edible mushroom species studied were found to be rich in Se (145 – 836 % towards the RDA) with *B. edulis* being rich in it, *C. cristata* in Cu, *S. Luteus* in Fe, and *H. crispa* in Zn and all contained low concentrations of toxic metals making them suitable for human consumption.

Keywords: Wild mushrooms . Elemental distribution . Toxic elements . Principal component analysis

3.2 Introduction

As described in Section 2.4, mushrooms are divided into three basic ecological groups, namely mycorrhizal, parasitic and saprophytic (Hall *et al*., 2003; Stamets, 2011). The first group forms a symbiotic relationship with living plants and assists the plant to absorb nutrients, nitrogen compounds and essential elements (Hall *et al*., 2003; Stamets, 2011). The second and third groups decompose the organic matter of living or dead plants respectively into carbon, hydrogen, nitrogen and minerals which are released into the ecosystem (Stamets, 2011).

Issues relating to food security and environmental impact have received a lot of attention by the scientific community. This led to the establishment of ten Millennium Development Goals (MDGs) by members of the United Nations, of which food security is the first DG. After taking stock of the progress made in attaining MDGs, South Africa came up with Vision 2030, commonly known as National Development Plan (NDP) in order to fast-track the attainment of all MDGs (Commission 2012). The lack of nutritious foods in many underdeveloped and developing countries poses a serious threat to food security. Food security is maintained when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life (World Food Summit 1996). In many underdeveloped countries, people scavenge for food from their immediate environment due to food shortages.

From ancient times, mushrooms have been consumed by man and for medicinal purposes, especially in China and many European countries from the southwest (e.g., Spain,

Italy), the Balkans to the northeast (e.g., Sweden, Poland, Belarus) of the continent (Miles & Chang, 2004). Modern studies have also revealed mushrooms to contain compounds that can be used in the treatment of cancers, cardiovascular disease and viral diseases (Levin & Branch, 1985; Liu *et al*., 2012; Miles & Chang, 2004).

Commercial growers of mushrooms (e.g. white button mushroom, *A. bisporus*; Shiitake, *L. edodes* and oyster, *P. ostreatus*) have seen this industry expanding in the last few centuries, with China (A.D. 1000) known to have cultivated Shiitake mushroom for a very long time and France being the first to cultivate the button mushrooms around 1600 (Miles & Chang, 2004). In South Africa, the mushroom industry started in the early 1940s (Eiker, 1990). By 1997, it was estimated that more than 6.5 million tons of mushrooms were cultivated, distributed and sold annually throughout the world (Hall *et al*., 2003). Despite the fact that this industry is well established and flourishing, only the working class (16.29 million) benefit from safe cultivated mushrooms in South Africa. Trading Economics (2018) has shown that the current unemployment rate in South Africa has risen to 27.2% (6.08 million people), with majority residing in rural areas where food scarcity is a norm. This number is steadily growing and rural communities supplement food shortages using wild growing mushrooms, while others use them as a source of income. Because of socioeconomic difficulties, people in underdeveloped countries cannot afford commercialised mushrooms and only eat wild growing mushrooms that grow during rainy seasons (Mdachi e*t al*., 2004). Majority of the people in developed countries collect wild mushrooms as a delicacy, whilst in poor countries they are consumed as an important dietary supplement (Levin & Branch, 1985).

Industrialisation and regional conflicts have left the environment in most communities severely polluted. Anthropogenic activities, such as mining and fossils (especially coal) power

stations, release substantial amounts of heavy metals into the environment (Claxton, 2015; Korb, 2011; Waste, 2010). Soils are contaminated with a variety of pollutants, particularly petroleum-based compounds, polychlorinated biphenyls (PCBs), heavy metals, pesticide-related compounds and radioactive wastes (Stamets, 2011). The vegetative part of the mushrooms, mycelium, is capable of absorbing different environmental pollutants including heavy metals, radioactive compounds coming largely from nuclear power plant accidents or pesticides which are accumulated in the fruiting bodies (Cocchi *et al.*, 2017; Gałgowska & Pietrzak-Fiećko, 2017; García *et.al.*, 2015; Kalač, 2009; Navarro *et al.*, 2017; Petkovšek & Pokorny, 2013; Szántó *et al.*, 2007; Zalewska *et.al.*, 2016). Unlike green plants, mushrooms can take up large concentrations of heavy metals such as Pb, Cd (Petkovšek & Pokorny, 2013) and Hg (Falandysz & Bielawski, 2007; Širić *et al*., 2016). This can be detrimental to the food chain, which also includes humans.

Several studies have revealed that wild growing mushroom affinities to some elements depend on the species as well as the total available amount in the soil substrate (Alonso *et al.*, 2003; Falandysz *et al.*, 2018; Kalač & Svoboda, 2000; Malinowska *et al.*, 2004). As it has been evidenced, for many metallic elements and metalloids which are not well regulated by fungus, the efficiency of bioconcentration and levels in fruiting bodies can substantially differ for the same species collected from different geographical areas (Falandysz *et al.*, 2017a; Falandysz *et al.*, 2017b). Therefore, it is important to know the identity and concentrations of essential and toxic elements in mushrooms, especially the edible ones. Such knowledge can be used to provide guidance on nutritional purposes as well as to warn local communities if a specific edible species of mushroom is suspected to contain high level of toxic metals and how to decrease the content in a mushroom meal. Unfortunately, cases of mushroom poisoning after

consumption of wild growing mushrooms have increased worldwide (Hall *et al*., 2003; Herrmann *et al*., 2012; Persson, 2012). Poisonous mushrooms can cause nausea, stomach cramps, hallucinations and allergies while deadly ones cause death. For these reasons, countries such as France and Finland employ mushroom inspectors to monitor markets for safe edible mushrooms (Hall *et al*., 2003).

Several studies on edible and inedible mushrooms have been published but fewer studies have focused on wild growing mushrooms from South Africa (Jonnalagadda *et al*., 2006; Sithole *et al.*, 2017). In this study, the elemental content (As, Be, Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se and Zn) in thirty wild growing mushrooms (edible and inedible) was determined as a function of soil quality. Bioconcentration factors (BCFs) for each analyte was also evaluated for each mushroom species. Using principal component analysis, trends, variations and similarities amongst the elements accumulated, were evaluated and cluster analysis on the mushroom species in relation to their metal content was performed. The estimated contribution of edible mushrooms to recommended dietary allowances (RDAs) and adequate intakes (AIs) was established using a consumption of 10 g, dry weight. After processing or cooking, the estimated levels of elemental concentration are expected to decrease as observed in recent studies (Drewnowska & Falandysz, 2015; Drewnowska *et al.*, 2017). Elemental levels obtained can therefore be used as a guide rather than absolute amount that can be taken in after consuming processed and cooked edible mushrooms (Falandysz & Borovička, 2013; Falandysz & Drewnowska, 2015).

3.3 Materials and methods

The samples of mature fruiting bodies of wild growing mushrooms were collected from different *Pine* or *Eucalyptus* plantations (Figure A1.1) owned by the Sappi, Mondi and Merensky companies in South Africa. The sampling points, Table 3.1 and Figure 3.1, were mainly limited to Pine or Eucalyptus plantations which are located in KwaZulu-Natal, South Africa. A few of the species were collected from areas closer to residential areas. Fruiting bodies (both pileus or cap and stipe or stalk) of fully-grown mushrooms were hand-picked from their natural environments. Of the collected species, thirty were positively identified and form the basis of this study with assistance from the mushroom expert at Cidara College of Agriculture. The fruiting bodies were cleaned of substrate and residues of plants, grass and soil using a plastic knife and/or a paint brush. Samples were then cut into pieces and dried to constant mass in an oven set at 40 °C. Dried mushrooms were pulverized using a porcelain mortar and pestle, Figure A1.3.

The powdered samples were stored in low density polyethylene (LPDE) plastic bottles, which were capped and then sealed with Parafilm^{TM} (Laboratory Film, Bemis Flexible Packaging, NEENAH, WI 54956) to prevent them from rehydrating. All glassware and plastic bottles were washed with a detergent, soaked in 10% HNO_3, overnight, rinsed with distilled water and finally with ultra-pure water. The corresponding mushroom soil substrate layer (0 – 18 cm depth) was sampled from six points (25 cm apart) around the mushrooms using a hand auger. Soils samples from the six points were mixed thoroughly to produce a representative sample. Soil samples were air dried at room temperature in the laboratory for 3 to 4 weeks, passed through a 2 mm mesh then crushed with a porcelain mortar and pestle before being

stored in LPDE plastic bottles. Soil and mushroom samples were stored in a dry cupboard (i.e. for less than a week) at room temperature until digestion was carried out.

Table 3. 1 Names of the sampling sites, elevation above sea level, soil pH and soil organic matter (SOM). The mushroom species as well as the number of fully-grown fruiting bodies collected.

No	Site name	Latitude	Longitude	Elevation (m)	pH	SOM%	Species**
1	Boston	29.40151ºS	29.58358ºE	1373	4.30	4.9	*AP*[33], *BM*[15]
2	Bulwer	29.84994ºS	29.76683ºE	1499	3.94	3.8	*AP*[11], *BE*[9], *BM*[7], *LD*[9], *RS*[77]
3	Cidara	29.33446ºS	30.16029ºE	1160	3.94	4.8	*BE*[5], *BM*[7], *LD*[10], *RS*[18]
4	Clemont	29.54787ºS	30.28405ºE	1450	3.96	4.5	*AP*[23], *BM*[23], *LD*[19]
5	Curry Post	29.41913ºS	30.20954ºE	1279	4.00	7.4	*RS*[33]
6	DonnyBrook	29.88539ºS	29.90538ºE	1339	3.88	4.5	*AP*[19], *BE*[7], *BM*[11], *RS*[28]
7	Gilboa farm	29.26079ºS	30.33490ºE	1497	4.14	13.7	*AP*[21], *BE*[6], *BM*[33], *LD*[5], *RS*[70]
8	Greytown	29.10037ºS	30.23575ºE	1385	3.87	7.6	*AP*[23], *BE*[4], *BM*[18], *RS*[55]
9	Karkloof	29.38443ºS	30.26320ºE	1092	4.31	4.1	*LD*[65], *RS*[45]
10	Lion Park	29.61790ºS	30.50220ºE	785	4.05	3.1	*TS*[113]
11	Mafakathini	29.65605ºS	30.07948ºE	1436	4.00	4.5	*AP*[27], *BM*[11], *RS*[71]
12	Richmond	29.85764ºS	30.19291ºE	988	4.16	2.7	*LD*[45], *RLion ParS*[51]
13	Singisi	29.59464ºS	30.26902ºE	1476	4.04	4.2	*AP*[35], *BE*[23], *BM*[28], *LD*[53], *RS*[47]
14	Stepmore A	29.55348ºS	29.52567ºE	1515	4.03	3.9	*BE*[15], *RS*[55]
15	Stepmore B	29.55348ºS	29.52567ºE	1545	4.03	1.5	*AP*[65], *BE*[11]
16	Twasamhlobo	29.38307ºS	29.37444ºE	1636	4.27	5.1	*AP*[17], *BE*[17]

* Superscript numbers on each species represent the number of mature fruiting bodies collected

**Species - *AP (Amanita pantherina), BE* (*Boletus edulis*)*, BM* (*Boletus mirabilis*)*, LD* (*Lactarius deliciosus*)*, RS* (*Rassula sardonia*) and *TS* (*Termitomyces sagittiformis*).

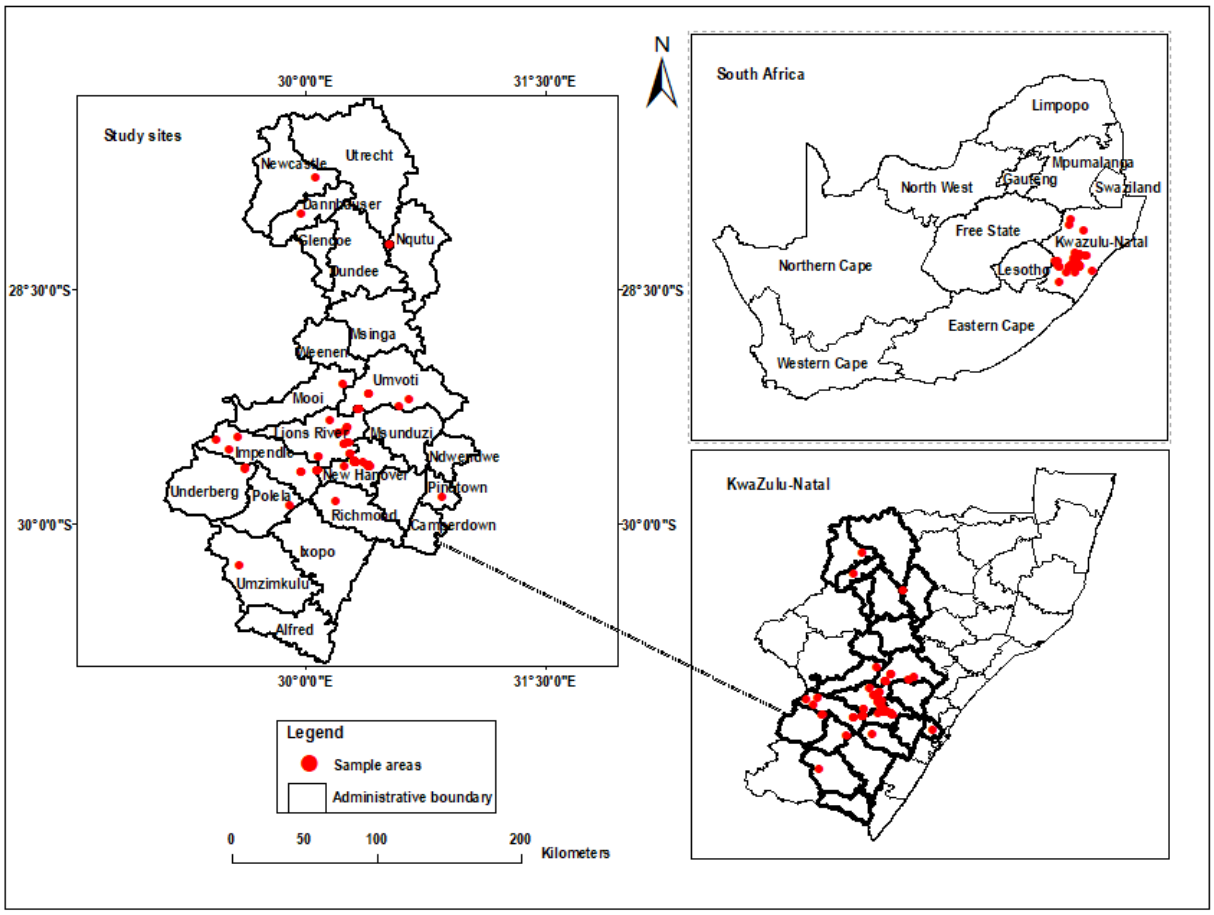

Figure 3. 1 A map showing sampling points were mushroom samples and soil samples were collected in KwaZulu-Natal, South Africa.

All polytetrafluoroethylene (PTFE) digestion vessels were soaked with 10% (v/v) HNO_3 solution overnight, and rinsed with ultra-pure water before digestion to avoid cross-contamination. The powdered mushroom samples (~0.25 g, accurately weighed) were digested with 10 mL of 69% HNO_3 and 2.5 mL of 30% hydrogen peroxide (H_2O_2). Both reagents were purchased from Merck (kGaA 64271 Darmstadt, Germany). The samples were predigested with

HNO_3 for 45 min in an open PTFE vessel prior to addition of H_2O_2. The vessels were closed and temperature-digested in an automatic microwave digestion system (MARS 6 CEM Corporation, Matthews, NC, USA) using Xpress Plus vessels. The microwave was set at 1600 W at 100% power, after which it was ramped to 180°C for 20 min, held for 45 min and cooled for 15 min. After digestion, mushroom samples cooled down to room temperature and then diluted to 50 mL using ultra-pure water. Samples were stored in high density polyethylene (HDPE) bottles and kept in the refrigerator at 4°C until analysis was carried out, within a week.

Soil samples (~0.25 g, accurately weighed) were digested similar to mushrooms. After digestion and cooling, samples were filtered using Whatman™ No. 1 qualitative filter paper and diluted to 50 mL using ultra-pure water. Samples were stored in HDPE bottles, kept in the refrigerator at 4°C until analysis was carried out within a week. All samples (mushroom and soil) and blanks were digested and analysed in triplicate.

3.4 Instrumentation Method

Analyses were carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES; the Perkin Elmer® Optima™ 5300 Dual View, Billerica, Massachusetts, USA). The RF power of the instrument was set at 1.30 kW with axial plasma observation. The plasma argon, auxiliary and nebulizer gas flow rates were 15.0, 0.20 and 0.80 L min^{-1}, respectively. The peristaltic pump and auto-sampler wash rates were both 1.50 mL min^{-1}. High-purity Ar (99.995%) supplied by Air Products South Africa (Pty) Ltd. (Kempton Park) was used to fuel the plasma and as carrier gas. Calibration standards were prepared by diluting 1000 mg L^{-1} ± 2 in 2% (w/w) HNO_3 of analytical grade standards (Trace**CERT**®; Fluka Analytical; Sigma-

Aldrich; St Louis, Mo USA) using ultra-pure water. Calibration standards are prepared by the manufacturer in either 2% or 12% HNO_3 solution. In order to match the matrix of the analytical grade standards, dilution was made using 2% or 12% HNO_3 solution.

3.5 Quality control

The certified reference material (CRM) used for elemental analysis of mushrooms was white clover (BCR® - 402) obtained from the Institute for Reference Materials and Measurements (IRMM), Belgium and for soil was metals in soil (D081-540), Sigma-Aldrich. The CRMs were digested according to the method employed for samples. For accuracy and precision of the analytical method, analyses of the CRMs were carried out at the beginning, middle and at the end of the analysis. In total, eight (for mushroom) and five (for soil) separate CRM solutions were prepared, in triplicate, and each solution analysed three times during each run. All measured values were within the quality control performance acceptance limits shown in Table 3.2 and Table 3.3.

Table 3. 2 Measurements of quality control of the analytical data using certified reference materials, white clover (BCR® - 402) (n = 24)

Analyte	Measured value (μg g^{-1})	Certified value (μg g^{-1})	% recovery
Se	6.06 ± 0.82	6.70 ± 0.25	90.4
Cr	5.43 ± 0.34	5.19	104.6
Fe	240.65 ± 18.9	244	98.6
Ni	8.28 ± 0.28	8.25	100.4
Zn	27.60 ± 8.78	25.2	109.5

Table 3. 3 Measurements of quality control of the analytical data using certified reference material, Metals in soil, D081-540 (n = 15)

Analyte	Wavelength (nm)	LOD* ($\mu g\ g^{-1}$)	LOQ** ($\mu g\ g^{-1}$)	Measured value ($\mu g\ g^{-1}$)	Certified value ($\mu g\ g^{-1}$)	Acceptance Limits ($\mu g\ g^{-1}$)	Recovery (%)
As	228.812	0.0830	0.2515	75 ± 7.2	88.4 ± 5.92	71.5 – 105	85.5
Be	313.042	0.0003	0.0009	50 ± 3.7	55.8 ± 6.26	45.8 – 65.9	89.1
Ca	422.673	0.0100	0.0303	7555 ± 550	7530 ± 7.28	6210 – 8850	100.3
Cd	228.802	0.0030	0.0082	120 ± 9.5	143 ± 5.60	116 – 169	83.5
Co	228.616	0.0070	0.0212	190 ± 14	199 ± 4.10	166 – 233	94.1
Cu	324.752	0.0054	0.0164	260 ± 18	268 ± 4.72	219 – 317	96.0
Fe	259.939	0.0062	0.0188	11130 ± 1347	12800 ± 18.0	5380 – 20100	86.9
K	766.490	45.407	138.50	2580 ± 312	2570 ± 7.55	1860 – 3290	100.3
Mg	285.213	0.0016	0.0048	2460 ± 252	2850 ± 5.51	2210 – 3490	86.2
Mn	259.372	0.0016	0.0048	412 ± 35	425 ± 9.69	347 – 502	96.9
Ni	341.476	0.0480	0.1455	226 ± 17	236 ± 4.17	194 – 279	95.7
Pb	217.000	0.0900	0.2727	90 ± 8.0	97.9 ± 11.3	80.0 – 116	88.8
Se	196.026	0.0750	0.2273	110 ± 11	127 ± 4.47	98.4 – 156	87.6
Zn	213.857	0.0018	0.0055	110 ± 10	130 ± 11.5	106 – 155	82.5

LOD* represents the limit of detection limit. LOQ** represents the limit of quantification.

3.6 Data analyses

Statistical analysis of data obtained was carried out using one-way analysis of variance (ANOVA), using full SPSS version 25 program (IBM Corp., USA). A post hoc test was performed using Tukey's HSD multiple comparison test at the 95% confidence level.

3.7 Results and discussion

The samples under study contained edible and inedible mushrooms collected during rainy season, September until April of year 2013 to 2016. Thirteen species of mature fruiting bodies, namely, *Amanita rubescens* (1)7*, Auricularia polytricha* (1)13*, Boletus edulis* (11)87*, Boletus mirabilis* (11)165*, Clavulina cristata* (5)countless*, Helvella crispa* (1)9*, Lacterious delicious* (9)335*, Suillus luteus* (6)37*, Termitomyces clypeatus* (4)69*, Termitomyces microcarpus* (2)countless*, Termitomyces reticulatus* (2)45*, Termitomyces umkowaanii* (4)12 and *Pleurotus ostreatus* (1)2 are considered edible without prior treatment, e.g. parboiling to weaken or destroy toxicity. The remaining seventeen species, namely, *Amanita foetidissima* (3)45*, Amanita muscaria* (3)17*, Amanita pantherina* (14)365*, Aseroe rubra* (1)75*, Chlorophyllum molybdites* (1)67*, Ganoderma lucidum* (1)13*, Gymnopilus junonius* (1)88*, Hypholoma fasciculare* (1)101*, Lentinus villosus* (3)25*, Lepista caffrorum* (1)65*, Pycnoporus sanguineus* (1)35*, Panaeolus papilionaceus* (2)19*, Pisolithus tinctorius* (3)15*, Podaxis pistillaris* (1)6*, Russula sardonia* (18)597*, Scleroderma citrinum* (1)7*, Scleroderma michiganense* (3)34 are considered inedible in this study. The analytes were categorised as macro-elements (primary to the growth of the mushroom), trace elements (essential elements needed in small amounts), and toxic elements (non-essential to growth of mushroom and harmful to human beings).

3.7.1 Macro-elements

Mushrooms are capable of taking up available elements found in their vicinity. In this study, five macro elements (Ca, Fe, K, Mg, and Na) were measured and are presented in Figure 3.2. Calcium in mushrooms ranged from 6.0 to 1880 mg kg^{-1}, with *L. villosus* (an inedible mushroom) having the highest amount of Ca. The bioconcentration factor (BCF) was found to be below 0.95 for all species. This shows that mushrooms have the ability to exclude uptake of Ca once the metabolic needs are met.

Iron in mushrooms ranged from 20 (*G. junonius*, inedible mushroom) to 1300 mg kg^{-1} (*S. luteu*s, an edible mushroom). These values were much lower than in mushrooms found in a study conducted in Turkey (above 9000 mg kg^{-1}) (Yamaç *et al.*, 2007). Iron in the soil substrate ranged from 10900 to 55610 mg kg^{-1} which is 27 times higher than Fe in *S. luteus*. The average BCF was 0.01 for mushrooms under study indicating that mushroom species tend to exclude Fe, once physiological needs are met. This finding agreed with the study carried out by Širić et al. (2016) in which they found that in relation to the underlying soils, wild edible mushrooms species were bio-exclusors of Fe, average BFC was 0.01.

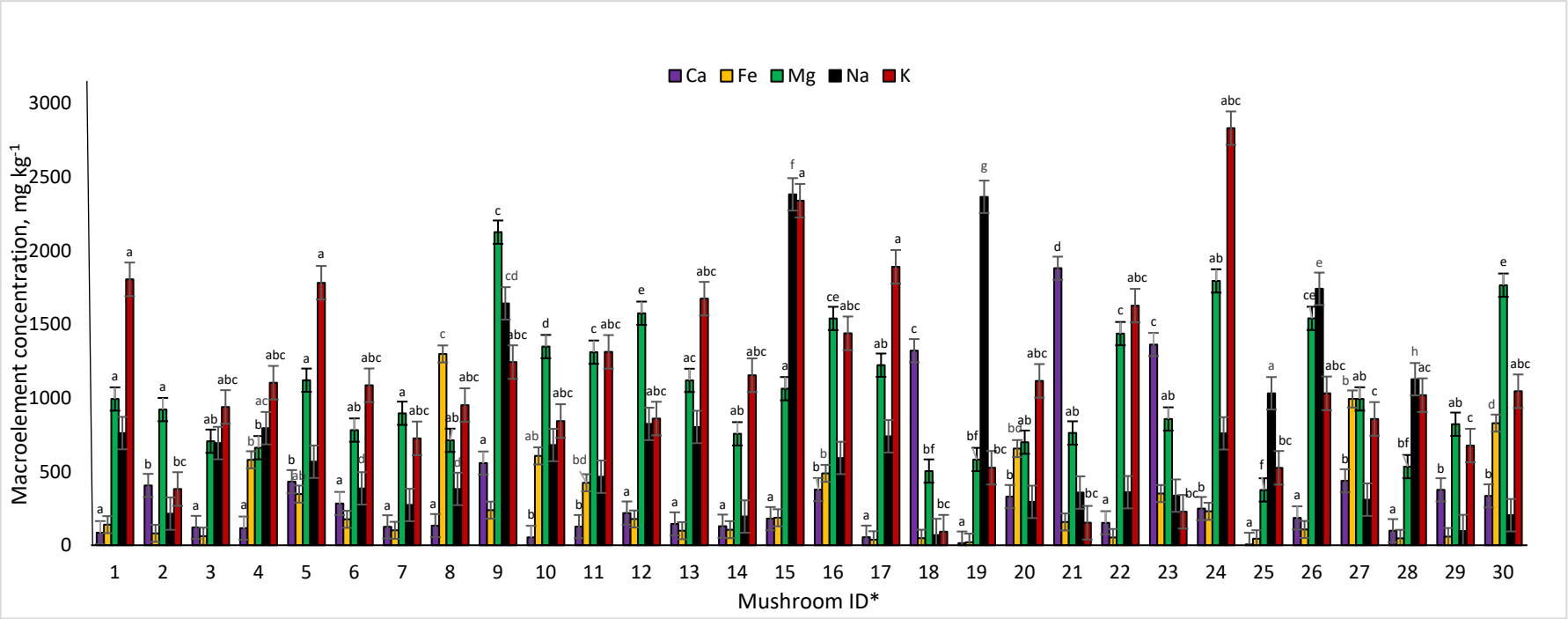

Figure 3. 2 Concentration of macro elements (Ca, Fe, Mg and Na) in (mg kg^{-1}) and K (g kg^{-1}) in mushrooms based on dry mass

***Edible mushrooms**: *Amanita rubescence*[1], *Auricularia polytricha*[2], *Boletus edulis*[3], *Boletus mirabilis*[4], *Clavulina cristata*[5], *Helvella crispa*[6], *Lacterious delicious*[7], *Pleurotus ostreatus*[26], *Suillus luteus*[8], *Termitomyces microcarpus*[9], *Termitomyces reticulatus*[10], *Termitomyces clypeatus*[11], *Termitomyces umkowaanii*[12].

Inedible mushrooms: *Amanita foetidissima*[13], *Amanita muscaria*[14], *Amanita pantherina*[15], *Aseroe rubra*[16], *Chlorophyllum molybdites*[17], *Ganoderma lucidum*[18], *Gymnopilus junonius*[19], *Hypholoma fasciculare*[20], *Lentinus villosus*[21], *Lepista caffrorum*[22], *Pycnoporus sanguineus*[23], *Panaeolus papilionaceus*[24], *Pisolithus tinctorius*[25], *Podaxis pistillaris*[27], *Russula sardonia*[28], *Scleroderma citrinum*[29], *Scleroderma michiganense*[30].

Different letter(s) (a - h) above the error bars for the same element, indicates statistically significant differences between means at $p < 0.05$ using one-way ANOVA.

Studies conducted by (Kemp, 2002) and (Gençcelep *et al.*, 2009) showed mushrooms, whether cultivated or wild, to accumulate high concentration of K, higher than other elements. This could be because K is required to contribute to the appropriate ionic environment for enzyme functioning (Jennings & Lysek, 1996). Potassium in mushrooms ranged from 2.3 (*G. lucidum*) to 70.8 g kg^{-1} (*P. papilionaceus)* (both inedible mushrooms). Our study was consistent with studies conducted on mushrooms gathered from other international countries such as Poland (Kojta & Falandysz, 2016; Malinowska *et al*., 2004). The highest BCF (170) was found in *C. molybdites*, which is inedible. This shows the mushroom's ability to accumulate K for physiological needs such as enzyme functioning.

The magnesium in mushrooms ranged from 375 (*P. tinctorius,* an inedible) to 2130 mg kg^{-1} (*T. microcarpus*, edible), with *T. microcarpus* having almost twice the amount found in other species of the same family, namely; *T. reticulatus*, *T. tylerianus*, and *T. umkowaanii*. Despite its tiny fruiting body, pileus (6-20 mm in diameter) and stipe (20-60 × 1-5 mm) small in size (Van Der Westhizen & Eicker, 1994), *T. microcarpus* can be beneficial as a supplement for Mg. In general, the BCF was found to be greater than 1.0, indicating the mushroom's ability to accumulate this essential element.

The mean concentration of Na in the species ranged from 70 (*G. lucidum*, inedible) to 2400 mg kg^{-1}, (*A. pantherina*, inedible). Mushrooms tended to accumulate Na, with high BCFs in most cases. This shows that mushrooms require Na for their physiological growth.

Generally, the concentration of macro elements in mushrooms was found to be in decreasing order of K > Na > Ca > Mg > Fe.

3.7.2 Trace elements

Seven trace elements, namely Be, Co, Cu, Mn, Ni, Se, and Zn were analysed in mushrooms and the results are presented in Table 3.5 and Table 3.6. The elements Be and Co (Table 3.5) were not detected in most species studied. The highest mean concentration of Be measured in the species wherein it was detected was 1.0 mg kg^{-1} (*A. muscaria,* inedible without parboiling). Co was only detected in 50% of the species studied and the highest mean concentration measured was 19 mg kg^{-1} (*A. polytricha*, edible).

The mean concentration of Cu in mushrooms ranged from 2.0 (*A. polytricha*, edible) to 170 mg kg^{-1} (*C. cristata*, edible). The BCF was found to be greater than one for most species. This shows that mushroom species are excellent accumulators of Cu.

The mean concentrations of Mn in mushrooms ranged from 1.5 (*P. tinctorius*, inedible) to 270 mg kg^{-1} (*A. rubra*, inedible) as shown in Table 3.6. The BCF was less than 0.2 for all species. This shows that mushrooms require low amounts of Mn for their physiological growth.

The mean concentrations of Ni in mushrooms ranged from 0.85 (*G. lucidum*, inedible) to 10 mg kg^{-1} (*A. muscaria,* inedible without parboiling). BCFs were generally very low, (< 1) indicating the mushroom's ability to exclude elements according to physiological needs.

Selenium concentrations ranged from 6.0 (*O. polypore*, inedible) to 50 mg kg^{-1} (*B. edulis*, edible). *B. edulis*, the highly sought-after species for cuisine, internationally, contained the highest amount of Se, more than two-folds higher than the other species. Mushrooms, particularly the *Boletus* species, are rich in Se (Cocchi *et al.*, 2006) which is consistent with our study. Mushrooms are known to be the most sensitive accumulators of Se compared to green plants, with concentrations sometimes a thousand times higher.

Table 3. 4 Concentration of trace elements (Be, Co, Cr, Cu) in mushrooms and soil (mean value (SD), mg kg^{-1}, dry weight) and bioaccumulation factor (BCF)

Sample ID*	Be			Co			Cr			Cu		
	Mushroom	**Soil**	**BCF**	**Mushroom**	**Soil**	**BCF**	**Mushroom**	**Soil**	**BCF**	**Mushroom**	**Soil**	**BCF**
1	ND**	0.37(0.05)	ND	ND	3.01(0.21)	ND	1.47(0.22)[a]	35.81(5.54)	0	54.41(1.84)[a]	10.17(0.51)	5.4
2	ND	NoD***	NoD	18.85(0.51)[b]	NoD	NoD	0.66(0.06)[ab]	NoD	NoD	1.59(0.11)[b]	NoD	NoD
3	ND	0.41(0.04)	ND	4.99(0.78)[a]	11.82(0.26)	0.4	2.62(1.09)[ab]	77.76(2.91)	0	79.71(3.85)[ad]	20.55(0.53)	5.6
4	0.33(0.04)[b]	0.49(0.05)	1.0	0.76(0.28)[a]	14.18(0.24)	0.1	1.56(0.25)[a]	84.51(2.28)	0	69.89(3.38)[ad]	28.64(0.42)	4.9
5	0.48(0.05)[b]	0.45(0.06)	0.9	0.21(0.08)[a]	13.54(0.33)	0	1.88(0.23)[ab]	126.23(5.53)	0	167.07(4.93)[c]	31.51(0.65)	8.9
6	ND	0.51(0.09)	ND	ND	5.23(0.21)	ND	1.94(0.21)[ab]	48.75(1.79)	0	28.61(1.07)[a]	9.98(0.85)	2.9
7	0.39(0.05)[b]	0.55(0.05)	1.0	0.16(0.07)[a]	11.99(0.39)	0	1.65(0.33)[ab]	91.64(4.72)	0	17.86(1.02)[b]	23.21(0.45)	1.1
8	ND	0.35(0.04)	ND	0.33(0.09)[a]	14.53(0.39)	0	1.65(0.11)[a]	98.67(3.08)	0	18.85(0.82)[b]	30.31(0.64)	1.7
9	ND	0.73(0.03)	ND	7.57(0.34)[a]	20.54(0.81)	0. 5	1.34(0.12)[ab]	55.22(5.36)	0	61.37(10.31)[a]	44.27(1.39)	1.7
10	ND	ND	ND	ND	4.88(0.21)	ND	2.68(0.34)[a]	5.41(2.41)	0.1	35.93(2.13)[ad]	10.39(0.23)	8.6
11	ND	1.24(0.04)	ND	ND	41.01(0.26)	ND	1.29(0.09)[b]	293.45(16.43)	0	25.32(3.61)[a]	119.47(0.31)	0.2
12	ND	0.77(0.08)	ND	9.91(1.47)[d]	21.94(0.33)	0.3	1.53(0.47)[ab]	183.04(6.61)	0	53.91(25.35)[ad]	65.18(5.81)	0.8
13	ND	0.49(0.04)	ND	ND	19.17(1.38)	ND	0.83(0.16)[ab]	127.02(3.29)	0	45.72(1.07)[a]	48.15(1.26)	1.0
14	1.31(0.09)[d]	0.41(0.07)	2.9	1.15(0.07)[a]	8.49(0.15)	0.1	2.11(0.58)[ab]	62.12(1.63)	0	32.41(1.49)[a]	17.72(0.38)	2.6
15	0.35(0.07)[b]	0.41(0.04)	1.1	0.53(0.66)[a]	11.55(0.27)	0.1	2.23(0.39)[a]	78.77(2.41)	0.1	57.76(2.07)[ad]	24.08(0.61)	4.1
16	ND)	0.46(0.05)	ND	0.89(0.07)[a]	31.79(0.91)	0	16.92(1.27)[c]	117.35(2.29)	0.1	25.17(1.81)[b]	34.19(0.85)	0.7
17	ND	ND	ND	ND	7.62(0.19)	ND	1.76(0.12)[a]	14.37(0.78)	0.1	68.84(3.67)[ad]	10.56(0.12)	6.5
18	ND	NoD	NoD	ND	NoD	NoD	0.42(0.04)[ab]	NoD	NoD	25.19(6.82)[b]	NoD	NoD
19	ND	0.51(0.04)	ND	ND	4.81(0.37)	ND	2.22(0.21)[a]	89.46(1.47)	0	8.92(1.08)[b]	14.09(0.21)	0.6

20	ND	0.46(0.05)	ND	ND	31.79(0.91)	ND	1.53(0.44)^{ab}	117.35(2.29)	0	41.02(2.16)^{a}	34.19(0.85)	1.2
21	0.32(0.03)^{b}	NoD	NoD	0.17(0.03)^{a}	NoD	NoD	1.58(0.32)^{ab}	NoD	NoD	11.55(0.77)^{b}	NoD	NoD
22	ND	0.77(0.16)	ND	ND	17.96(1.37)	ND	0.63(0.11)^{ab}	124.13(0.52)	0	21.13(0.87)^{b}	27.22(0.64)	0.8
23	ND	NoD	NoD	ND	NoD	NoD	1.31(0.22)^{ab}	NoD	NoD	4.78(1.01)^{b}	NoD	NoD
24	ND	NoD	NoD	ND	NoD	NoD	1.68(0.22)^{ab}	NoD	NoD	18.17(1.18)^{b}	NoD	NoD
25	ND	0.51(0.04)	ND	ND	4.81(0.37)	ND	1.74(0.91)^{a}	89.46(1.47)	0	3.78(0.84)^{b}	14.09(0.21)	0.3
26	ND	NoD	NoD	0.83(0.08)^{a}	NoD	NoD	1.37(0.12)^{a}	NoD	NoD	65.91(1.23)^{ad}	NoD	NoD
27	ND	0.28(0.14)	ND	17.74(7.16)^{b}	15.71(1.35)	1.1	2.86(1.34)^{ab}	75.46(20.91)	0	33.15(2.72)^{a}	20.52(2.56)	1.6
28	0.35(0.05)^{b}	0.52(0.04)	1.0	0.57(0.09)^{a}	10.65(0.29)	0.1	1.46(0.14)^{a}	85.76(2.99)	0	31.72(1.24)^{a}	23.96(0.55)	1.9
29	ND	0.46(0.05)	ND	ND	30.92(2.08)	ND	0.91(0.18)^{ab}	162.58(51.71)	0	49.06(2.02)^{a}	34.16(2.91)	1.4
30	ND	0.61(0.06)	ND	ND	19.97(1.27)	ND	3.13(2.33)^{b}	124.15(5.07)	0	76.51(4.46)^{d}	49.01(2.73)	1.9

Different superscript letter(s) (a – h) in each column for the same element, indicates statistically significant differences between means at p < 0.05 using one-way ANOVA.
Edible mushrooms:– *Amanita Rubescence[1], Auricularia polytricha[2], Boletus Edulis[3], Boletus Mirabilis[4], Clavulina Cristata[5], Helvella crispa[6], Lacterious Delicious[7], Suillus Luteus[8], Termitomyces microcarpus[9] Termitomyces reticulatus[10], Termitomyces clypeatus[11], Termitomyces umkowaanii[12],*
Inedible mushrooms:– *Amanita foetidissima[13], Amanita Muscaria[14], Amanita Pantherina[15], Aseroe rubra[16], Chlorophyllum molybdites[17], Ganoderma Lucidum[18], Gymnopilus junonius[19], Hypholoma fasciculare[20], Lentinus Villosus[21], Lepista caffrorum[22], Pycnoporus sanguineus[23], Panaeolus papilionaceus[24], Pisolithus tinctorius[25], Pleurotus[26], Podaxis Pistillaris[27] Russula Sardonia[28], Scleroderma citrinum[29], Scleroderma michiganense[30].*

**ND – below instrument detection limit. ***NoD – No available data.

Table 3. 5 Concentration of trace elements (Mn, Ni, Se, Zn) in mushrooms and soil (mean value (SD), mg kg^{-1}, dry weight) and bioaccumulation factor (BCF)

Sample ID*	Mn			Ni			Se			Zn		
	Mushroom	Soil	BCF	Mushroom	Soil	BCF	Mushroom	Soil	BCF	Mushroom	Soil	BCF
1	7.34(0.36)[a]	76.81(1.18)	0.1	3.74(0.21)[a]	13.38(0.35)	0.3	14.41(3.06)[a]	ND**	ND	140.65(4.59)[a]	8.72(0.47)	16.1
2	6.04(0.35)[a]	NoD***	NoD	0.99(0.05)[b]	NoD	NoD	9.81(0.76)[b]	NoD	NoD	12.01(4.06)[b]	NoD	NoD
3	11.62(1.15)[a]	312.19(6.21)	0.1	4.27(1.15)[a]	30.84(0.54)	0.2	46.33(2.05)[c]	16.78(3.55)	2.9	72.94(3.89)[c]	25.34(1.44)	4.0
4	17.19(1.34)[a]	311.77(6.83)	0.1	4.83(1.41)[a]	34.46(0.51)	0.2	9.55(0.92)[b]	17.25(2.55)	0.8	90.76(3.84)[d]	27.78(1.49)	4.2
5	25.18(1.57)[a]	228.92(11.83)	0.1	5.56(0.72)[b]	45.85(0.47)	0.2	8.71(1.08)[bd]	22.91(2.03)	0.9	133.06(4.85)[a]	31.81(1.87)	5.9
6	10.64(1.84)[a]	155.06(17.16)	0.1	4.01(0.33)[a]	13.89(0.81)	0.3	13.05(1.25)[ae]	6.71(2.09)	1.9	153.98(6.78)[ah]	9.74(3.84)	15.8
7	6.19(0.37)[a]	242.61(6.09)	0	3.31(0.24)[a]	34.81(0.68)	0.1	10.87(1.47)[b]	17.57(3.04)	0.7	99.78(6.67)[d]	30.46(1.22)	4.4
8	29.53(1.17)[a]	393.63(12.48)	0.1	3.28(0.13)[a]	33.54(0.45)	0.2	8.36(1.38)[d]	ND	ND	49.76(1.77)[e]	24.86(1.05)	2.6
9	67.92(15.45)[a]	948.61(38.46)	0.1	2.69(0.61)[a]	53.21(2.61)	0.1	11.61(2.09)[b]	13.31(2.01)	2.5	100.16(2.56)[cd]	57.95(1.75)	1.8
10	16.67(1.12)[b]	152.99(7.03)	0.1	4.79(0.13)[ab]	18.67(0.17)	0.3	12.03(1.57)[b]	0.99(1.91)	11.8	103.91(10.33)[d]	28.47(2.83)	4.0
11	6.25(0.37)[a]	406.49(4.44)	0	3.13(0.11)[a]	3.94(1.19)	1.2	10.77(2.13)[d]	52.65(3.03)	0.2	72.54(2.25)[ad]	47.91(2.23)	1.4
12	8.04(0.78)[a]	752.13(11.63)	0	2.21(0.21)[ab]	67.35(2.51)	0.1	9.41(0.89)[b]	32.11(2.01)	0.3	89.28(4.72)[d]	89.43(4.01)	1.2
13	7.73(0.33)[a]	837.35(11.85)	0	2.84(0.53)[bd]	54.11(1.61)	0.1	11.06(0.65)[b]	20.72(4.51)	0.8	172.83(9.57)[f]	41.53(1.81)	4.5
14	11.53(0.69)[a]	235.93(4.42)	0.1	9.41(0.81)[a]	28.61(0.65)	0.5	13.39(1.81)[a]	13.45(3.58)	1.1	112.91(5.98)[cd]	26.45(1.05)	5.4
15	18.75(0.83)[a]	285.02(5.68)	0.1	3.71(0.36)[a]	33.08(0.51)	0.2	11.41(1.46)[b]	16.55(3.33)	0.8	128.69(5.97)[a]	26.73(1.21)	7.1
16	264.06(7.61)[c]	1332.51(32.62)	0.2	8.18(0.24)[c]	56.27(0.53)	0.2	8.77(0.75)[bd]	19.47(3.73)	0.5	58.61(3.46)[c]	45.92(1.38)	1.3
17	6.41(0.42)[a]	468.35(12.71)	0	3.58(0.71)[a]	7.68(0.33)	0.5	12.86(1.82)[ae]	ND	ND	91.44(2.26)[ce]	11.67(1.18)	7.8
18	17.17(2.87)[a]	NoD	NoD	0.85(0.09)[b]	NoD	NoD	10.44(0.29)[b]	NoD	NoD	74.39(4.86)[cd]	NoD	NoD
19	4.43(0.15)[a]	167.23(3.38)	0	5.42(0.11)[a]	19.01(0.91)	0.3	14.51(0.86)[a]	19.11(5.29)	0.8	20.28(1.42)[b]	10.29(1.48)	2.0
20	24.08(3.91)[a]	1332.51(32.62)	0	1.52(0.37)[bd]	56.27(0.53)	0	12.21(0.52)[b]	19.47(3.73)	0.6	86.31(6.93)[d]	45.92(1.38)	1.9
21	140.53(12.3)[b]	NoD	NoD	3.85(0.18)[a]	NoD	NoD	6.71(1.75)[d]	NoD	NoD	23.21(2.61)[b]	NoD	NoD

Sample ID*	Mn			Ni			Se			Zn		
	Mushroom	Soil	BCF	Mushroom	Soil	BCF	Mushroom	Soil	BCF	Mushroom	Soil	BCF
22	12.43(1.03)^{a}	1230.31(60.92)	0	0.98(0.21)^{b}	26.95(1.16)	0	11.05(0.77)^{b}	18.61(0.52)	0.6	57.38(7.42)^{ce}	45.31(2.36)	1.3
23	95.81(40.89)^{d}	NoD	NoD	2.14(0.31)^{d}	NoD	NoD	5.43(1.26)^{d}	NoD	NoD	36.83(6.56)^{e}	NoD	NoD
24	45.29(4.55)^{a}	NoD	NoD	2.01(0.31)^{b}	NoD	NoD	8.17(0.91)^{b}	NoD	NoD	77.75(2.96)^{ce}	NoD	NoD
25	1.43(0.25)^{a}	167.23(3.38)	0	5.32(0.28)^{a}	19.01(0.91)	0.3	19.69(0.87)^{e}	19.11(5.29)	1.0	21.15(1.68)^{b}	10.29(1.48)	2.1
26	12.41(0.19)^{a}	NoD	NoD	4.34(0.31)^{a}	NoD	NoD	7.11(0.83)^{bd}	NoD	NoD	85.13(9.21)^{d}	NoD	NoD
27	32.06(11.81)^{a}	375.95(17.12)	0.1	2.63(0.45)^{d}	28.09(2.54)	0.1	10.68(0.86)^{b}	10.48(3.77)	1.0	64.84(7.24)^{cd}	27.25(1.95)	2.4
28	12.14(0.61)^{a}	290.66(6.11)	0.1	3.61(0.19)^{a}	32.27(0.61)	0.1	9.91(1.29)^{b}	16.67(3.22)	0.7	50.82(2.29)^{ce}	26.25(1.07)	2.7
29	14.29(3.04)^{a}	1719.04(86.76)	0	1.94(0.22)^{d}	47.28(3.19)	0	9.47(1.63)^{b}	20.79(2.74)	0.6	214.58(13.95)^{g}	54.66(6.57)	3.9
30	19.69(3.35)^{a}	789.64(35.61)	0.1	1.71(0.32)^{d}	51.68(1.11)	0	13.19(1.45)^{a}	11.75(2.32)	1.4	134.52(13.22)^{h}	47.54(3.64)	3.0

Different superscript letter(s) (a – h) in each column for the same element, indicates statistically significant differences between means at p < 0.05 using one-way ANOVA.

Edible mushrooms:– *Amanita Rubescence[1], Auricularia polytricha[2], Boletus Edulis[3], Boletus Mirabilis[4], Clavulina Cristata[5], Helvella crispa[6], Lacterious Delicious[7], Suillus Luteus[8], Termitomyces microcarpus[9] Termitomyces reticulatus[10], Termitomyces clypeatus[11], Termitomyces umkowaanii[12],*

Inedible mushrooms:– *Amanita foetidissima[13], Amanita Muscaria[14], Amanita Pantherina[15], Aseroe rubra[16], Chlorophyllum molybdites[17], Ganoderma Lucidum[18], Gymnopilus junonius[19], Hypholoma fasciculare[20], Lentinus Villosus[21], Lepista caffrorum[22], Pycnoporus sanguineus[23], Panaeolus papilionaceus[24], Pisolithus tinctorius[25], Pleurotus[26], Podaxis Pistillaris[27] Russula Sardonia[28], Scleroderma citrinum[29], Scleroderma michiganense[30].*

**ND – below instrument detection limit. ***NoD – No available data.

In nature, Se species can either be in organic or inorganic forms. Organic forms include selenomethionine (Semet) and selenocysteine (Secys) while inorganic forms exist in the environment as elemental Se, selenide (Se^{2-}), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (Hatfield *et al.*, 2011; Mehdi *et al.*, 2013). Of the edible wild growing mushrooms, species of *boletus* are considered rich in Se (Costa-Silva *et al.*, 2011; Falandysz, 2008, 2013). Selenium is reported to have anticancer properties, which makes edible species of *boletus* mushrooms an excellent nutraceutical (Ferreira *et al*., 2010).

In South Africa, this mushroom species is not well-known by indigenous people as it was brought to the country when pine trees were planted. Besides being alien, it is only available in pine plantations which are private entities, well-guarded and only available to private companies (Figure A1.1) that collect this species for commercial purposes. Several mechanisms (including DNA cytosine methyltransferase inhibition, antioxidant protection, enhanced immune surveillance, and inhibition of angiogenesis) have been proposed to account for the anticancer potential of Se (Zaidman *et al.*, 2005). The BCF was high in most species, especially in *T. reticulatus*, 12, an edible mushroom, that forms a symbiotic relationship with termites (Van der Westhuizen & Eicker, 1990) and one species that is a favourite to most indigenous people, indicating that mushrooms are excellent accumulators of Se in their fruiting bodies.

Zinc concentrations in mushrooms studied ranged from 12 (*A. polytricha*, edible) to 215 mg kg^{-1} (*S. citrinum*, inedible). The BCF was greater than one for all species, with *A. rubescens*, an edible mushroom, having a BCF of 16. This shows that mushroom species are excellent accumulators of Zn metal. The concentration of trace elements in mushrooms was found to be in decreasing order of Mn > Zn > Cu > Se > Co > Ni > Be.

3.7.3 Toxic elements

Three toxic metals, namely; As, Cd, and Pb, were analysed in this study and the results are presented in Figure 3.3.

The mean concentration of As in mushrooms was below 3.0 mg kg^{-1}. The BCFs for As were below 0.7 for all species studied indicating exclusion of this toxic element, which is consistent with a study carried out by (Melgar *et al.*, 2014). Both arsenical insecticides and combustion of fossil fuel are anthropogenic sources of As and it occurs in natural waters in two forms, namely arsenous acid (H_3AsO_3) and its salt, and arsenic acid (H_3AsO_5) and its salt (Falandysz & Borovička, 2013; Falandysz & Rizal, 2016; Morais *et al.,* 2012). Studies conducted on As concentration in mushrooms show it to be regulated by genetics, type of nutrition and environment. This means that certain species of the same genus (e.g. *Agaricus calvatia*, *Laccaria*, *Langermannia*, *Lepista*, *Collybia*, *Lycoperdon* and *Macrolepiota*) will most likely contain higher amounts of As than the others, i.e. collected from the same habitat (Vetter, 2004).

Cadmium concentrations in mushrooms were below 3.0 mg kg^{-1}, with *A. rubra* (inedible) containing the highest amount of Cd metal. Several studies have shown mushrooms to have a high affinity for Cd from the growing substrate even if the area is unpolluted (A Kabata-Pendias, 2011). *Amanita muscaria* was found to contain high levels of Cd, 29.9 µg g^{-1} dwt (Lepp *et al.*, 1987).

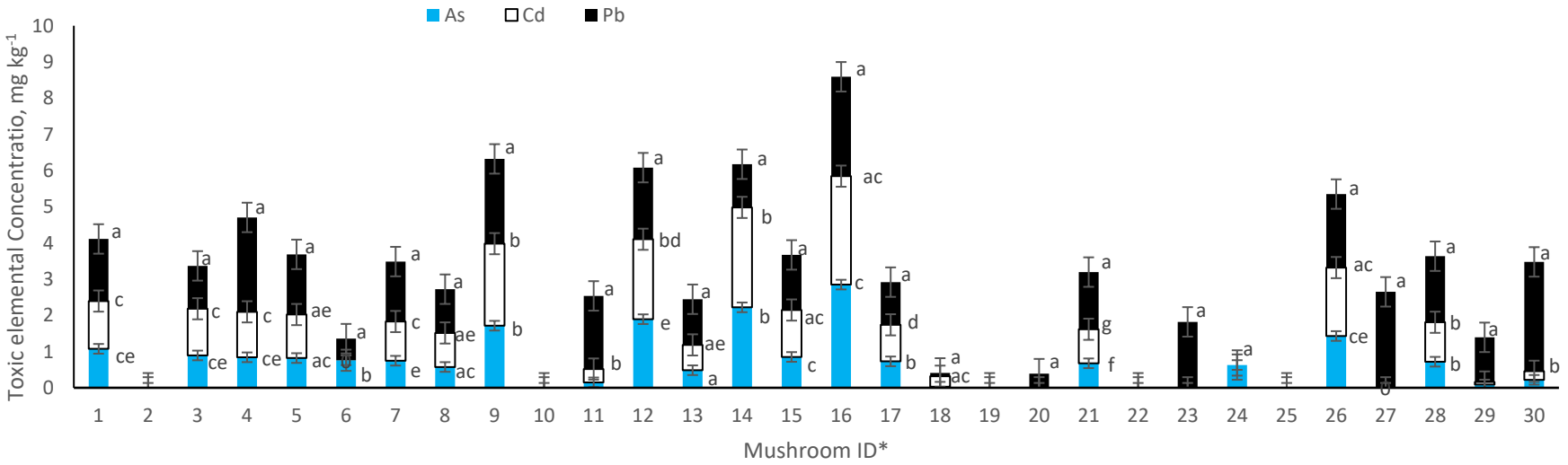

Figure 3. 3 Concentration of toxic elements (As, Cd and Pb) in mushrooms (mg kg^{-1}, dry mass)

*Edible mushrooms: *anita rubescence*[1], *Auricularia polytricha*[2], *Boletus edulis*[3], *Boletus mirabilis*[4], *Clavulina cristata*[5], *Helvella crispa*[6], *Lacterious delicious*[7], *Pleurotus ostreatus*[26], *Suillus luteus*[8], *Termitomyces microcarpus*[9], *Termitomyces reticulatus*[10], *Termitomyces clypeatus*[11], *Termitomyces umkowaanii*[12].
*Inedible mushrooms: *Amanita foetidissima*[13], *Amanita muscaria*[14], *Amanita pantherina*[15], *Aseroe rubra*[16], *Chlorophyllum molybdites*[17], *Ganoderma lucidum*[18], *Gymnopilus junonius*[19], *Hypholoma fasciculare*[20], *Lentinus villosus*[21], *Lepista caffrorum*[22], *Pycnoporus sanguineus*[23], *Panaeolus papilionaceus*[24], *Pisolithus tinctorius*[25], *Podaxis pistillaris*[27], *Russula sardonia*[28], *Scleroderma citrinum*[29], *Scleroderma michiganense*[30].
Different letter(s) (a - g) above the error bars for the same element indicates statistically significant differences between means at $p < 0.05$ using one-way ANOVA.

A study conducted in pristine areas of Northern Poland showed BCFs for Cd to range between 3.3 and 36 in *Parasol* mushrooms (*Macrolepiota procera*) (Gucia *et al*., 2012). A recent study conducted on 28 samples of mushrooms (15 Mycorrhizals and 13 Saprophytes) showed that bioaccumulation of Cd is species dependent and that Agaricus species strongly bio-accumulate this element. Their results showed that *A. macrosporus* contained high levels of Cd, with a mean concentration of 52.9 mg kg^{-1} in the hymenophore and 28.3 mg kg^{-1} in the rest of the fruiting body and a BCF value as high as 1399 followed by *A. sylvicola* (Melgar *et al.*, 2016).

Our study did not include species of the genus *Agaricus* and it might be a good idea to carry out such a study in future. Concentration values from our study were in the range (0 to 3.0 mg kg^{-1}) and average BCF value of 2.0, which only compares with that found in two species (*F. hepatica* and *P. ostreatus*) from the study conducted by Melgar *et al*. (2016). Our findings were however consistent with those obtained from other studies conducted in Turkey, where Cd in mushrooms ranged from 0.26 to 3.24 mg kg^{-1} dry matter (Tuzen *et al.*, 2007; Yamaç *et al*., 2007) and Sweden with values ranging between 0.46 and 5.1 mg kg^{-1} (Mędyk *et al.*, 2017). The concentration of Cd in all but one species (*A. rubra*) was below the maximum permissible level for Cd in cultivated mushrooms (3.0 mg kg^{-1}, dry weight) (European Union 2008).

The mean concentrations of Pb in mushrooms was below 3.0 mg kg^{-1} with *S. michiganense,* an inedible mushroom, having the highest concentration. As shown in Figure 3.3, there is no statistically significant difference in the accumulation of Pb by both edible and inedible mushrooms. Lower BCFs, ranging between 0.01 and 0.05 indicate exclusion of this toxic metal (García *et al.*, 2009). The Food and Agriculture Organization and World Health Organization (2000) has set the provisional tolerable weekly intake of Pb at 25 μg kg^{-1}

bodyweight. For a person with a bodyweight of 60 kg these mushrooms do not pose a health risk.

The concentration of toxic elements in mushrooms was found to be in decreasing order of Cd ≥ Pb > As. The results of this study indicate that elemental accumulation in mushrooms is species dependent and independent on soil concentrations as mushrooms tend to exclude or accumulate elements based on physiological needs.

3.8 Principal Component Analysis

Principal component analysis (PCA) is a procedure which is used to reduce dimensions of multivariate data allowing to understand trends and variations in the data set by a reduced number of latent variables (Tazikeh *et al.*, 2018). In order to determine whether there exist trends and variations amongst the elements accumulated, PCA was carried out. PCA showed three components accounting for 55% of the variability as shown in Figure 3.4. A component loading of greater than 0.7 matrix indicates elements which are closely related to each other.

The first principal component (25%) showed Mn (0.94), Ni (0.94), and Pb (0.83) to have the greatest proximity amongst them, indicating common anthropogenic sources. The second principal component (19%) showed a closer relationship amongst As (0.86), Be (0.77) and Cd (0.86). These metals share a common mobility in soil, greatly limited, hence they owe their mobility to their salts (Kabata-Pendias, 2011). The third principal component (11%) showed Cu (0.80) and Zn (0.85) to have a common association, perhaps it is because of the observed high levels of both elements in mushrooms (Kabata-Pendias, 2011). Mushroom studies, especially on species of *Boletaceae* family (Blanuša *et al.*, 2001), have shown Cu and Zn to have relatively the same transfer factor (mushroom/soil ratio) which could explain the

association between these two elements. Besides this, in a study conducted by Tazikeh *et al*. (2018) they found that these two elements (Cu and Zn) positively correlated to Fe and Al owing to their ability to effectively adsorb to Fe oxides.

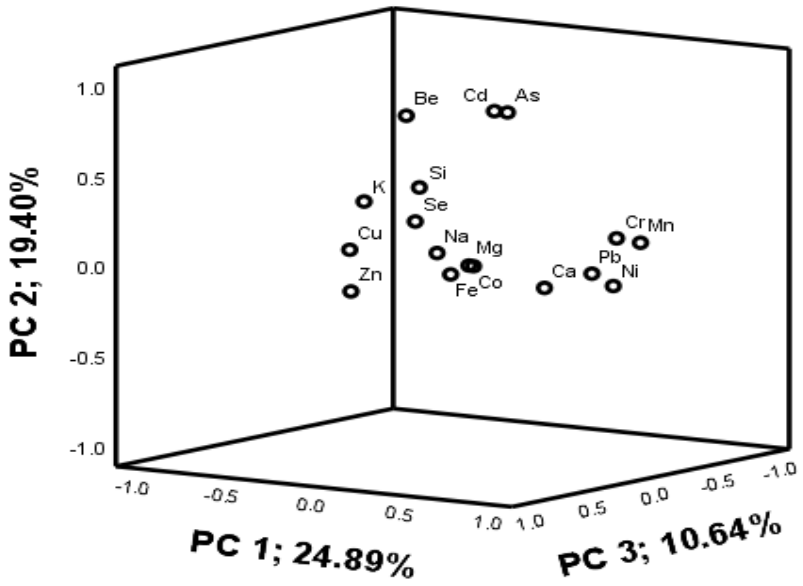

Figure 3. 4 Relationship amongst elemental concentrations in the fruiting bodies projected on a 3D-plane.

Principal component analysis was also conducted on different mushrooms. However, the analysis showed no significant difference in elemental accumulation by edible or inedible mushrooms indicating that accumulation is independent of the edibility or inedibility of mushrooms.

3.9 Nutritional value

The estimated contribution of mushrooms to recommended dietary allowances (RDAs) and adequate intakes (AIs) using 10 g, dry weight, are presented in Table 3.6. In South Africa, mushrooms are prepared in many different ways, including, but not limited to, frying in oil, boiling to make a soup or seasoning for chicken and beef stews (Levin & Branch, 1985; Sangvichien & Taylor-Hawksworth, 2001). After processing or cooking, the estimated elemental concentrations decrease as observed in recent studies (Drewnowska & Falandysz, 2015; Drewnowska *et al*., 2017; Falandysz & Drewnowska, 2015). Our study shows edible mushrooms, especially *B. edulus,* to be rich in Se, contributing an estimated 836 % towards the RDA for this element without exceeding the tolerable upper intake limit (UL).

It is estimated that consumption of 10 g of edible mushrooms would contribute between 2.0 – 10 % towards the AI for K whilst it would contribute 1.0 % towards the IA for Na. The study conducted by Gençcelep *et al*., (2009) found similar results for K hence, mushrooms were recommended for an anti-hypertensive diet. *S. luteus* was found to be rich in Fe contributing 162 % towards the RDA for the element without exceeding the upper UL thereby making the species a good alternative to Fe rich herbs as supplements. *C. cristata* and *H. crispa* were found to be rich in Cu (186 %) and Zn (193%) towards the RDAs, respectively. Elemental levels obtained in this study only serve to provide a guide rather than absolute amount ingested after consuming processed and cooked edible mushrooms (Falandysz & Borovička, 2013).

Table 3.6 Mean contribution to Recommended Dietary Allowances and Adequate Intakes from consumption of 10 g, dry weight of wild growing edible mushrooms

Sample	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn
1	0.85(0.08)	0.02(0.02)	0.54(0.02)	1.40(0.08)	451.39(11.22)	9.93(0.22)	0.07(0.00)	7.62(0.49)	0.04(0.00)	0.14(0.03)	14.07(0.06)
2	4.06(0.29)	0.01(0.01)	0.02(0.00)	0.80(0.01)	95.51(1.62)	9.21(0.06)	0.06(0.00)	2.15(0.01)	0.01(0.00)	0.10(0.01)	1.20(0.04)
3	1.21(0.09)	0.03(0.01)	0.80(0.04)	0.61(0.02)	234.69(12.68)	7.06(0.42)	0.12(0.01)	6.94(0.56)	0.04(0.00)	0.46(0.02)	7.29(0.04)
4	1.16(0.72)	0.02(0.03)	0.70(0.03)	5.80(0.03)	275.91(12.57)	0.66(0.20)	0.17(0.01)	7.95(0.59)	0.05(0.01)	0.10(0.01)	9.08(0.04)
5	4.32(0.09)	0.02(0.02)	1.67(0.05)	3.47(0.61)	445.55(8.91)	11.21(0.34)	0.25(0.02)	5.67(0.25)	0.06(0.01)	0.09(0.01)	13.31(0.05)
6	2.84(0.15)	0.02(0.02)	0.29(0.01)	1.76(0.27)	271.51(12.98)	7.82(0.30)	0.11(0.02)	3.86(0.14)	0.04(0.00)	0.13(0.01)	15.40(0.07)
7	1.25(0.14)	0.02(0.03)	0.18(0.01)	1.01(0.21)	181.46(2.49)	8.96(0.35)	0.06(0.00)	2.74(0.21)	0.03(0.00)	0.11(0.01)	9.98(0.07)
8	1.33(0.06)	0.02(0.01)	0.19(0.01)	12.99(0.40)	237.91(10.30)	7.13(0.16)	0.30(0.01)	3.82(0.24)	0.03(0.00)	0.08(0.01)	4.98(0.02)
9	5.58(0.04)	0.01(0.01)	0.61(0.10)	2.32(0.04)	310.90(18.09)	21.26(0.40)	0.68(0.15)	16.42(0.52)	0.03(0.01)	0.12(0.02)	10.02(0.03)
10	0.54(0.02)	0.03(0.03)	0.36(0.02)	6.07(0.29)	210.87(5.00)	13.49(0.43)	0.17(0.01)	6.81(0.27)	0.05(0.00)	0.12(0.02)	10.39(0.10)
11	1.27(0.04)	0.01(0.01)	0.25(0.04)	4.24(0.03)	328.22(5.20)	13.11(0.24)	0.06(0.00)	4.65(0.08)	0.03(0.00)	0.11(0.02)	7.25(0.02)
12	2.19(0.10)	0.02(0.05)	0.54(0.25)	1.78(0.03)	215.07(8.99)	15.75(0.73)	0.08(0.01)	8.24(0.63)	0.02(0.00)	0.09(0.01)	8.93(0.05)
26	1.85(0.08)	0.01(0.00)	0.66(0.01)	1.10(0.03)	257.90(3.55)	15.40(0.07)	0.12(0.00)	17.41(1.55)	0.04(0.00)	0.07(0.01)	0.85(0.09)
					DRI (mg day^{-1})*						
RDAs/AIs	1.3 x 10^3	0.025 – 0.035	**0.9**	**8 – 18**	4.7 x 10^3	**310**	1.8 - 2.3	1.5 x 10^3	–	**0.055**	**8 - 11**
ULs	2.5 x 10^3	ND	10	45	ND	350	11	2.3 x 10^3	1.0	400	40

Amanita rubescens[1], *Auricularia polytricha*[2], *Boletus edulis*[3], *Boletus mirabilis*[4], *Clavulina cristata*[5], *Helvella crispa*[6], *Lacterious delicious*[7], *Pleurotus ostreatus*[26], *Suillus luteus*[8], *Termitomyces microcarpus*[9], *Termitomyces reticulatus*[10], *Termitomyces clypeatus*[11], *Termitomyces umkowaanii*[12]. RDAs are the Recommended Dietary Allowance, shown in bold, while AI are the Adequate Intakes shown in normal type for age group 19 – 50 years.
ULs are the maximum level of daily intake that is likely to pose no risk of adverse effect. NDs is not detectable due to concentrations being below the instrument's detection limit. DRI represents the Dietary Reference Intakes and are expressed in mg day^{-1}. * Sourced from: Food and nutrition board, Institute of Medicine, National Academies, 2011

3.10 Conclusion

The elemental content in mushrooms was found to be K >> Na > Ca > Mg > Fe; Mn > Zn > Cu > Se > Co > Ni > Be; and Cd ≥ Pb > As. The inedible species, *Aseroe rubra,* was found to contain high amounts of As, and Cd. Principal component analysis revealed that the accumulation of metals by mushrooms was independent of species (edible and inedible) and dependent on soil quality parameters and the nature of the environment. We can therefore conclude that inedibility of mushrooms is not dependent on levels of toxic metals but probably on secondary metabolites. Edible mushroom can accumulate toxic metals if grown in contaminated sites which may make them unsafe for human consumption if eaten in excess. The thirteen edible mushroom species studied were found to be rich in Se (145 – 836 % towards the RDA) with *C. cristata* being rich in Cu, *S. luteus* in Fe, and *H. crispa* in Zn.

3.11 Acknowledgements

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3.12 Conflict of Interest

The authors declare that they have no conflict of interest.

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CHAPTER 4
Elemental bioaccumulation and nutritional value of five species of wild growing mushrooms from South Africa

4.1 Abstract

The collection of wild edible mushrooms for use is an ancient practice. In this study, the elemental concentrations in five mushrooms were compared as a function of species and geographical location. The accumulation of metals from the substrate was found to be species dependent. Mushrooms excluded As, Be, Ca, Cd, Co, Cr, Fe, Mn, Ni and Si, and accumulated elements in the following order: *Amanita pantherina* (K>Na>Zn>Cu>Mg); *Boletus edulis* (edible) (K>Cu >Zn>Se >Na>Mg); *Boletus mirabilis* (edible) (K>Cu>Zn>Na>Mg); *Lactarius deliciosus* (edible) (K>Zn>Mg); and *Russula sardonia* (K>Na>Zn>Cu>Mg). Statistical analysis showed soil parameters to affect elemental accumulation by edible mushrooms more than inedible ones, especially for uptake of Se by *B. edulis*, which indicated an antagonistic relationship with As and Pb in the soil. The results also showed the edible mushrooms to contain proteins (25 – 55 %), carbohydrates (34 – 69 %), ash (3 - 6.5 %) and lipids (0.8 - 5.3 %).

Keywords *Boletus edulis*. *Boletus mirabilis*. *Lactarius deliciosus. Amanita pantherina*. *Russula sardonia*.

4.2 Introduction

Collection of wild growing mushrooms for edible consumption, recreation or treatment of various ailments is an ancient activity in many developed and underdeveloped countries (Bertelsen, 2013; Money, 2017). In South Africa, the knowledge on which this activity is based has usually been passed from one generation to the next by word of mouth; no documentation of mushroom collection is available from pre-colonial times. In the early 1900s, collection and documentation of wild growing mushrooms began in South Africa, with the sole purpose of preserving the species. This culminated in the formation of the South African National Collection of Fungi (PREM), which celebrated its centenary in 2005 (Rong & Baxter, 2006). Collection and preservation information promotes knowledge on biodiversity in a region including hosts or substrates on which they can be found, location, and growth conditions (Rong & Baxter, 2006). Documentation on parasitic fungi that damage crops, trees and foodstuffs, as well as those that cause wood-rot in mining props was included (Levin & Branch, 1985).

Fungi are a highly biodiverse group of organisms and play various roles in nature, the economy, environmental science, food science and health, and are involved in soil mineral weathering, organic substrate decomposition and elemental recycling. For centuries, edible mushrooms have been collected from forests, or later cultivated, and consumed due to their nutritional value, medicinal utility and unique flavor (Deshmukh *et al.*, 2016; Falandysz & Borovička, 2013; Ouzouni *et al.*, 2009). Mushrooms are known to have many of the essentials of a balanced diet, such as proteins, vitamins, and minerals, while at the same time, having low fat and carbohydrate content (Deshmukh *et al*., 2016; Miles & Chang, 2004; Ouzouni *et al*., 2009). As a consequence of differing substrata, atmospheric conditions, age and part of the fructification, the chemical composition of edible mushrooms, their nutritional value and sensory properties differ according to species (Alam *et al*., 2008; Ayodele & Okhuoya, 2009;

Ouzouni *et al*., 2009). Aside from their nutritional value, some mushrooms have medicinal benefits thereby making them nutraceuticals or functional foods (Miles & Chang, 2004).

Wild edible mushrooms may contain relatively high concentrations of essential elements, even if growth soils concentrations are low due to a large surface area to volume ration that promotes high nutrient uptake (Agerer, 2001). This contrasts with vascular plants, for which high concentrations of metals are mostly related to metal-rich soils (Falandysz & Borovička, 2013; Kabata-Pendias, 2011). It has been reported that the elemental content in mushrooms constitutes between 56 to 70% of the ash, generally in the order K > P > Na > Ca > Mg (Miles & Chang, 2004).

Wild mushrooms of the genus *Termitomyces* are considered superior by most indigenous people with the species *Termitomyces Heim* (known to have a symbiotic relationship with termites) being commonly consumed by the indigenous people of South Africa (Deshmukh *et al*., 2016). Alien mushrooms, which were introduced during the establishment of eucalyptus and pine plantations (Joubert, 2012), including *Amanita pantherina, Boletus edulis, Boletus mirabilis, Lactarius deliciosus* and *Russula sardonia* are also found in South African environments. Although three of these alien mushrooms are edible and considered a delicacy in many European countries, South Africans do not collect them due to unfamiliarity and lack of access as they are only found on private farms or in plantations owned by large corporations (Clarke & Isaacs, 2005).

Though *Boletus edulis* is exported to other international countries, this mushroom is not sold at low-income South African food markets (Joubert, 2012). The main pine species grown in KwaZulu-Natal plantations (*Pinus patula, Pinus elliottii* and *Pinus taeda*) have symbiotic relationships with four mycorrhizae mushrooms (*A. pantherina, B. edulis, L. deliciosus* and *R. sardonia)* while the fifth species (*B. mirabilis*) is a saprophyte as it decomposes dry pine stumps. Application of fertilizers containing N, P, K, Mg, Cu and B increases the nutrient

content of pine needles and the potassium content of the soil, which enhances mycorrhizal development. These mushrooms, in turn, transport immobile or scarce elements to the plant, which are vital to their optimum growth (Donald *et al.*, 1987).

The five wild growing mushroom species, especially *B. edulis*, have extensively been studied, Table 2.1 (Alaimo *et al*., 2018; Falandysz *et al*., 2017; Kojta & Falandysz, 2016; Proskura *et al.*, 2017; Zhang *et al*., 2010). However, no study on the bioaccumulation of elements and nutritional content of the species growing under uncontrolled environments in South Africa has ever been conducted. Previously, we reported on the elemental distribution in wild growing mushrooms (Rasalanavho *et al.*, 2019). The aim of this study was to investigate the bioaccumulation of elements from the environment and hence, evaluate the impact of geographical location on uptake by wild growing mushrooms found in the pine plantations of KwaZulu-Natal, South Africa, and also to assess for nutritional value.

4.3 Materials and methods

4.3.1 Collection, storage and treatment of mushroom species

The samples of mature fruiting bodies of wild growing mushrooms were collected from different Pine or Eucalyptus plantations owned by the Sappi, Mondi and Merensky companies in South Africa, as described in Appendix A1. The sampling points for the five-mushroom species, listed in Table 3.1, were mainly limited to pine plantations located in KwaZulu-Natal, South Africa. Each mushroom species was sampled at no less than seven sites. Fruiting bodies (both the pileus/cap and stipe/stalk) of fully-grown mushrooms were hand-picked from their natural environment. The fruiting bodies were cleaned of soil substrate, plant and grass residue using a plastic knife and/or a paint brush. Samples were then cut into pieces using a plastic

knife and dried to constant mass in an oven set at 40 °C. Dried mushrooms were then pulverized using a porcelain mortar and pestle as illustrated in Figures A1.2 to Figure A1.3.

The powdered samples were stored in low density polyethylene (LPDE) plastic bottles, which were capped and then sealed with Parafilm^{TM} (Laboratory Film, Bemis Flexible Packaging, NEENAH, WI 54956) to prevent rehydration. All glassware and plastic bottles had been previously soaked overnight in a detergent, then rinsed thoroughly, then soaked in 10% HNO_3, then rinsed with distilled water and finally with ultra-pure water.

The corresponding mushroom soil substrate layer (0 – 18 cm depth) was also sampled from six points (25 cm apart) around the mushrooms, using a hand auger. Soil samples were thoroughly mixed together to produce a representative sample. This was air dried at room temperature in the laboratory for several weeks, passed through a 2 mm mesh then crushed with a porcelain mortar and pestle before being stored in LPDE plastic bottles. Soil and mushroom samples were stored in a dry cupboard at room temperature until needed for the digestion phase.

All polytetrafluoroethylene (PTFE) digestion vessels were soaked overnight in 10% (v/v) HNO_3 solution and rinsed with ultra-pure water before digestion, to avoid cross-contamination. The powdered mushroom samples (~0.25 g, accurately weighed) were predigested with 10.0 mL of HNO_3 (69 %) for 45 min in open PTFE vessels prior to addition of 2.5 mL H_2O_2 (30 %) before digestion. All analytical reagents used had been purchased from Merck (kGaA 64271 Darmstadt, Germany). The vessels were closed and temperature-digested in an automatic microwave digestion system (MARS 6 CEM Corporation, Matthews, NC, USA) using Xpress Plus vessels. The microwave was set at 1600 W (100% power), after which the temperature was increased up to 180°C for 20 min, held at this temperature for 45 min and then allowed to cool naturally in the microwave for 15 min. After digestion, mushroom samples were allowed to cool to room temperature in the fume hood, before being diluted to 50.0 mL

using ultra-pure water. Samples were stored in high density polyethylene (HDPE) bottles and kept in the refrigerator at 4 °C until analysis was carried out, which was within a week.

Soil samples (~0.25 g, accurately weighed) were digested similarly to the mushrooms. After digestion and cooling, samples were filtered using Whatman^{TM} No. 1 qualitative filter paper and diluted to 50.0 mL using ultra-pure water. Samples were stored in HDPE bottles and refrigerated at 4°C until analysis was carried out within a week. All samples (mushroom and soil) and blanks were digested and analysed in triplicate.

4.3.2 Instrumentation method for elemental analysis

Analyses were carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES; the Perkin Elmer® Optima™ 5300 Dual View, Billerica, Massachusetts, USA). The RF power of the instrument was set at 1.30 kW with axial plasma observation. The plasma argon, auxiliary and nebulizer gas flow rates were 15.0, 0.20 and 0.80 L min^{-1}, respectively. The peristaltic pump and auto-sampler wash rates were both 1.50 mL min^{-1}. High-purity Ar (99.995%) supplied by Air Products South Africa (Pty) Ltd. (Kempton Park) was used to fuel the plasma and as a carrier gas. ICP-OES was the chosen instrument for elemental analysis due to its multi-element capabilities, extremely low detection limits and large linear range. Method used for determining selenium in mushrooms was modified following a study conducted by Falandysz (2013) in this field. Calibration standards were prepared by diluting 1000 mg L^{-1} ± 2 in 2% (w/w) HNO_3 of analytical grade (TraceCERT®; Fluka Analytical; Sigma-Aldrich; St Louis, Mo, USA), using ultra-pure water.

4.3.3 Quality control

The certified reference material (CRM) used for elemental analysis of mushrooms was white clover (BCR® - 402) obtained from the Institute for Reference Materials and Measurements (IRMM), Belgium and for soil it was metals in soil (D081-540), Sigma-Aldrich. The CRMs were digested using the same method as employed for the samples. For accuracy and precision of the analytical method, analyses of the CRMs were carried out at the beginning, middle and end of sample analysis. In total, eight BCR® - 402 and five D081-540 CRM solutions were prepared, and each solution was analysed three times during each run. Measured values were within the quality control performance acceptance limits as shown in Table 4.1 and Table 4.2. Our results for the controlled experiments were comparable to those of a similar study obtained for BCR-381 (Ouzouni *et al*., 2009).

Table 4. 1 Method validation parameters for elemental analysis of the certified reference material, white clover (BCR® - 402) (mean (SD), n = 24)

Analyte	Measured value ($mg\ kg^{-1}$)	Certified value ($mg\ kg^{-1}$)	Recovery (%)
Se	6.0(0.82)	6.7(0.25)	90.4
Fe	240.0(19)	244	98.6
Ni	8.3(0.28)	8.25	100.4
Zn	28.0(8.8)	25.2	109.5

Table 4. 2 Method validation parameters for elemental analysis in soil certified reference material, D081-540 (mean (SD), n = 15)

Analyte	Wavelength (nm)	LOD* (mg kg^{-1})	LOQ** (mg kg^{-1})	Measured value (mg kg^{-1})	Certified value (mg kg^{-1})	Acceptance Limits (mg kg^{-1})	Recovery (%)
As	228.812	0.0830	0.2515	75(7.2)	88.4(5.92)	71.5 – 105	85.5
Be	313.042	0.0003	0.0009	50(3.7)	55.8(6.26)	45.8 – 65.9	89.1
Ca	422.673	0.0100	0.0303	7555(550)	7530(7.28)	6210 – 8850	100.3
Co	228.616	0.0070	0.0212	190(14)	199(4.10)	166 – 233	94.1
Cu	324.752	0.0054	0.0164	260(18)	268(4.72)	219 – 317	96.0
Fe	259.939	0.0062	0.0188	11130(1347)	12800(18.0)	5380 – 20100	86.9
K	766.490	45.407	138.50	2580(312)	2570(7.55)	1860 – 3290	100.3
Mn	259.372	0.0016	0.0048	412(35)	425(9.69)	347 – 502	96.9
Ni	341.476	0.0480	0.1455	226(17)	236(4.17)	194 – 279	95.7
Se	196.026	0.0750	0.2273	110(11)	127(4.47)	98.4 – 156	87.6
Zn	213.857	0.0018	0.0055	110(10)	130(11.5)	106 – 155	82.5

LOD* represents the limit of detection limit.
LOQ** represents the limit of quantification.

4.3.4 Bioaccumulation factor (BAF)

Bioaccumulation refers to the gradual accumulation of substances in a living organism. These include toxic substances, of which, heavy metals and polychlorinated biphenyls are of major concern. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate faster than that at which the substance is lost (Arnot & Gobas, 2006). Accumulation of elements from the soil substrate by the fruiting body of mushrooms was evaluated through the calculation of BAFs according to Equation 4.1 (Mahlangeni *et al.*, 2019).

$$BAF = \frac{C_m}{C_s} \quad \text{Equation 4. 1}$$

where *Cm* is the mean concentration of an element in the mushroom and *Cs* is the mean concentration of the same element in the soil (dry weight).

4.3.5 Protein content in mushrooms

The crude protein in the mushrooms were determined by the macro-Kjeldahl method. The Kjeldahl method can conveniently be divided into three steps: digestion, neutralization and titration. The powdered mushroom samples (~0.50 g, accurately weighed) were digested in a Kjeldahl flask with 25 mL of 98% H_2SO_4 solution, potassium sulfate (~10 g, accurately weighed) and one spatula of mercury (II) oxide catalyst while heating in a mantle heater under a fume hood. After completion of the digestion process, seen as a clear solution, 240.0 mL of ultra-pure water was added to the flask. Thereafter concentrated sodium hydroxide solution (80.0 mL), prepared by dissolving solid NaOH pellets (~45 g, accurately weighed in 75.0 mL of ultra-pure water) was added into the flask. About 5 grains of Zn metal catalyst were then added and the contents of the flask were mixed thoroughly and immediately distilled with the delivery tube being immersed below the surface of 25.0 mL of 0.1 M HCl solution. Distillation was deemed complete after approximately 270 mL of distillate were collected. The distillate was then titrated with 0.1 M NaOH solution with the aid of methyl orange indicator. The nitrogen content (% N) in the sample was calculated using Equation 4.2.

$$\%\, Nitrogen = Molarity\ of\ acid \times \frac{(V_a - V_b)}{10 \times mass\ used} \times 14.07 \quad \text{Equation 4. 2}$$

Where V_a and V_b represent volume of acid and titration volume of the alkaline solution respectively. A conversion factor of 4.38 (0.7 x 6.25) based on the presence of 70% of digestible

protein was used to convert % N to % crude protein in the mushroom sample. This is because mushrooms contain a considerable amount of non-protein nitrogen in the form of chitin in their cell walls (Chang & Hayes, 2013).

4.3.6 Lipids content in mushrooms

The amount of lipids in the mushrooms was determined according to the soxhlet extraction method, EPA 3540C. Briefly, 10.0 g of the powdered mushroom sample was weighed into a thimble, which was then covered with a filter paper. The thimble and its contents were inserted into a soxhlet extractor, and extracted with 250.0 mL of hexane for 9 hours. The solution in the round bottom flask was filtered into a clean pre-weighed flask. The extraction procedure was repeated with fresh 250.0 mL hexane. Both extracts were combined and concentrated using a rotary evaporator. The lipid was dried in a vacuum oven set at 50 ºC. Percent lipid was calculated using Equation 4.3.

$$\%\ lipids\ in\ mushrooms = \frac{mass\ of\ lipid}{mass\ of\ dry\ sample} \times 100 \quad \text{.....Equation 4. 3}$$

4.3.7 Ash content in mushrooms

The ash content in the mushroom was determined by accurately weighing 5.0 g of dry powdered mushroom sample into a clean pre-weighed crucible. The mushroom was incinerated in an oven set at 600 ºC for 24 hours. The crucible and its contents were cooled in a desiccator and thereafter weighed. The amount of ash was determined by difference using Equation 4.4.

Ash content = mass of crucible and contents after heating – mass of empty crucible …… Equation 4. 4

4.3.8 Moisture content, carbohydrate estimation, and total energy in mushrooms

The moisture content was determined by drying pre-weighed fresh mushroom samples to constant mass in an oven set at 40 ºC. Percentage moisture was calculated using Equation 4.5.

$$\%\ moisture\ in\ mushrooms = \frac{M_s - M_d}{M_s} \times 100 \quad \text{.....Equation 4. 5}$$

where M_s is the mass of sample mushroom used and M_d the mass of dry mushrooms.

The carbohydrate content present was estimated using Equation 4.6.

% Carbohydrate = 100 – (protein + lipids content + ash content + moisture content) …. Equation 4. 6

Total energy was calculated using Equation 4.7.

Energy (Kcal) = 4 × (protein + carbohydrate) + 9 × lipid content ……. Equation 4.7

4.3.9 Soil pH and soil organic matter (SOM)

A soil slurry was prepared by thoroughly mixing 10.0 g of dried soil with 25 mL of 1M KCl solution which was allowed to stand for 30 minutes before the pH was measured using a standard glass electrode (MeterLab® PHM210, Radiometer Analytical). SOM was determined using the wet chemistry extraction procedure (Walkley & Black, 1934) with minor modifications. Briefly, approximately 0.30 g of the dried soil sample was measured into a dry conical flask. Thereafter, 10.0 mL of 1 N $K_2Cr_2O_7$ and 20.0 mL of 98% H_2SO_4 were added to the sample. While monitoring with a thermometer, the mixture was heated to 135 ºC. The resulting solution was cooled under the fume hood and thereafter ultra-pure water was added to the mixture to give a total volume of 200 mL. After adding 4 drops of Ferroin indicator, the mixture was titrated using 0.4 N ferrous sulfate; titrations were done in triplicate. SOM was determined using Equation 4.8.

$$\%\ SOM = 3\ \times \frac{[1 - (sample\ titration \frac{volume}{blank} titration\ volume)]}{mass\ of\ dry\ sample} \quad \text{.....Equation 4. 8}$$

4.4 Data analyses

Statistical data analysis involved one-way analysis of variance (ANOVA), using Statistical Package for the Social Sciences (SPSS) version 25 program (IBM Corp., USA). This was followed by Tukey's post-hoc test to compare means of mineral content from each mushroom species collected from each site at a significant level of $p < 0.05$.

4.5 Results and discussions

4.5.1 Soil parameters, pH and SOM

The effect of soil parameters such as pH and soil organic matter (SOM) on the solubility of elements needed for plant or mushroom growth and development is well documented (Kabata-Pendias, 2011). SOM is the organic fraction of the soil that influences numerous soil properties and comprises of decomposed plant and animal material as well as microbial organisms (Chan, 2008). SOM is the key component in the creation and maintenance of high-quality soil; it increases its cation exchange capacity and water-holding capacity (Dick & Gregorich, 2004). Equilibrium levels of SOM depend on climate, landscape, texture, inputs and disturbances. In this study, the pH of the soil samples ranged from 3.87 and 4.31 as shown in Table 3.1, indicating that mushrooms thrive or grow in acidic environments. The SOM values ranged between 1.50 and 13.7 %, Table 3.1 which falls within most agricultural soils in South Africa. Soil samples were, to a large extent, considered rich in SOM, which can be explained by samples being obtained where there was absence of non-tillage activities which results in low oxidation levels of organic matter (Fey & Mills, 2003; Lehmann & Kleber, 2015).

4.5.2 Nutritional content of edible mushrooms

The moisture and nutritional (protein, lipid, ash, carbohydrates and energy, based on dry weight) content in different parts (pileus and stipe) of the mature fruiting bodies of the three species of wild growing edible mushrooms (*B. edulis, B. mirabilis* and *L. deliciosus*) are shown in Table 4.3. The moisture content ranged from 90 to 93 %, with *B. mirabilis* containing the highest amount in the stipe. There was no statistically significant difference in the moisture content between either of the morphological parts of the mushroom body or amongst the species studied. Our results were similar to those previously reported (Di Anibal *et al.*, 2015).

Table 4. 3 Nutritional content (protein, lipid, ash, carbohydrates) based on dry mass, energy content (kcal/100 g, dry weight) and moisture content in different parts of the mushrooms (mean (SD), n=3)

Species	Part	Moisture	Protein content		Lipid	Ash	Carbohydrates	Energy
		%	Instrumental	Kjeldahl	%	%	%	Kcal/100 g
B. edulis	Pileus	90.0(0.4)^{a*}	39.0(0.5)^{d}	37.0(0.8)^{e}	5.0(1.0)^{c}	6.5(0.1)^{d}	51.7(0.8)^{a}	398.5(5.0)^{c}
	Stipe	90.3(1.7)^{a}	22.5(0.8)^{b}	21.3(0.9)^{b}	1.5(0.0)^{a}	6.0(0.1)^{e}	57.2(0.2)^{d}	390.8(1.0)^{a}
B. mirabilis	Pileus	91.5(0.3)^{a}	31.2(2.1)^{c}	33.0(0.4)^{d}	3.3(0.0)^{b}	6.5(0.3)^{de}	70.0(1.2)^{b}	410.4(0.4)^{b}
	Stipe	92.5(1.2)^{a}	22.2(0.7)^{b}	27.4(0.6)^{c}	0.8(0.0)^{a}	4.6(0.2)^{c}	71.3(1.0)^{c}	383.0(0.3)^{ab}
L. deliciosus	Pileus	91.2(0.5)^{a}	21.0(0.5)^{b}	20.8(1.2)^{b}	5.3(0.1)^{b}	4.0(0.0)^{b}	67.2(0.8)^{d}	385.7(1.0)^{d}
	Stipe	90.3(0.2)^{a}	17.5(1.3)^{a}	18.0(0.7)^{a}	3.0(0.0)^{c}	3.0(0.1)^{a}	76.0(0.8)^{e}	403.0(0.4)^{c}

*Different superscript letters in a column represents significant differences between means ($P < 0.05$), Tukey's post hoc test for multiple comparisons

Generally, mushroom species were found to be rich in protein, ranging between 17.5 (*L. deliciosus*) to 39.0 % (*B. edulis*), with the pileus being richer than the stipe. Our results were slightly higher than those reported in some mushroom species (Alam *et al*., 2008; Ayodele &

Okhuoya, 2009; Heleno *et al*., 2009) but comparable to others (Barros *et al*., 2007; Heleno *et al*., 2015). These findings indicate that protein content is dependent on both species and morphology (pileus or stipe).

The lipid content of mushrooms ranged between 0.8 (*B. mirabilis*) and 5.3 % (*L. deliciosus*) showing that mushrooms contain very small amount of lipids in the fruiting body, although generally, the pileus is richer in lipids than the stipe. These findings are similar to those previously reported for other mushroom species (Heleno et al., 2009; Heleno et al., 2015). The ash content in the mushrooms ranged between 3.0 (*L. deliciosus*) to 6.5 % (*B. edulis*). Similar to lipid content, the pileus contained higher amounts of ash than the stipe. Our results were slightly higher than those previously reported for *B. edulis* (Heleno *et al*., 2015). The differences could be attributed to geographical origin and effects of soil quality.

The higher the protein content the lower will be the carbohydrate content, as they are arithmetically related by difference. The results showed the stipe to contain higher amounts of carbohydrates than the pileus. In particular, *L. deliciosus* contained the highest amount of carbohydrates (67.2 g in the pileus and 76.0 g in the stipe). The lowest amount (51.7 g in the pileus and 57.2 g in the stipe) was obtained in *B. edulis.* Our results were lower than those previously reported on *B. edulis* (Heleno et al., 2015). The energy profile ranged between 383.0 and 410.4 Kcal per 100 g of dry mushrooms. In the mushrooms, *B. edulis* and *B. mirabilis*, the general pattern was that the pileus showed a higher energy profile than the stipe which was not the case with *L. deliciosus*.

Results from ANOVA ($p < 0.05$) and Tukey's post hoc test showed significant differences between means for protein, lipid, ash, carbohydrate and energy content with regards to morphological parts (pileus and stipe) and species. Translocation of minerals in mushrooms (from mycelium to stipe to pileus) is simpler to those of vascular plants; it was expected that the pileus (for storage) would contain relatively higher concentrations of minerals. Similar

patterns of results were observed for *B. edulis, L. edodes* and *X. badius* in a previous study (Heleno *et al*., 2015). However, our results differ from those obtained by Di Anibal *et al.* (2015) for different mushroom species. Studies conducted on edible leafy green vegetables that are consumed by rural and semi-urban residents in South Africa show that the nutrient content in vegetables is considerably less than that found in the three edible mushroom species in this study (Mavengahama, 2013). During their time of abundance (rainy season), the nutrient content of these mushrooms suggest that they would be excellent food supplements, which can go a long way in combating food insecurity in the country.

4.5.3 Elemental profile in all studied mushrooms

The elemental composition for the edible *B. edulis* (Table 4.4), *B. mirabilis* (Table 4.5) and *L. deliciosus* (Table 4.6) and the inedible *A. pantherina* (Table 4.7) and *R. sardonia* (Table 4.8) are presented below. Potassium was found to be highly accumulated by all five species, with *A. pantherina* containing the highest amount (71.08 g kg^{-1}) with a corresponding BAF value of 144.84 from samples collected at Singisi. The results showed accumulation of K to be environmentally dependent as a huge range of variation within a species collected from different geographical sites (Figure A2.1 through Figure A2.5) was observed. Amongst the species, *L. delicious* had low accumulation of K with samples collected from Gilboa farm having the highest concentration (20.75 g kg^{-1}). The order of accumulation of K amongst species, through the use of BAF values, was found to be *A. pantherina* > *B. mirabilis* > *R. sardonia* > *B. edulis* > *L. deliciosus*, in the ratio of 1:3:2:2:3, respectively. Three edible species (*B. mirabilis, B. edulis* and *L. deliciosus)* accumulated appreciable amounts of K, as such, can be used as a source of this essential element.

Table 4. 4 Elemental composition (mean (SD), dry weight) in the fruiting bodies of *B. edulis* species (M) and soil substrate (S) collected from eight different sites as well as the corresponding bioaccumulation factor (BAF)

Element	Type	SITES							
		Bulwer (9)* **	**Cidara (5)**	**Donnybrook (7)**	**Greytown (4)**	**Singisi (23)**	**Stepmore A (15)**	**Stepmore B (11)**	**Twasamhlobo (17)**
As*	M	0.89(0.03)^{bc}	1.71(0.02)^{d}	1.68(0.3)^{d}	0.38(0.1)^{a}	1.22(0.1)^{c}	0.68(0.2)^{ab}	0.67(0.1)^{ab}	0.69(0.1)^{ab}
	S	9.88(1.3)	6.38(2.1)	1.85(2.9)	8.92(5.2)	1.85(1.1)	8.02(2.3)	3.66(4.6)	4.80(2.5)
Ca*	M	126.62(23)^{cd}	26.47(4.0)^{a}	33.25(8.0)^{a}	69.28(3.0)^{ab}	97.81(12)^{bc}	206.28(20)^{e}	168.29(41)^{de}	106.26(2.0)^{bc}
	S	425.99(8.9)	401.71(5.7)	575.29(16)	524.73(9.3)	280.87(13)	444.27(8.8)	314.86(8.9)	676.64(16)
Cd*	M	1.39(0.0)^{bc}	1.85(0.1)^{d}	1.74(0.2)^{d}	1.03(0.1)^{a}	1.64(0.1)^{cd}	1.15(0.1)^{ab}	1.16(0.0)^{ab}	0.96(0.1)^{a}
	S	7.90(0.1)	8.17(0.4)	3.57(0.2)	9.39(0.2)	4.06(0.4)	7.51(0.2)	4.70(1.0)	7.13(0.1)
Co*	M	2.08(0.1)^{c}	0.17(0.1)^{a}	1.17(0.1)^{b}	2.00(0.2)^{c}	0.30(0.1)^{a}	1.69(0.3)^{bc}	1.31(0.2)^{b}	4.44(0.3)^{d}
	S	16.72(0.1)	9.58(0.9)	3.01(0.2)	11.31(0.1)	3.91(0.2)	15.13(0.2)	10.67(0.2)	17.04(0.3)
Cr*	M	2.35(0.2)^{a}	9.47(16)^{a}	0.05(0.1)^{a}	1.52(0.0)^{a}	0.47(0.2)^{a}	0.73(0.2)^{a}	0.68(0.2)^{a}	2.43(0.1)^{a}
	S	86.89(4.0)	104.40(7.0)	35.81(6.0)	112.44(2.0)	45.56(2.0)	91.96(2.0)	57.40(1.0)	107.59(2.0)
Cu*	M	101.09(3.5)^{f}	39.71(4.0)^{a}	76.17(2.0)^{de}	63.62(3.0)^{bcd}	81.98(2.0)^{e}	52.77(16)^{abc}	51.62(2.5)^{ab}	70.22(1.0)^{cde}
	S	27.76(0.4)	10.41(0.6)	10.17(0.5)	33.22(0.9)	7.36(0.4)	27.17(0.4)	18.08(2.0)	26.95(0.5)
	BAF	3.64	3.82	7.49	1.92	11.14	1.94	2.85	2.61
Fe**	M	0.04(0.0)^{ab}	0.04(0.0)^{ab}	0.02(0.0)^{a}	0.13(0.0)^{d}	0.03(0.0)^{a}	0.07(0.0)^{bc}	0.08(0.0)^{c}	0.05(0.0)^{abc}
	S	41.45(0.4)	42.94(0.1)	25.40(1.0)	45.69(0.4)	27.55(0.1)	39.69(0.4)	26.82(0.5)	38.49(0.8)
K**	M	23.59(0.2)^{bcde}	18.18(0.5)^{a}	19.37(0.5)^{ab}	21.43(0.6)^{abcd}	20.52(0.3)^{abc}	26.47(0.4)^{de}	27.09(0.4)^{e}	24.77(0.5)^{cde}
	S	6.16(0.3)	0.35(0.0)	0.48(0.0)	4.32(0.1)	0.49(0.0)	4.66(0.1)	4.65(0.3)	0.46(0.0)
	BAF	3.83	52.42	40.70	4.96	41.82	5.68	5.83	53.87
Mg**	M	0.76(27)^{b}	0.54(12)^{a}	0.56(4.0)^{a}	0.60(15)^{a}	0.74(26)^{b}	0.76(29)^{b}	0.75(79)^{b}	0.86(9.0)^{c}
	S	2.15(29)	0.22(15)	0.41(15)	1.26(23)	0.68(33)	1.98(11)	1.28(38)	1.15(26)
Mn*	M	7.42(0.5)^{abc}	5.24(0.4)^{ab}	3.53(0.3)^{a}	10.72(0.3)^{bc}	11.00(0.4)^{bc}	22.69(4.0)^{d}	17.24(4.0)^{d}	11.21(0.5)^{c}
	S	216.75(2.0)	169.67(7.0)	76.81(1.0)	204.33(4.0)	141.75(4.0)	144.98(2.0)	135.97(2.0)	609.71(13)
Na**	M	0.43(0.0)^{ab}	0.93(0.1)^{d}	0.62(0.0)^{bc}	0.30(0.0)^{a}	0.43(0.0)^{ab}	1.05(0.3)^{d}	0.77(0.0)^{cd}	0.35(14)^{ab}
	S	0.27(0.0)	0.28(0.0)	0.27(0.0)	0.28(0.0)	0.27(0.0)	0.28(0.0)	0.28(0.0)	0.28(0.9)
	BAF	1.63	3.33	2.33	1.04	1.63	3.75	2.79	1.26
Ni*	M	0.17(0.4)^{a}	ND	ND	3.28(0.1)^{b}	0.75(0.9)^{a}	0.56(0.5)^{a}	0.57(0.1)^{a}	3.61(0.2)^{b}
	S	51.04(0.2)	22.54(1.0)	13.38(0.4)	46.19(0.6)	15.83(0.5)	48.52(0.6)	34.03(1.0)	35.66(0.7)
Pb*	M	ND	0.31(0.6)^{a}	0.80(1.5)^{ab}	2.02(1.0)^{a}	ND	ND	ND	0.06(0.6)^{b}
	S	87.40(6.0)	60.86(2.0)	43.44(4.0)	97.84(2.0)	48.76(0.4)	76.76(1.5)	51.40(5.0)	55.28(0.1)
Se*	M	37.45(1.0)^{ab}	40.30(3.0)^{bc}	47.98(3.0)^{cd}	30.54(1.0)^{a}	53.00(0.8)^{d}	42.34(6.0)^{bc}	42.85(4.0)^{bc}	46.85(2.0)^{bc}
	S	14.92(4.0)	21.20(2.0)	ND	22.77(2.0)	ND	9.32(4.0)	7.14(2.0)	17.57(6.0)
	BAF	2.51	1.90	ND	1.34	ND	4.54	6.00	2.67
Si*	M	63.84(7.0)^{b}	15.68(3.0)^{a}	15.57(1.0)^{a}	ND	191.17(22)^{c}	74.83(25)^{b}	92.13(17)^{b}	ND
	S	231.45(32)	191.20(18)	195.98(21)	298.77(62)	166.51(15)	458.49(212)	428.49(12)	92.56(18)
Zn*	M	75.76(2.0)^{c}	59.86(2.5)^{a}	56.43(2.5)^{a}	82.61(0.6)^{c}	74.42(6.5)^{bc}	53.63(9.0)^{a}	63.39(3.0)^{ab}	107.07(2.0)^{d}
	S	52.08(2.0)	10.45(0.4)	8.70(0.5)	31.38(2.0)	16.39(2.0)	48.86(2.0)	36.02(4.0)	22.45(1.0)
	BAF	1.45	5.73	6.47	2.63	4.54	1.10	1.76	4.77

* Values expressed in mg kg^{-1}. ** Values expressed in g kg^{-1}. *** Value in bracket represents the number of fruiting bodies collected at a specified sampling site.

Potassium plays a crucial role in controlling blood pressure in the human body, and an increased intake of K reduces the risk of cardiovascular and kidney disease through the lowering of Na^+ - Cl^- cotransporter (NCC) activities (McDonough *et al.*, 2017). The amounts of K in *B. edulis* and *B. mirabilis* measured in this study were similar to those found in other studies among other *Boletus* species (Alaimo *et al*., 2018; Brzezicha-Cirocka *et al.*, 2016; Falandysz & Borovička, 2013; Proskura *et al*., 2017; Zhang *et al*., 2010). The amounts of K in *A. pantherina* (this study) were higher than those reported for another *Amanita* species found in Poland (Falandysz *et al*., 2018). Correlation analysis (Table A3.1 through Table A3.5) showed no influence on the accumulation of K by pH, SOM and altitude. Being an important element in mushrooms, K is accumulated in large concentrations regardless of its availability in the soil substrate. The growth of pine trees is significantly enhanced by the availability of both K and P (Donald *et al*., 1987), hence the significant amount of K in mushroom species owing to the symbiotic relationship with vascular plants.

All five species were found to accumulate Zn, albeit to varying degrees. In general, the amount of Zn varied within a species collected from different sites (Figure A2.1 through Figure A2.5), as well as amongst mushroom species. *A. pantherina* was found to contain the highest concentrations of Zn (96.61 to 212.53 mg kg^{-1}), followed by *L. deliciosus* (59.37 to 150.53 mg kg^{-1}), *B. mirabilis* (47.39 to 130.44 mg kg^{-1}), *B. edulis* (53.63 to 107.07 mg kg^{-1}), and *R. sardonia* (39.44 to 65.76 mg kg^{-1}). At Bulwer and Singisi, where all five species were sampled, concentrations of Zn (in mg kg^{-1}) were found to be in decreasing order of *A. pantherina* (120.23) > *B. mirabilis* (94.71) > *L. deliciosus* (82.94) > *B. edulis* (75.76) > *R. sardonia* (47.20) and *A. pantherina* (136.83) > *B. mirabilis* (130.44) > *L. deliciosus* (119.42) > *B. edulis* (74.42) > *R. sardonia* (48.60), respectively. These findings indicate that accumulation of Zn by mushrooms are species and site dependent.

Table 4. 5 Elemental composition (mean (SD), dry weight) in the fruiting bodies of *B. mirabilis* species (M) and soil substrate (S) collected from eight different sites as well as the corresponding bioaccumulation factor (BAF)

Element	Type	SITES							
		Boston (15)* **	**Bulwer (7)**	**Cidara (7)**	**Donnybrook (11)**	**Gilboa Farm (33)**	**Greytown (18)**	**Mafakathini (11)**	**Singisi (28)**
As*	M	0.51(0.1)^{b}	0.90(0.0)^{cd}	ND	0.77(0.1)^{bc}	1.17(0.2)^{d}	0.59(0.1)^{b}	1.10(0.2)^{d}	0.61(0.2)^{b}
	S	6.50(7)	9.88(1)	6.38(2)	4.89(2)	5.20(2)	8.92(5)	14.35(0.3)	1.85(1)
Ca*	M	61.50(3.0)^{b}	135.22(12)^{d}	14.80(0.4)^{a}	88.36(8.0)^{c}	121.40(4.0)^{d}	121.26(4.0)^{d}	72.28(4.0)^{bc}	166.17(12)^{e}
	S	869.15(8)	425.99(9)	401.71(6)	497.15(7)	386.05(15)	524.73(9)	411.92(18)	280.87(13)
Cd*	M	0.88(0.1)^{b}	1.37(0.0)^{de}	ND	1.51(0.1)^{ef}	1.33(0.1)^{d}	0.95(0.1)^{b}	1.57(0.1)^{f}	1.12(0.1)^{c}
	S	11.11(0.1)	7.90(0.1)	8.20(0.4)	3.75(0.2)	8.40(0.1)	9.40(0.2)	9.90(0.1)	4.00(0.4)
Co*	M	ND	0.50(0.1)^{bc}	0.10(0.1)^{ab}	0.30(0.1)^{abc}	0.30(0.1)^{abc}	0.65(0.3)^{c}	1.80(0.3)^{d}	0.10(0.0)^{ab}
	S	13.99(0.1)	16.72(0.1)	9.58(0.9)	5.23(0.2)	25.33(0.2)	11.31(0.1)	14.36(0.1)	3.91(0.2)
Cr*	M	1.50(0.1)^{b}	0.11(0.1)^{a}	ND	1.79(0.3)^{b}	ND	1.28(0.1)^{b}	0.18(0.1)^{a}	1.93(0.8)^{b}
	S	113.50(1)	86.98(4)	104.39(7)	48.75(2)	102.54(1)	112.44(2)	100.78(1)	45.56(2)
Cu*	M	63.76(6)^{bcd}	59.08(1)^{bc}	66.92(5)^{cd}	154.90(7)^{f}	37.50(4)^{a}	53.89(2)^{b}	64.08(6)^{bcd}	74.28(2)^{de}
	S	37.24(2)	27.76(0.4)	10.41(0.6)	9.98(0.8)	53.67(0.2)	33.22(1)	29.04(0.2)	7.36(0.4)
	BAF	1.71	2.13	6.43	15.53	0.70	1.62	21.2	10.10
Fe**	M	0.03(0.0)^{a}	0.04(0.0)^{a}	0.20(0.0)^{c}	0.04(0.0)^{a}	3.16(0.2)^{d}	0.03(0.0)^{a}	0.13(0.0)^{b}	0.12(0.0)^{b}
	S	51.21(0.5)	41.45(0.4)	42.94(1.2)	25.64(1.4)	43.02(0.2)	45.69(0.4)	48.11(0.2)	27.55(1.1)
K**	M	27.85(0.2)^{bcd}	35.00(0.2)^{e}	29.55(1.0)^{cd}	16.46(0.7)^{a}	24.13(0.2)^{b}	26.41(0.6)^{bc}	30.33(0.2)^{cde}	29.35(1.0)^{cd}
	S	2.58(0.0)	6.16(0.3)	0.35(0.0)	0.33(0.0)	0.32(0.0)	4.32(0.1)	3.20(0.1)	0.49(0.0)
	BAF	10.80	5.68	85.20	50.80	76.72	6.11	9.50	59.80
Mg**	M	0.66(28)^{c}	0.82(26)^{f}	0.60(10)^{b}	0.53(17)^{a}	0.65(6.0)^{c}	0.65(6.0)^{c}	0.74(14)^{d}	0.81(19)^{ef}
	S	1.44(10)	2.15(29)	0.22(15)	0.25(13)	0.34(5.0)	1.26(23)	1.53(27)	0.68(33)
	BAF	0.46	0.38	2.77	2.15	1.90	0.52	0.48	1.19
Mn*	M	7.61(0.4)^{ab}	14.23(0.4)^{bc}	7.97(0.3)^{ab}	5.42(0.2)^{a}	52.30(6.0)^{d}	16.33(0.1)^{c}	10.98(0.6)^{abc}	12.35(0.8)^{abc}
	S	571.38(11)	216.75(2.0)	169.67(7.0)	155.06(17)	653.99(9.0)	204.33(4.0)	194.35(4.0)	141.75(4.0)
Na**	M	0.57(0.1)^{bc}	0.61(0.1)^{c}	0.77(0.1)^{d}	0.33(0.0)^{a}	1.77(0.3)^{f}	1.02(0.1)^{e}	0.93(0.0)^{e}	0.45(0.0)^{ab}
	S	0.26(0.0)	0.27(0.0)	0.28(0.0)	0.29(0.0)	0.26(0.0)	0.28(0.0)	0.26(0.0)	0.27(0.0)
	BAF	2.26	2.29	2.74	1.16	6.93	3.67	3.59	1.68
Ni*	M	1.99(0.2)^{a}	ND	ND	2.14(0.3)^{a}	6.37(0.8)^{b}	2.14(0.2)^{a}	ND	2.74(0.5)^{a}
	S	55.91(0.7)	51.05(0.2)	22.54(1.0)	13.89(0.8)	34.28(0.4)	46.19(0.6)	54.17(0.6)	15.83(0.5)
Pb*	M	2.95(0.8)^{bc}	ND	ND	1.40(1.0)^{ab}	4.48(1.0)^{c}	2.73(0.6)^{bc}	ND	1.42(1.0)^{ab}
	S	104.50(3.0)	87.40(6.0)	60.86(2.0)	45.49(2.0)	63.10(2.0)	97.84(2.0)	99.78(2.0)	48.76(0.4)
Se*	M	11.59(2.0)^{bc}	6.75(0.2)^{a}	14.38(0.1)^{c}	9.08(1.0)^{ab}	12.07(1.0)^{bc}	6.94(2.0)^{a}	8.86(2.0)^{ab}	10.20(0.8)^{ab}
	S	14.88(7.0)	14.92(4.0)	21.21(2.0)	6.71(2.0)	27.06(3.0)	22.77(2.0)	10.293(2.)	ND
Si*	M	ND	63.58(13)^{c}	18.21(3.0)^{ab}	6.00(23)^{ab}	29.01(5.0)^{b}	ND	128.22(22)^{d}	ND
	S	344.04(96)	231.45(32)	191.20(18)	227.46(27)	553.66(43)	298.77(62)	275.09(212)	166.51(15)
Zn*	M	91.57(1.0)^{b}	94.71(1.0)^{bc}	104.76(3.0)^{cd}	55.84(4.0)^{a}	47.39(10)^{a}	114.68(0.6)^{d}	114.29(6.0)^{d}	130.44(4.0)^{e}
	S	41.93(2.0)	52.08(2.0)	10.45(0.4)	9.74(4.0)	23.14(0.5)	31.38(2.0)	38.35(0.8)	16.39(2.0)
	BAF	2.18	1.82	10.02	5.73	2.05	3.65	2.98	7.96

* Values expressed in mg kg^{-1}. ** Values expressed in g kg^{-1}. *** Value in bracket represents the number of fruiting bodies collected at a specified sampling site.

This observation was consistent with findings from other studies conducted across several species of mushrooms (Brzezicha-Cirocka *et al*., 2016; Falandysz & Borovička, 2013). Correlation analysis (Table A3.2 and Table A3.6), indicates a strong positive correlation between Mn in the soil and accumulation of Zn by *B. edulis* (0.89). The mobility of both these elements is increased in acidic soil solutions, hence they become bioavailable to mushrooms and plants species (Kabata-Pendias, 2011).

The results obtained for Mg show a similar trend to Zn, with concentrations (in g kg^{-1}) being in decreasing order of *A. pantherina* (0.98) > *L. deliciosus* (0.83) > *B. mirabilis* (0.82) > *B. edulis* (0.76) > *R. sardonia* (0.58) at Bulwer and *A. pantherina* (1.33) > *L. deliciosus* (1.01) > *B. mirabilis* (0.81) > *B. edulis* (0.74) > *R. sardonia* (0.61) at Singisi. Correlation analysis (Table A3.2) for *B. edulis*, showed a positive correlation between Mg concentration in mushrooms and altitude (0.94), significant at the 0.01 level (2-tailed); this was not observed for the other studied species. It has been observed that *B. edulis* is generally found in pine plantations at altitudes of between 900 and 1 500 m, supporting the positive correlation observed between Mg and altitude (Forestry SA online, 2009). Previous studies reported similar ranges for Mg in mushrooms (Alaimo *et al*., 2018; Brzezicha-Cirocka *et al*., 2016; Di Anibal *et al*., 2015).

The results showed Cu to accumulate in all species, but to varying degrees. The highest concentration was found in *B. mirabilis* (154.90 mg kg^{-1}) from samples collected at Donnybrook. When the site was common (Bulwer and Singisi), *B. edulis* was found to accumulate more Cu than the other species, unlike for Zn and Mg. The concentration (in mg kg^{-1}) of Cu for species collected at Bulwer was found to be *B. edulis* (101.09) > *B. mirabilis* (59.08) > *A. pantherina* (48.62) > *R. sardonia* (29.18) > *L. deliciosus* (19.86). This pattern was similar for the species collected from Singisi.

Table 4. 6 Elemental composition [mean(SD), dry weight] in the fruiting bodies of *L. deliciosus* species (M) and soil substrate (S) collected from seven different sites as well as the corresponding bioaccumulation factor (BAF)

Element	Type	SITES						
		Bulwer (9)***	**Cidara (10)**	**Clemont (19)**	**Gilboa Farm (5)**	**Karkloof (65)**	**Richmond (45)**	**Singisi (53)**
As*	M	0.88(0.2)^{c}	0.39(0.1)^{ab}	0.57(0.0)^{abc}	0.39(0.2)^{ab}	0.36(0.1)^{a}	0.58(0.2)^{abc}	0.77(0.2)^{bc}
	S	9.88(1.0)	6.38(2.0)	6.00(5.0)	5.20(2.0)	4.00(3.0)	7.40(7.0)	3.40(8.0)
Ca*	M	78.37(4.0)^{a}	86.06(8.0)^{a}	165.16(15)^{d}	127.16(5.0)^{bc}	106.91(14)^{ab}	136.58(3.0)^{cd}	139.54(11)^{cd}
	S	426.00(9.0)	401.71(6.0)	592.40(8.0)	386.05(15)	792.75(5.0)	965.59(85)	294.05(11)
Cd*	M	1.13(0.0)^{b}	0.90(0.1)^{a}	0.90(0.1)^{a}	0.78(0.1)^{a}	0.78(0.1)^{a}	0.90(0.1)^{a}	1.15(0.1)^{b}
	S	7.90(0.1)	8.20(0.4)	7.00(0.0)	8.40(0.2)	4.50(0.4)	10.55(0.2)	4.00(1.0)
Co*	M	0.05(0.0)^{a}	ND	ND	0.70(0.2)^{b}	ND	ND	0.01(0.1)^{a}
	S	16.72(0.1)	9.58(0.9)	10.00(0.4)	25.33(0.2)	4.30(0.4)	18.48(0.3)	3.12(0.1)
Cr*	M	0.15(0.1)^{a}	1.45(0.1)^{b}	1.75(0.4)^{bc}	1.70(0.2)^{bc}	2.35(0.4)^{c}	1.85(0.2)^{bc}	0.15(0.1)^{a}
	S	86.89(4.0)	104.40(6.0)	76.60(3.0)	102.54(1.0)	72.05(2.0)	127.87(10)	41.74(2.0)
Cu*	M	19.86(0.4)^{bc}	16.67(0.8)^{a}	21.79(1.5)^{c}	22.14(1.4)^{c}	20.00(1.3)^{c}	14.85(0.8)^{a}	16.96(1.0)^{ab}
	S	27.76(0.4)	10.41(0.6)	23.01(0.5)	53.67(0.2)	11.35(0.8)	37.87(0.2)	8.37(0.3)
	BAF	0.72	1.60	0.95	0.41	1.76	0.39	2.03
Fe**	M	0.05(0.0)^{a}	0.04(0.0)^{a}	0.06(0.0)^{ab}	0.06(0.0)^{ab}	0.08(0.0)^{b}	0.04(0.0)^{a}	0.08(0.0)^{b}
	S	41.45(0.4)	42.94(1.0)	38.00(0.6)	43.02(0.2)	28.79(1.0)	49.73(0.3)	23.46(0.7)
K**	M	17.40(0.1)^{c}	16.29(0.4)^{a}	19.17(0.2)^{d}	20.75(0.1)^{e}	17.02(0.3)^{bc}	16.47(0.2)^{ab}	20.32(0.2)^{e}
	S	6.17(0.3)	0.35(0.0)	6.50(0.1)	0.32(0.0)	0.35(0.0)	2.92(0.2)	0.68(0.0)
	BAF	2.82	46.96	2.95	65.95	49.15	5.64	29.90
Mg**	M	0.83(12)^{ab}	0.86(40)^{ab}	1.11(80)^{d}	0.93(54)^{bc}	0.84(26)^{ab}	0.78(24)^{a}	1.01(48)^{cd}
	S	2.15(29)	0.22(15)	2.63(29)	0.34(5.0)	0.28(12)	1.61(21)	0.89(20)
	BAF	0.39	4.02	0.42	2.69	3.04	0.48	1.13
Mn*	M	6.65(0.3)^{bc}	7.20(0.5)^{c}	8.65(0.6)^{d}	11.91(0.3)^{e}	3.85(0.2)^{a}	5.80(0.3)^{b}	6.35(0.6)^{bc}
	S	216.75(2.0)	169.67(7.0)	270.82(2.0)	653.99(9.0)	219.33(9.0)	203.76(8.0)	99.02(2.0)
Na**	M	0.43(0.0)^{c}	0.23(0.0)^{b}	0.14(0.0)^{a}	0.22(0.0)^{b}	0.14(0.0)^{a}	0.22(0.1)^{b}	0.38(0.1)^{c}
	S	0.27(0.0)	0.28(0.0)	0.27(0.0)	0.26(0.0)	0.28(0.0)	0.27(0.0)	0.27(0.0)
Ni*	M	ND	3.00(0.4)^{b}	2.75(0.3)^{b}	3.00(0.4)^{b}	2.80(0.3)^{b}	2.25(0.1)^{b}	ND
	S	51.05(0.2)	22.54(1.0)	41.16(0.2)	34.28(0.4)	18.47(1.0)	62.24(0.4)	15.10(0.7)
Pb*	M	ND	3.25(0.9)^{c}	0.65(0.9)^{ab}	0.85(1.0)^{ab}	1.10(1.0)^{abc}	2.50(1.0)^{bc}	ND
	S	87.40(6.0)	60.86(2.0)	81.20(2.0)	63.10(2.0)	53.17(3.0)	101.93(1.0)	45.25(2.0)
Se*	M	8.50(0.4)^{a}	11.3(1.0)^{a}	12.5(2.0)^{a}	9.00(2.0)^{a}	9.70(5.0)^{a}	10.5(2.0)^{a}	9.20(0.5)^{a}
	S	14.92(4.0)	21.21(2.0)	8.23(3.0)	27.06(3.0)	13.75(1.0)	19.00(6.0)	ND
Si*	M	98.43(30)^{a}	ND	ND	6.60(20)^{a}	ND	132.41(65)^{a}	360.62(135)^{b}
	S	231.46(32)	191.20(18)	317.95(50)	553.66(43)	169.88(12)	151.43(32)	199.70(14)
Zn*	M	82.94(1.0)^{b}	103.07(4.0)^{cd}	150.53(11)^{e}	107.01(5.0)^{cd}	94.62(4.0)^{bc}	59.37(4.0)^{a}	119.42(9.0)^{d}
	S	52.08(2.0)	10.45(0.4)	49.23(1.0)	23.14(0.5)	9.79(3.0)	35.48(2.0)	17.26(0.4)
	BAF	1.59	9.86	3.06	4.63	9.67	1.67	6.92

*Values expressed in mg kg^{-1}. ** Values expressed in g kg^{-1}. *** Value in bracket represents the number of fruiting bodies collected at a specified sampling site.

On average, BAFs for Cu ranged between 1 and 5, with *B. mirabilis* showing the highest BAF and *L. deliciosus* the lowest. The BAF for *Boletus* species in this study, especially for samples collected from Singisi, were comparable to those obtained from a study conducted on *Boletus badius* (Fr.) (Proskura *et al*., 2017).

Of the five mushroom species studied, all but *L. deliciosus* accumulated Na metal. The highest concentration of Na was found in *A. pantherina* (2.70 g kg^{-1}, Bulwer) and lowest in *B. edulis* (0.30 g kg^{-1}, Greytown). At Bulwer (common sampling point), the concentrations of Na (in g kg^{-1}) was found to be *R. sardonia* (0.70) > *B. mirabilis* (0.61) > *A. pantherina* (0.52) > *B. edulis* (0.43) while at Singisi (common sampling point) it was found to be *A. pantherina* (2.19) > *R. sardonia* (1.18) > *B. mirabilis* (0.45) > *B. edulis* (0.43). For samples collected at Singisi, the accumulation of Na amongst inedible mushrooms was more than three times that of edible ones. Correlation analysis (Table A3.3) showed that SOM (0.95) and Si (0.85) positively influenced the accumulation of Na by *B. mirabilis* species. Since this mushroom is a saprophyte, this explains the positive correlation between Na and SOM.

Selenium was accumulated only by the edible mushroom, *B. edulis*, with concentrations ranging between 30.54 and 53.00 mg kg^{-1} and the highest BAF value of 6 being obtained from samples collected at Stepmore B. Samples collected from Singisi were found to contain the highest amount of Se. The concentration variation of Se in mushroom species depends on the physio-chemical properties of the soil (composition of bed rock, redox status, pH and microbial activities) (Malinowska *et al*., 2004; Mehdi *et al*., 2013). Figure A2.1 shows variation of elemental uptake expressed in terms of percent bioaccumulation at each sampling site. A strong negative correlation was observed between the amount of Se in mushrooms and that of As (-0.85) and Pb (-0.87) in the soil (Table A3.2), indicating that the toxic metals (As and Pb) inhibited uptake of Se by *B. edulis*.

Table 4. 7 Elemental composition [mean (SD), dry weight] in the fruiting bodies of *A. pantherina* species (M) and soil substrate (S) collected from ten different sites as well as the corresponding bioaccumulation factor (BAF)

Element	Type	SITES									
		Boston (33)***	**Bulwer (11)**	**Clemont (23)**	**Donnybrook (19)**	**Gilboa Farm (21)**	**Greytown (23)**	**Mafakathini (27)**	**Singisi (35)**	**Stepmore B (65)**	**Twasamhlobo (17)**
As*	M	1.10(0.2)^{a}	0.75(0.1)^{a}	1.10(1.0)^{a}	0.56(0.1)^{a}	0.33(0.1)^{a}	0.61(0.1)^{a}	0.89(0.1)^{a}	0.65(0.1)^{a}	1.00(0.2)^{a}	0.50(0.1)^{a}
	S	6.50(7.0)	10.0(1.0)	6.00(6.0)	1.85(3.0)	5.20(2.0)	8.92(5.0)	14.5(0.3)	1.85(3.0)	3.66(3.0)	4.80(3.0)
Ca*	M	225.52(2.0)^{a}	225.48(4.0)^{a}	149.48(3.0)^{a}	311.34(11)^{a}	164.75(1.0)^{a}	334.11(11)^{a}	177.51(5.0)^{a}	245.26(9.0)^{a}	228.33(5.0)^{a}	255.40(15)^{a}
	S	869.15(8.0)	425.99(9.0)	592.40(8.0)	575.29(16)	386.05(15)	524.73(9.0)	411.92(19)	280.87(13)	314.86(11)	676.64(16)
Cd*	M	1.45(0.1)^{a}	1.15(0.1)^{a}	1.45(1.0)^{a}	0.90(0.0)^{a}	0.92(0.0)^{a}	1.77(2.0)^{a}	1.32(0.1)^{a}	1.11(0.1)^{a}	1.45(0.0)^{a}	0.80(0.1)^{a}
	S	11.11(0.1)	7.90(0.1)	7.00(0.0)	3.57(0.2)	8.41(0.2)	9.40(0.2)	9.86(0.1)	4.06(0.4)	4.70(1.0)	7.15(0.2)
Co*	M	0.25(0.3)^{ab}	0.28(0.1)^{ab}	ND	ND	ND	ND	0.70(0.1)^{c}	0.33(0.1)^{b}	2.06(0.1)^{d}	ND
	S	13.99(0.3)	16.72(0.1)	10.00(0.4)	3.00(0.2)	25.32(0.3)	11.31(0.1)	14.36(0.1)	3.91(0.2)	10.67(0.2)	17.04(0.3)
Cr*	M	2.25(0.1)^{cd}	0.08(0.1)^{a}	1.64(0.3)^{bcd}	1.73(0.0)^{cd}	1.41(0.1)^{bcd}	2.11(0.2)^{cd}	1.00(0.3)^{abc}	2.48(1.0)^{d}	0.35(0.1)^{ab}	2.32(0.4)^{d}
	S	113.50(1.0)	86.89(4.0)	76.60(2.0)	35.81(6.0)	102.54(1.0)	112.44(2.0)	100.78 (1.0)	45.56(2.0)	57.40(1.0)	107.59(2.0)
Cu*	M	66.23(4.0)^{de}	48.62(2.0)^{a}	70.65(0.4)^{e}	56.77(1.0)^{bc}	61.28(2.0)^{cd}	60.86(3.0)^{cd}	66.34(4.0)^{de}	70.30(2.0)^{e}	53.23(0.7)^{ab}	50.85(2.0)^{ab}
	S	37.24(2.0)	27.76(0.4)	23.01(0.5)	10.17(0.5)	53.67(0.2)	33.22(0.9)	29.04(0.2)	7.35(0.4)	18.08(2.0)	26.95(0.4)
	BAF	1.78	1.75	3.07	5.58	1.14	1.83	2.28	9.55	2.94	1.89
Fe**	M	0.32(0.0)^{d}	0.11(0.0)^{abc}	0.08(0.0)^{a}	0.07(0.0)^{a}	0.06(0.0)^{a}	0.3(0.0)^{d}	0.45(0.0)^{e}	0.16(0.0)^{bc}	0.11(0.0)^{ab}	0.17(0.0)^{c}
	S	51.21(0.5)	41.45(0.4)	38.00(0.6)	25.40(1.0)	43.02(0.2)	45.70(0.2)	48.11(0.2)	27.55(1.1)	26.82(0.5)	38.50(0.8)
K**	M	68.44(5.1)^{cd}	57.38(3.6)^{ab}	60.35(5.4)^{abc}	65.16(2)^{bcd}	53.54(0.5)^{a}	57.92(1.8)^{ab}	56.61(0.6)^{ab}	71.08(1.7)^{d}	61.32(4)^{abcd}	60.57(3.4)^{abc}
	S	2.58(0.0)	6.16(0.3)	6.50(0.1)	0.48(0.0)	0.32(0.0)	4.32(0.1)	3.19(0.1)	0.49(0.0)	4.65(0.1)	0.46(0.0)
	BAF	26.55	9.30	9.30	136.89	170.21	13.41	17.73	144.84	13.19	131.71
Mg**	M	1.15(31)^{cd}	0.98 (39)^{a}	1.15(31)^{bcd}	1.22(27)^{de}	1.08(27)^{abc}	1.15(62)^{cd}	1.25(28)^{def}	1.33(73)^{ef}	1.37(52)^{f}	1.03(12)^{ab}
	S	1.44(10)	2.15(29)	2.63(29)	0.41(15)	0.34(5.0)	1.26(23)	1.53(27)	0.68(33)	1.28(23)	1.15(26)
	BAF	0.80	0.46	0.43	2.98	3.14	0.91	0.81	1.95	1.07	0.89
Mn*	M	16.34(2.0)^{ab}	16.72(0.5)^{ab}	23.00(1.0)^{c}	17.12(0.5)^{b}	14.42(0.3)^{a}	28.74(1.0)^{d}	24.22(0.5)^{c}	21.77(0.5)^{c}	29.230(0.8)^{d}	16.94(0.5)^{ab}
	S	571.38(11)	216.75(2.0)	270.82(2.0)	76.81(1.0)	654.00(9.0)	204.33(4.0)	194.35(4.0)	141.75(4.0)	136.00(2.0)	609.71(13)
Na**	M	2.70(0.8)^{d}	0.52(0.1)^{a}	0.62(0.1)^{a}	1.51(0.2)^{bc}	1.32(0.1)^{ab}	1.18(0.2)^{ab}	2.55(0.5)^{d}	2.19(0.0)^{cd}	2.27(0.2)^{cd}	1.19(0.1)^{ab}
	S	0.25(0.0)	0.27(0.0)	0.27(0.0)	0.27(0.0)	0.26(0.0)	0.28(0.0)	0.26(0.0)	0.26(0.0)	0.28(0.0)	0.28(0.0)
	BAF	10.72	1.95	2.32	5.67	5.16	4.26	9.81	8.25	8.23	4.30
Ni*	M	2.50(0.2)^{b}	ND	2.52(0.2)^{b}	3.42(1.0)^{b}	2.70(0.1)^{b}	2.52(0.2)^{b}	ND	3.31(0.6)^{b}	ND	2.35(0.4)^{b}
	S	55.91(0.7)	51.05(0.2)	41. 16(0.2)	13.38(0.4)	34.28(0.4)	46.19(0.6)	54.17(0.6)	15.83(0.5)	34.03(0.4)	35.67(0.7)
Pb*	M	1.22(1.0)^{a}	ND	0.20(1.0)^{a}	1.40(1.0)^{a}	1.75(2.0)^{a}	0.14(0.8)^{a}	ND	0.50(1.0)^{a}	ND	1.95(0.4)^{a}
	S	104.50(3.0)	87.40(6.0)	81.20(2.0)	43.44(4.0)	63.10(2.0)	97.84(2.0)	99.78(2.0)	48.76(0.4)	51.40(0.4)	55.28(0.1)
Se*	M	11.29(1.0)^{ab}	8.18(1.0)^{ab}	11.01(1.0)^{ab}	12.00(2.0)^{b}	9.23(2.0)^{ab}	7.30(1.0)^{a}	8.85(0.2)^{ab}	11.38(1.0)^{ab}	11.22(2.0)^{ab}	10.21(2.0)^{ab}
	S	14.88(7.0)	14.92(4.0)	8.23(3.0)	ND	27.06(3.0)	22.77(2.0)	10.29(2.0)	ND	7.15(4.0)	17.57(6.0)
Si*	M	ND	147.70(10)^{c}	ND	ND	ND	5.65(0.3)^{a}	348.86(0.3)^{d}	ND	95.02(14)^{b}	ND
	S	344.04(19)	231.45(32)	317.95(17)	195.98(17)	553.66(11)	298.77(29)	275.09(11)	166.51(15)	428.49(11)	92.56(17)
Zn*	M	96.61(5.0)^{a}	120.23(2.0)^{b}	212.53(2.0)^{e}	134.32(5.0)^{ab}	138.39(2.0)^{c}	139.43(6.0)^{c}	158.84(3.0)^{d}	136.83(9.0)^{c}	97.45(7.0)^{a}	131.07(4.0)^{ab}
	S	41.93(2.0)	52.08(2.0)	49.23(1.0)	8.70(0.5)	23.14(0.5)	31.38(2.0)	38.35(0.8)	16.39(2.0)	36.02(1.0)	22.45(1.0)
	BAF	2.30	2.31	4.32	15.41	5.98	4.44	4.14	8.35	2.71	5.84

*Values expressed in mg kg^{-1}. ** Values expressed in g kg^{-1}. *** Value in bracket represents the number of fruiting bodies collected at a specified sampling site.

Concentration levels were consistent with those found in an earlier study, which showed *B. edulis* to contribute in excess of 836% towards the recommended dietary allowance for Se (Rasalanavho *et al*., 2019). Mushrooms in this study were found to be bioexcluders of the elements As, Ca, Cd, Co, Cr, Fe, Mn, Ni, Pb and Si (BAF < 1). The small amounts of these elements that were accumulated varied according to the species, and within a species according to the sampling site. The species *L. deliciosus* was also found to exclude Na metal, making this mushroom the lowest accumulator of minerals.

Correlation analysis (Table A3.2 and Table A3.3) shows that the accumulation of As by *B. edulis* (edible) was negatively affected by Cu in soil (-0.85). Cobalt in *B. edulis* positively correlated with Mn in soil (0.87) while Cd and Cu were both negatively correlated with Cu (-0.85) and Na (-0.85) in the soil, respectively. Silicon (0.87) and SOM (0.92) in the soil positively influenced the accumulation of Fe by *B. mirabilis* while for *L. deliciosus* (edible) the accumulation of Fe was negatively affected by Cd (-0.92), Cr (-0.86) and Fe (-0.94) in the soil. Accumulation of Mn by *B. mirabilis* correlated positively with both SOM (0.95) and Si (0.86) in the soil (Table A3.3) whilst accumulation of Mn by *L. deliciosus* positively correlated with Si (0.93) in the soil. Correlation analysis (Table A3.5), showed a positive correlation between Mg in the soil and accumulation of Ca by *R. sardonia* (0.87). Both these elements exhibited common chemical properties.

The differences in the observed concentration levels of elements amongst these ectomycorrhizal species could be attributed to their morphological growth, especially the mycelium, and their interaction with symbiotic plant species as well as the soil solution. It is well known that ectomycorrhizal mushrooms form beneficial relationships with some plant species, wherein the mushroom benefits in nutrient uptake while transferring soil derived minerals (transfer capacities) to promote plant growth (Kabata-Pendias, 2011; Varma & Hock, 2013).

Table 4. 8 Elemental composition [mean (SD), dry weight] in the fruiting bodies of *R. sardonia* species (M) and soil substrate (S) collected from eleven different sites as well as the corresponding bioaccumulation factor (BAF)

Element	Type	SITES										
		Bulwer (77)* **	**Cidara (18)**	**Curry Post (33)**	**Donnybrook (28)**	**Gilboa Farm (70)**	**Greytown (55)**	**Karkloof (45)**	**Mafakathini (71)**	**Richmond (51)**	**Singisi (47)**	**Stepmore A (55)**
As*	M	0.85(0.0)^{ab}	0.35(0.1)^{a}	ND	1.25(0.1)^{ab}	1.35(0.2)^{ab}	0.30(0.3)^{a}	2.10(0.1)^{b}	0.70(0.2)^{ab}	0.90(0.1)^{ab}	1.00(0.2)^{ab}	0.60(0.1)^{a}
	S	9.90(1.0)	6.40(2.0)	9.85(4.0)	1.85(3.0)	5.20(2.0)	8.90(5.0)	9.30(6.0)	14.5(0.3)	7.40(7.0)	3.40(8.0)	3.70(3.0)
Ca*	M	145.05(6.0)^{h}	78.69(8.0)^{cd}	9.05(0.4)^{a}	84.41(5.0)^{de}	34.06(7.0)^{b}	114.13(7.0)^{fg}	54.41(2.0)^{bc}	124.41(20)^{ef}	132.49(7)^{gh}	92.22(3.0)^{def}	115.37(3.0)^{fg}
	S	425.99(9.0)	401.71(6.0)	497.63(3.0)	575.29(16)	386.05(15)	524.73(9.0)	523.37(7.0)	411.92(19)	965.59(85)	294.05(11)	314.86(9.0)
Cd*	M	1.15(0.0)^{ab}	0.90(0.0)^{a}	ND	1.45(0.1)^{ab}	1.30(0.0)^{ab}	0.76(0.0)^{a}	2.70(0.1)^{b}	1.15(0.1)^{ab}	1.23(0.0)^{ab}	1.30(0.1)^{ab}	1.07(0.0)^{a}
	S	7.90(0.1)	8.17(0.4)	4.90(0.4)	3.57(0.2)	8.40(0.2)	9.40(0.2)	5.90(0.4)	9.90(0.1)	10.55(0.2)	3.95(1.0)	4.70(1.0)
Co*	M	0.10(0.0)^{ab}	0.30(0.0)^{bc}	ND	2.05(0.2)^{g}	0.90(0.1)^{f}	ND	0.60(0.2)^{de}	0.80(0.1)^{ef}	0.45(0.0)^{cde}	0.35(0.0)^{bcd}	2.00(0.1)^{g}
	S	16.72(0.1)	9.60(1.0)	4.80(0.4)	3.00(0.2)	25.32(0.3)	11.31(0.1)	3.10(0.4)	14.36(0.1)	18.48(0.3)	3.10(0.4)	10.67(0.2)
Cr*	M	0.12(0.1)^{ab}	1.40(0.0)^{d}	ND	ND	ND	1.25(0.0)^{d}	ND	0.15(0.2)^{ab}	0.30(0.2)^{bc}	ND	0.50(0.3)^{c}
	S	86.89(4.0)	104.40(7.0)	89.46(1.0)	35.81(6.0)	102.54(1.0)	112.44(2.0)	80.88(4.0)	100.78(1.0)	127.86(10)	41.74(2.0)	57.40(1.0)
Cu*	M	29.18(0.7)^{ab}	36.68(1.0)^{cd}	28.88(0.6)^{ab}	34.75(2.0)^{cd}	29.55(0.2)^{ab}	37.26(1.0)^{d}	26.82(3.0)^{a}	30.35(2.0)^{cd}	32.63(0.4)^{bc}	35.80(1.0)^{cd}	29.37(0.7)^{ab}
	S	27.76(0.4)	10.41(0.6)	14.09(0.2)	10.17(0.5)	53.67(0.2)	33.22(1.0)	15.07(0.6)	29.04(0.2)	37.87(0.2)	8.40(0.3)	18.08(2.0)
	BAF	1.05	3.52	2.05	3.42	0.55	1.12	1.78	1.05	0.86	4.28	1.62
Fe**	M	0.04(0.0)^{ab}	0.03(0.0)^{ab}	0.02(6.0)^{a}	0.03(0.0)^{ab}	0.03(0.0)^{a}	0.04(0.0)^{ab}	0.02(0.0)^{a}	0.08(0.0)^{c}	0.07(0.0)^{b}	0.04(0.0)^{ab}	0.15(0.0)^{c}
	S	41.45(0.4)	42.94(1.2)	30.53(1.2)	25.40(1.0)	43.02(0.2)	45.69(0.4)	33.83(1.5)	48.11(0.2)	49.73(0.3)	23.46(0.7)	26.82(0.5)
K**	M	31.24(0.5)^{e}	25.21(0.4)^{bc}	23.00(0.3)^{ab}	25.47(1.4)^{bc}	19.75(0.8)^{a}	30.50(1.4)^{de}	25.51(1.6)^{bc}	30.94(1.1)^{cd}	25.63(0.3)^{bc}	27.34(1.1)^{cd}	26.78(0.3)^{cd}
	S	6.16(0.3)	0.35(0.0)	0.35(0.0)	0.48(0.0)	0.32(0.0)	4.32(0.1)	0.44(0.0)	3.23(0.1)	2.92(0.2)	0.68(0.0)	4.65(0.1)
	BAF	5.07	72.66	66.36	53.50	62.77	7.06	57.86	9.69	8.77	40.21	5.75
Mg**	M	0.58(7.0)^{cd}	0.57(11)^{bcd}	0.40(3.0)^{a}	0.55(21)^{bc}	0.40(7.0)^{a}	0.66(13)^{d}	0.49(17)^{b}	0.46(38)^{cd}	0.58(11)^{cd}	0.61(6.0)^{cd}	0.60(13)^{cd}
	S	2.15(29)	0.22(15)	0.29(11)	0.41(15)	0.34(5.0)	1.26(23)	0.40(9.0)	1.54(27)	1.61(21)	0.89(20)	1.28(23)
Mn*	M	11.89(0.5)^{cd}	13.17(0.6)^{d}	6.46(0.0)^{a}	9.87(1.0)^{c}	7.65(0.5)^{ab}	16.00(0.3)^{e}	10.80(1.0)^{c}	14.35(0.7)^{e}	9.70(0.3)^{bc}	13.10(0.7)^{d}	16.71(0.7)^{e}
	S	216.75(2.0)	169.67(7.0)	167.23(3.0)	76.80(1.0)	654.00(9.0)	204.33(4.0)	253.00(9.0)	194.34(4.0)	203.76(8.0)	99.02(1.0)	135.98(2.0)
Na**	M	0.70(0.0)^{a}	1.03(0.1)^{b}	0.78(0.0)^{a}	1.32(0.1)^{cd}	1.22(0.0)^{bc}	1.09(0.1)^{b}	2.23(0.1)^{e}	1.41(0.1)^{cd}	1.09(0.0)^{b}	1.18(0.1)^{bc}	0.60(0.0)^{a}
	S	0.27(0.0)	0.28(0.0)	0.28(0.0)	0.27(0.0)	0.26(0.0)	0.28(0.0)	0.27(0.0)	0.26(0.0)	0.27(0.0)	0.27(0.0)	0.28(0.0)
	BAF	2.61	3.69	2.79	4.97	4.78	3.94	8.38	5.42	4.10	4.43	2.16
Ni*	M	4.54(0.1)^{a}	2.26(0.3)^{b}	5.31(0.0)^{a}	2.37(0.2)^{a}	3.10(0.2)^{a}	2.00(0.2)^{b}	2.33(0.5)^{a}	4.75(0.3)^{a}	4.70(0.1)^{a}	2.05(0.1)^{a}	1.90(0.2)^{a}
	S	51.04(0.2)	22.54(1.0)	19.01(1.0)	13.38(0.4)	34.28(0.4)	46.19(0.6)	14.45(0.3)	54.17(0.6)	62.23(0.4)	15.10(0.7)	34.03(0.4)
Pb*	M	ND	2.70(1.0)^{b}	ND	1.20(0.1)^{ab}	1.32(0.8)^{ab}	2.20(0.5)^{ab}	1.77(0.9)^{a}	ND	ND	2.53(0.8)^{a}	ND
	S	87.40(6.0)	60.86(2.0)	56.40(5.0)	43.44(4.0)	63.10(2.0)	97.84(2.0)	54.54(2.0)	99.78(2.0)	101.93(1.0)	45.25(2.0)	51.40(3.0)
Se*	M	7.20(0.6)^{a}	10.13(2.0)^{ab}	13.67(0.6)^{bc}	12.60(1.0)^{bc}	14.00(1.0)^{c}	7.45(0.7)^{a}	6.50(3.0)^{a}	8.40(1.0)^{a}	7.95(1.0)^{a}	8.60(0.8)^{a}	6.50(1.0)^{a}
	S	14.92(4.0)	21.21(2.0)	19.10(5.0)	ND	27.06(3.0)	22.77(2.0)	9.15(4.0)	10.29(2.0)	19.00(6.0)	ND	7.15(3.0)
Si*	M	320.00(7.0)^{c}	ND	2.20(0.9)^{a}	40.13(3.0)^{a}	20.00(10)^{a}	ND	251.10(53)^{c}	180.95(12)^{bc}	95.76(27)^{ab}	512.20(12)^{d}	481.65(27)^{d}
	S	231.46(32)	191.20(14)	388.92(29)	195.98(14)	553.66(11)	298.77(11)	209.26(18)	275.09(32)	151.43(11)	199.70(14)	428.49(12)
Zn*	M	47.20(0.3)^{ab}	60.10(2.0)^{cd}	51.60(0.2)^{bc}	42.32(8.0)^{ab}	39.95(2.0)^{a}	59.60(1.0)^{cd}	40.11(0.3)^{a}	65.76(9.0)^{cd}	41.10(0.3)^{a}	48.60(2.0)^{ab}	39.44(0.3)^{a}
	S	52.08(2.0)	10.45(0.4)	10.30(2.0)	8.72(0.5)	23.14(0.5)	31.38(2.0)	12.85(0.7)	38.35(0.8)	35.50(2.0)	17.26(0.4)	36.02(1.0)
	BAF	0.91	5.75	5.01	4.85	1.73	1.90	3.12	1.71	1.16	2.81	1.10

* Values expressed in mg kg^{-1}. ** Values expressed in g kg^{-1}. *** Value in bracket represents the number of fruiting bodies collected at a specified sampling site.

The nature of ion activity in the soil solution influences the uptake of important minerals and it is more usually controlled by metabolic processes, especially at low external cation concentrations, than through passive diffusion. How far the mycelia can extend in the soil matrix has an effect on the amount of elements they can absorb from the soil. Several soil exploration systems have been suggested, namely contact exploration type, medium exploration type (fringe, smooth and mat subtypes), long distance exploration type and pick-a-back exploration type (Agerer, 2001). The *Lactarius* and *Rassula* species form contact exploration systems while most *Boletus* species form long distance exploration systems (Varma & Hock, 2013). This means that the mycelia of *Boletus* species are able to reach considerable distance and hence absorb more minerals than those for the *Lactarius* and *Rassula* species.

Correlation analysis enables us to find relationships between two variables. In this study, the interactions between chemical elements accumulated by mushrooms and soil parameters was evaluated using correlation analysis. The interaction can either be antagonistic or synergistic in nature (Kabata-Pendias, 2011), which means that the interaction can either inhibit or stimulate the absorption of other elements, as illustrated by the findings in this study. The uptake of Se by *B. edulis* was found to be high and an antagonistic relationship was observed with As and Pb in the soil solution (Table A3.2 and Table A3.6). This interaction is important, especially in the edible species (*B. edulis*) which showed high amounts of Se and Cu to inhibit accumulation of the heavy minerals As, Cd and Pb, which are considered toxic. Accumulation of essential elements is enhanced by the large area covered by the mycelium of *B. edulis* in the soil medium. The antagonistic relationship between Fe and the accumulation of Cd and Cr by the species of *L. deliciosus* is also a positive attribute (Table A3.4), as it inhibits the absorption of these heavy metals. Soil organic matter was found to enhance (synergistic) the absorption of Fe, Mn and Na by *B. mirabilis* species (Table A3.2 and Table A3.6). This could be attributed to its habitat, as it is known to grow on highly decomposed stumps of pine

trees. No correlation was observed between soil parameters and the uptake of minerals by the mushroom species, *A. pantherina* (Table A3.1).

4.5 Conclusion

This study investigated the bioaccumulation of elements and the nutritional content of five wild growing mushrooms collected from pine plantations in KwaZulu-Natal, South Africa. The fruiting bodies of the three edible mushrooms (*B. edulis, B. mirabilis, L. deliciosus*) contained proteins (25 – 55 %), carbohydrates (34 – 69 %), ash (3 - 6.5 %) and lipids (0.8 - 5.3 %) by dry weight. The results showed elemental concentrations to be species and habitat dependent. All mushroom species were bioexcluders of As, Be, Ca, Cd, Co, Cr, Fe, Mn, Ni and Si, and accumulated elements in the following order: *Amanita pantherina* (K > Na > Zn > Cu > Mg); *Boletus edulis* (K > Cu > Zn > Se > Na > Mg); *Boletus mirabilis* (K > Cu > Zn > Na > Mg); *Lactarius deliciosus* (K > Zn > Mg); and *Russula sardonia* (K > Na > Zn > Cu > Mg). Correlation analysis showed accumulation of elements by the edible mushrooms to be affected by soil parameters more than inedible ones, with As and Cd accumulation being inhibited by available Cu in the soil substrate, indicating the self-preservation tendency of wild mushrooms. This study validates the ethno-medicinal use and consumption of wild growing mushrooms due to low accumulation of toxic metals therefore, their promotion as food supplements should be encouraged to overcome nutritional deficiencies in low income markets.

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4.7 Conflict of Interest

The authors declare that they have no conflict of interest.

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CHAPTER 5
Chemical Constituents of the wild growing mushroom, *Termitomyces sagittiformis* fruiting bodies: nutritional, mineral intake analysis and structural elucidation of mycochemical compounds.

5.1 Abstract

Termitomyces mushrooms, that form symbiotic relationship with wood-destroying termites are found in many parts of the African continent and East Asia. In this chapter we report the nutritional and mineral intake analysis as well as the extraction and structural elucidation of mycochemical compounds from dried samples of the rare species of *Termitomyces sagittiformis* mushroom. Nutritional value for the pileus and stipe parts of the mushrooms was determined and found to be 93.8 and 94.3 % moisture; while the dry weight contained 30.0 and 21.5 % protein; 5.5 and 1.4 % lipids; 10.3 and 5.4 % ash; 54.6 and 71.8 % carbohydrates; 386.2 and 385.3 kcal/100g respectively. Mycochemical compounds isolated and characterised using TLC, ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, and HMBC analytical techniques includes, ergosterol, glycosphingolipid, linoleic acid, mannitol, oleic acid, and uracil. GC-MS was used to establish volatile profiles of the fractions. Antioxidant activities of methanolic extracts were profiled using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and Ferric reducing power. The effective concentration at which 50% of DPPH radicals are scavenged (EC_{50}) was 27760 μg mL^{-1} while the effective reducing power concentration at which the absorbance is 0.5 (EC_{50}) ranged between 5.6 and 22.1 μg mL^{-1}, (crude extract and fractions). The total phenolic content, dry weight, in the pileus and stipe part of the mushroom were 5.40 and 7.38 mgGAE/g respectively.

Keywords *Termitomyces, T. sagittiformis*. Ergosterol. Glycosphingolipid. Linoleic acid. Mannitol. Oleic acid. Uracil. Antioxidant. Total Phenolic content.

5.2 Introduction

Species of the genus *Termitomyces* belong to the family *Lyophyllaceae* (Borkar *et al*., 2015; Hsieh & Ju, 2018). *Termitomyces* mushrooms are found in many parts of Africa (e.g. South Africa, Zimbabwe, Zambia, Uganda, Kenya etc), and East Asia (China, India etc) (Borkar *et al*., 2015; Karun & Sridhar, 2013; Pegler & Vanhaecke, 1994; Sangvichien & Taylor-Hawksworth, 2001; Van der Westhuizen & Eicker, 1990; Wei *et al*., 2009). The species of *Termitomyces* mushrooms form symbiotic relationship with wood-destroying termites, *Odontotermes badius, Odontotermes latericius, Odontotermes transvaalensis* or *Odontotermes vulgaris* (Van Der Westhizen & Eicker, 1994). In South Africa, seven species of *Termitomyces* are well-described and documented (Levin & Branch, 1985; Van Der Westhizen & Eicker, 1994; Van der Westhuizen & Eicker, 1990). The seven species includes, *Termitomyces microcarpus, Termitomyces clypeatus, Termitomyces striatus, Termitomyces schimperi, Termitomyces sagittiformis, Termitomyces reticulatus and Termitomyces umkowaanii.* These mushrooms are distributed in many parts of the country, including KwaZulu-Natal Province, South Africa.

During rainy season (early September through late April) *Termitomyces* mushrooms are consumed by majority of indigenous South African people who resides at rural and semi-urban areas. The most abundant species of this genus are the *T. umkowaanii*, *T. clypeatus*, *T. microcarpus* and *T. reticulatus*. With the exception of *T. umkowaanii* that appear as a single or less than five fruiting bodies, all the other species appear in large colonies when they sprout out from the underground (Borkar *et al*., 2015; Van der Westhuizen & Eicker, 1990). The size of the pileus and stipe of *Termitomyces* species differs enormously with *T. umkowaanii* having the

largest diameter compared to the other species while *T. microcarpus* has the smallest size. *T. reticulatus* has the longest stipe, pseudorrihiza, (most of it found underground) and *T. microcarpus* the smallest. *T. umkowaanii* has the fleshiest and thick stipe compared to other species of this genus. These mushrooms are very delicious and more comparable with chicken meat, perhaps the reason why mushrooms are more closely related to animal than plant kingdom.

Besides their nutritional value and richness in minerals, see Subsection 2.6.1, (Alam *et al*., 2008; Liu *et al*., 2012; Rasalanavho *et al*., 2019), mushrooms, like plant species are also able to biosynthesize bioactive secondary metabolites that are responsible for defence mechanism against fungivores and mycoparasitic fungi, chemical communication between same or different species, animal & plant-pathogenic, entomopathogenic and attraction or signalling (Fogarasi *et al*., 2018; Spiteller, 2008; Spiteller, 2015) and as potential beneficial for medicinal purposes (Cruz *et al.*, 2016; Hsieh & Ju, 2018; Shikongo, 2012; Stojković *et al*., 2014; Takaku *et al.*, 2001; Wasser, 2002). These compounds, described in Subsection 2.6.2 are mainly polysaccharides, alkaloids, terpenoids, steroids, fatty acids and phenolic in nature (Athanasakis *et al.*, 2013; Bernhoft *et al*., 2010; Fogarasi *et al*., 2018; Spiteller, 2008; Taofiq *et al.*, 2016).

Although species of *Termitomyces* are well-known mycorrhizal fungus for many generations and consumed by indigenous people in South Africa, there are no conclusive studies carried on mineral, chemical composition, total phenolic content and antioxidant activities of these species. Our study is based on the mushroom species, *T. sagittiformis,* fully described under Subsection 2.5.5. This species of *Termitomyces* was first described by Kalchbrener and Cook in Natal, South Africa in 1881 as *Agaricus sagittiformis* and in 1975 renamed *Termitomyces sagittiformis* by Reid (Vrinda & Pradeep, 2009). Therefore, the aim of this work was to isolate secondary metabolites, evaluate the proximate mineral, nutritional content and

chemical composition profiles and antioxidant activities of collected, dried fruiting bodies of the mushroom *T. sagittiformis.*

5.3 Materials and methods

5.3.1 Equipment, chemicals and reagents

Silica gel 60 (0.040 – 0.063 mm, 230 – 400 mesh ASTM) for column chromatography (CC) and thin-layer chromatography (TLC) plates, Silica gel 60 F_{254} were purchased from Merck KGaA, Darmstadt, Germany.

Analytical grade methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT), Gallic acid, ascorbic acid (purity >99.7%), sodium phosphate buffer (pH 6.6), potassium ferricyanide [$K_3Fe(CN)_6$], Trichloroacetic acid, ferric chloride ($FeCl_3$), Folin-Ciocalteu's phenol reagent (FCR) and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich (Germany). ultra-pure water (UPW). All other chemicals and reagents were of analytical grade.

5.3.2 Collection, storage and treatment of mushroom species

The samples of mature fruiting bodies of wild growing mushrooms, *Termitomyces sagittiformis* shown in Figure 2.11, were collected from the wild game park, Lion Park near Pietermaritzburg City in South Africa (site details given on Table 3.1). Both the pileus (cap) and stipe (stalk) of juvenile and fully-grown fruiting bodies were hand-picked from their natural environment on a termite hill. In total, +300 samples of mature fruiting bodies were collected. All samples were subjected to preservations protocols described in Appendix 1.

5.3.3 Nutritional content of T. sagittiformis

The crude protein in the mushroom was determine by the macro-Kjeldahl method described in Section 4.3.5. The amount of lipids in the mushroom was determined through the use of a soxhlet extraction method EPA 3540C, fully described in Section 4.3.6. Ash content was determined as described in Section 4.3.7, while moisture content was determined following the procedure described in Section 4.3.8. Percent carbohydrates content present was estimated using Equation 4.6 and total energy in kilocalories was calculated using Equation 4.7.

The soil pH and soil organic matter (SOM) was determined as described in Section 4.3.9.

5.3.4 Sample digestion

Dried powdered samples i.e. pileus, stipe and soil substrate (0.25 g) each were digested using a microwave acid-assisted digestion system (Anton Paar, Multiwave Go, MFD 2014, Austria) as described in Section 3.3.

5.3.5 Instrumentation Method

Instrument parameters followed were similar to those given in Section 3.4. Briefly, analyses were carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Varian 720-ES, Varian Inc, Palo Alto, CA, USA). The RF power of the instrument was set at 1.0 kW with axial plasma observation. The plasma argon, auxiliary and nebulizer gas flow rates were 15.0, 0.20 and 0.80 L min^{-1}, respectively. The peristaltic pump and auto-sampler wash rates were both 1.50 mL min^{-1}. High-purity Ar (99.995%) supplied by Air Products South Africa (Pty) Ltd. (Kempton Park) was used to fuel the plasma and as carrier gas. Calibration standards were prepared by diluting 1000 mg L^{-1} ± 2 in 2% (w/w) HNO_3 of analytical grade

standards (Trace**CERT**®; Fluka Analytical; Sigma-Aldrich; St Louis, Mo USA) using ultra-pure water.

5.3.6 Solvent Extraction

Dried powdered samples of the pileus part of the mushroom, 53.8000 g was weighed in a 1 L conical flask and subjected to exhaustive extraction with 2 x 500 mL of distilled hexane, with constant agitation using a magnetic stirrer for 24 hours. The extract was carefully filtered using WhatmanTM No. 1 qualitative filter paper. The combined filtrate was stored in the refrigerator set at 4 ºC until needed. The residue was further extracted with 2 x 500 mL DCM/MeOH (1:1) and finally with 2 x 500 mL 100% MeOH. Extraction portions from each solvent system were pooled and concentrated under reduced pressure at 40 °C in a rotary vacuum evaporator. The three extracts were dried and weighed. The percent yields of the extracts were calculated based on dry biomass using Equation 5.1.

$$Yield(\%) = (m_e \times 100)/m_s \quad \text{..... Equation 5.1}$$

where m_e = mass of extract after solvent evaporation and drying, m_s = mass of dry powdered mushroom sample used.

Spatula tip (about 500 mg, not accurately weighed) of the hexane extract was dissolved in hexane and monitored with a TLC plate to check available components and also to determine a suitable solvent system to separate components. Grace Reveleris™ machine, shown in Figure A1.4 (c), was used to separate components of the fraction with the aid of gradient solvent system, 100% hexane to hexane:ethyl acetate (9:1). The solvent mixture was gradually increased by introducing ethyl acetate at 1% after every 3 minutes.

Six separations were obtained and all were subjected to GC-MS as well as ^{1}H NMR. The fifth component yielded Compound **1**, a carboxylic acid and the sixth component yielded Compound **2**, a sterol.

The DCM/MeOH and MeOH extracts were monitored using TLC plate and because of some similarities with the retention values, both extracts were pooled together, a portion was subjected to Solid Phase Extraction (SPE) using a DIOL SPE Cartridge (6 cc/2g) following a sequential vacuum elutions, shown in Figure A1.4 (a). Briefly the procedure involved dissolving 160 mg of the extract in 1.6 mL of DCM/MeOH (1:1) and the solution was allowed to percolate down the SPE column and left overnight to dry under the hood. Slight vacuum was applied to the column before DIOL SPE protocol was commenced using the following solvent systems:

- 6 mL hexane/DCM (9:1) – Fraction A
- 6 mL DCM/EtOAc (20:1) – Fraction B
- 6 mL EtOAc – Fraction C
- 6 mL EtOAc/MeOH (5:1) – Fraction D
- 6 mL MeOH – Fraction E

Each partition step was repeated twice before proceeding to the next and pooled into separate pre-weighed vial. The contents of the vial were dried using a stream of liquid nitrogen under the hood. Masses of each dry fractions were recorded and analysed using ^{1}H NMR spectroscopy.

The remaining amount of the DCM/MeOH crude (7.7357 g) was dissolved in 100 mL DCM/MeOH solvent. The solution was adsorbed on a 20.6300 g of silica and left in the hood to dry. The crude on the silica was partitioned using vacuum liquid chromatography (VLC) method (Maurya *et al.*, 2018) while applying protocols mentioned under DIOL SPE. Based on these protocols, five fractions labelled A, B, C, D and E were obtained, as presented in

Figure A1.5. The fractions were subjected to exhaustive normal phase column chromatography over silica gel to yield four compounds **3, 4, 5** and **6**.

5.3.7 Determination of antioxidant activities

Antioxidant activities of the mushroom extracts were evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and ferric reducing antioxidant power (FRAP) mechanism.

5.3.7.1 DPPH radical scavenging assays

Several dilute solutions of the standard (Ascorbic acid) and extract fractions A, B, C, D, and E were prepared using analytical grade methanol. The following concentrations were prepared: 25000, 8000, 4000, 2000, 1000, 500, 250, 125, and 62.5 μg mL^{-1}. DPPH solution was prepared by dissolving 2 mg of the dry reagent in 50 mL methanol. Each prepared sample concentration (150 μL) was mixed with 2850 μL of 0.1 mM DPPH solution. The mixture was incubated in the dark for 30 minutes and thereafter the absorbance was measured at 517 nm. The radical scavenging activity (RSA) was calculated using Equation 5.2.

$$\%RSA = \frac{(A_B - A_S)}{A_B} \times 100 \quad \text{..... Equation 5.2}$$

where A_B is the absorbance of blank/DPPH and A_s is the absorbance of the extract solution. A graph of % RSA against extract concentration was plotted and this was used to determine the effective concentration providing 50% of radical scavenging activity (EC_{50}). Ascorbic acid was used as a positive control. Measurements were done in triplicates.

5.3.7.2 Ferric Reducing Power (FRAP analysis)

Solutions of ascorbic acid (vitamin C) and Butylated hydroxytoluene (BHT) were prepared by dissolving 50.0 mg of the dry reagent in 50 mL of methanol, giving a 1000 mg L^{-1} working standards. Dried extract samples were subjected to the same preparation treatment. Several dilutions (i.e. 10, 25, 125, 250, 500 and 750 mg L^{-1}) of methalonic solutions were prepared. The procedure followed was based on the method described in the literature with minor modifications (Barros *et al*., 2008). Briefly 1 mL of each dilute methalonic solution was pipetted into a separate test tube. Thereafter 1 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] (w/v) were added into each solutions. The mixture in the test tube was incubated in a 50 ºC warm bath for 20 minutes. Trichloroacetic acid (10%, 1 mL) was added to each mixture and then incubated for 10 minutes at room temperature. Only 2 mL of the solution was pipetted into a clean test tube to which 7 mL of ultra-pure water and 1 mL of 1% ferric chloride ($FeCl_3$) were added. The absorbance of each dilution was measured spectrophotometrically at 700 nm.

5.3.8 Determination of total phenolic content (TPC)

TPC of the methanolic extract was evaluated following the method used by Waterhouse with minor modifications (Waterhouse, 2002). Briefly, 1.5 mL of ultra-pure water was added to a plastic cuvette containing 50 µL of the sample extract. To the mixture, 100 µL of the Folin-Ciocalteu's (F – C) phenol reagent was added. The resulting yellowish solution was mixed thoroughly in an ultra-sonic bath at room temperature for less than 8 minutes. Thereafter 300 µL of saturated sodium carbonate (Na_2CO_3) was added to the solution, resulting in a blueish mixture that was incubated for 2 hours at 20 ºC. The absorbance was measured at 765 nm. The

total phenolic content of the samples was calculated using Equation 5.3. Analyses was performed in triplicate.

$$Total\ phenolic\ content = \frac{c \times V}{m} \quad \text{..... Equation 5. 3}$$

where c is the extract concentration from the calibration curve (mg/L), V is the volume (mL) of the solvent used for the extraction, and m represents the weight (g) of the dried sample used.

5.4 Quality control

The certified reference material (CRM) used for elemental analysis of mushrooms was Lyophilised brown bread (BCR® - 191) obtained from the Institute for Reference Materials and Measurements (IRMM), Belgium and for soil was metals in soil (D081-540), Sigma-Aldrich. The CRMs were digested according to the method employed for samples. CRM solutions were prepared in triplicates, and each solution analysed three times during each run. All measured values were within the quality control performance acceptance limits shown in Table 5.1.

Table 5. 1 Elemental Method validation parameters for elemental analysis certified reference materials, Lyophilised brown bread (BCR® - 191) (n = 3)

Analyte	Measured value (mg kg^{-1})	Certified value (mg kg^{-1})	Recovery (%)
Fe	75.3(1.4)	40.7(2.3)	185
Mn	22.7(0.35)	20.3(0.7)	112
Zn	18.9(0.35)	19.5(0.5)	97

5.5 Data analyses

Statistical analysis of data obtained was carried out using one-way analysis of variance (ANOVA), using full SPSS version 25 program (IBM Corp., USA). A post hoc test was performed using Tukey's HSD multiple comparison test at the 95% confidence level.

5.6 Results and discussions

5.6.1 Nutritional content of T. sagittiformis

The moisture and nutritional (protein, lipid, ash, carbohydrates and energy, based on dry weight) content in different parts (pileus and stipe) of the mature fruiting bodies of the mushroom species *T. sagittiformis* are shown in Table 5.2. Statistically, the % moisture content for the pileus and the stipe shows significant difference ($p < 0.05$). This difference was observed in the study conducted on *T. robustus* (Ugbogu *et al.*, 2018). The % moisture values (93.8% in the pileus and 94.3% in the stipe) were however higher than those obtained for the morphological parts of the other mushroom species studied earlier in this project, shown in Table 5.2. These values were higher than the average norm of 90% moisture in mushroom samples.

Table 5. 2 Nutritional content (protein, lipid, ash, carbohydrates, g/100 g) and energy in a dry weight as well as % moisture in different parts of *T. sagittiformis* mushroom, (mean ± SD; n=3)

Species	Part	Moisture	Protein content		Lipid	Ash	Carbohydrates	Energy
			Instrumental	Kjeldahl				Kcal/100 g
T.	pileus	93.8±0.4^{ab}	30.0±0.2^{c}	29.6±0.9^{cd}	5.5±0.1^{bc}	10.3±0.2^{de}	54.6±0.8^{bc}	386.2±0.9^{ab}
sagittiformis	stipe	94.3±0.9^{a}	21.4±0.9^{b}	21.5±1.2^{bc}	1.4±0.2^{ab}	5.4±0.1^{a}	71.8±1.0^{de}	385.3±1.1^{c}

In each column, different letters mean significant differences ($p < 0.05$) for multivariate Tukey Post Hoc Multiple comparison for observed means.

Proximate composition (protein, lipid, ash, carbohydrates) and estimated energy observed for different parts of the mushroom, pileus and stipe, were also found to be significantly different at $p < 0.05$. The rich amount of protein (30.0 g/100 g pileus and 21.5 g/100 g stipe) found in this mushroom species conforms with most findings on other basidiomycetes species studied elsewhere as well as falling within the same range (Akyüz & Kirbağ, 2010; Alam *et al*., 2008; Ayodele & Okhuoya, 2009; Ijeh *et al.*, 2016; Liu *et al*., 2012; Nabubuya *et al.*, 2010). From the studies conducted by Botha and Eicker (1992) on *Termitomyces* mycelial protein, *T. sagittiformis w*as found to contain high crude protein content of superior quality with an equally high nutritional index value than other species. This made them to conclude that "the high nutritional value of *T. umkowaanii* and *T. sagittiformis* protein warrant the commercial cultivation of fruit bodies" (Botha & Eicker, 1992). This shows that *T. sagittiformis* can be used as a protein supplement by vegetarian individuals.

The total lipids (Crude fat), 5.5 g/100 g and 1.4 g/100 g in the pileus and stipe respectively, was within the range observed by Kalač (2009). The ash content found in the pileus part of the mushroom (10.3 g/100 g) was twice that obtained in the stipe (5.4 g/100 g). Our values were however consistent with results found in other studies conducted on mushroom species. As observed from other studies, the carbohydrate account for the most part of the dry component of the mushrooms (Ijeh *et al*., 2016; Kalač, 2009; Liu *et al*., 2012). Our findings show that the pileus contain less carbohydrates (54.6 g/100 g) than the stipe (71.8 g/100 g). Since *Termitomyces* species form symbiotic relationship with termites that feed on the mushroom, this could explain why most of the carbohydrate is stored in the stipe rather than in the pileus part of the mushroom. From the mycochemical studies conducted on this species, mannitol (Compound **4**, isolated and characterised from the extract) was found to be the main representative of the monosaccharide. The nutritional pattern (total carbohydrates > crude protein > ash > crude fat) was similar to that observed by Liu *et al*. (2012). The energy profile

(386.2 Kcal/100 g in the pileus and 385.3 Kcal/100 g in the stipe) was similar to that obtained in other mushroom studies (Heleno *et al*., 2015).

5.6.2 Elemental content in T. sagittiformis

This is the first study involving the elemental constituent composition of *T. sagittiformis* collected from the natural environment in South Africa. No previous data on the accumulation of elemental concentration is available in the literature. A total of thirty (30) analytes' (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, In, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Si, Sr, Tl, V and Zn) concentration in caps and stipes of *T. sagittiformis* were evaluated. The results for the analysis are given in Table 5.3. Analytes that were not detected (Ag, Al, As, B, Be, Bi, Cd, Co, Ga, Hg, In, Mo, Ni, Pb, Se, Sr and V) in both the pileus and stipes part of the mushroom were omitted in the table of results. Both the pileus and stipes of *T. sagittiformis* were found be good accumulator of the elements K > Cu > Si > and Zn, with the highest amount found on the pileus part of this species.

The amount of K in the pileus ranged from 31201 – 32180 mg kg^{-1} db while in the stipe it was 17817 – 19040 mg kg^{-1}db and is in the same range as findings from studies conducted on other mushroom species (Mędyk *et al*., 2017). Astonishingly the amount of K was by far higher that obtained from a *Termitomyces* species, *T. intermedius* (Reyes *et al*., 2016). We also noticed that values for K were more than 3 – 5 times higher than what was obtained in other mushroom species (Falandysz *et al.*, 2017; Ijeh *et al*., 2016; Quarcoo & Adotey, 2013; Ugbogu *et al*., 2018). The concentration range of Cu (31.7 – 33.4 mg kg^{-1} db) and Zn (81.0 – 81.6 mg kg^{-1} db) were in the same range as that found in other non- *Termitomyces* mushroom species studied internationally (Falandysz & Borovička, 2013). Silicon was found to be the second most abundant element after oxygen in a study conducted on *T. intermedius* while our

study showed that it is the fourth abundant element (543.4 – 714.3 mg kg^{-1} db) after K, Mg and Na (Reyes *et al*., 2016).

It was however able to regulate the absorption and accumulation of other elements (Ba, Ca, Cr, Fe, Li, Mg, Mn and Na) even though the geochemical site is rich in these elements. These elements were not bioaccumulated (BAF < 1) by this species by the elemental concentrations differed significantly at $p < 0.05$, with Mg (1869.3 – 1946.3 mg kg^{-1}) > Na (824.9 – 854.1 mg kg^{-1}) > Fe (411.3 – 574.7 mg kg^{-1}) > Ca (143.6 – 144.7 mg kg^{-1}) > Mn (29.4 – 31.8 mg kg^{-1}) > Li (13.9 – 35.0 mg kg^{-1}) > Ba (1.4 – 16.2 mg kg^{-1}) > Cr (1.6 – 1.8 mg kg^{-1}). Our results on these analytes differed significantly with accumulated concentrations in other mushroom species (Falandysz & Borovička, 2013). We also noticed that the elements Ca, Li and Na were more concentrated in the stipe than in the pileus part of the fruiting body.

The most consumed part of the mushroom fruiting body is the pileus than the stipe. It is therefore important to determine the translocation factors between these two parts of the mushroom in order to evaluate deposition of elemental content (Gupta *et al*., 2019). It is widely known that natural and anthropogenic activities release vast amounts of trace and toxic elements which are ultimately absorbed and accumulated by mushroom species. In this study it was found that the translocation factor for Ba (5.7) was by far the highest compared to the values for Cr, Cu, Fe, K, Mg, Mn, Si and Zn which ranged between 1.1 and 1.7. This means that the pileus and the stipe contain comparable amounts of accumulated element as shown by similar letters on each row at ($p < 0.05$) for multivariate Tukey Post Hoc Multiple comparison on Table 5.3.

Table 5. 3 Elemental concentration, in mg kg^{-1}, dry biomass (db) in both parts (pileus and stipes) of *T. sagittiformis* mushroom, (mean ± SD; n = 3)

Element	Wavelength	Elemental concentration, median and range			TF	BAF	
		Pileus (p)	Stipe (s)	Soil substrate (ss)	p/s	p/ss	s/ss
		7.9±7.5[a]*	1.4±0.6[a]	228.5±34.0	5.7	0.0	0.0
Ba	455.403	6.9**	1.2	206.4			
		1.4 – 16.2***	0.9 – 2.1	206.4 – 267.6			
		144.1±0.5[b]	177.0±21.6[b]	2692.6±360.7	0.8	0.1	0.1
Ca	422.673	144.2	178.0	2572.7			
		143.6 – 144.7	155.0 – 198.1	2407.1 – 3097.9			
		1.7±0.1[c]	1.6±0.1[c]	55.4±10.0	1.1	0.0	0.0
Cr	267.716	1.6	1.6	54.2			
		1.6 – 1.8	1.5 – 1.7	46.0 – 65.9			
		32.4±0.9[d]	22.1±1.5[d]	13.6±4.0	1.5	2.4	1.6
Cu	324.754	32.0	21.9	11.6			
		31.7 – 33.4	20.7 – 23.6	11.0 – 18.2			
		476.8±86.4[e]	313.6±54.7[e]	42166.6±7814.8	1.5	0.0	0.0
Fe	259.940	444.4	305.7	41689.5			
		411.3 – 574.7	263.3 – 371.8	34601.3 – 50209.0			
		31698.7±489.9[f]	18516.0±629.8[f]	3721.1±965.1	1.7	8.5	5.0
K	766.491	31713.8	18691.3	3603.2			
		31201 – 32180	17817 – 19040	2820.4 – 4739.7			
		27.5±11.7[g]	42.5±20.8[g]	141.9±18.6	0.6	0.2	0.3
Li	460.289	33.5	54.5	135.4			
		13.9 – 35.0	18.5 – 54.6	127.4 – 162.8			
		1897.6±42.3[h]	1100.4±39.6[h]	3535.7±630.3	1.7	0.5	0.3
Mg	279.078	1877.3	1077.8	3478.2			
		1869.3 – 1946.3	1077.3 – 1146.1	2936.1 – 4192.8			
		30.5±1.2[i]	26.5±1.1[i]	504.5±54.1	1.2	0.1	0.1
Mn	257.610	30.4	26.4	488.5			
		29.4 – 31.8	25.5 – 27.6	460.1 – 564.8			
		842.9±15.8[j]	1343.8±114.3[j]	1284.0±62.4	0.6	0.7	1.0
Na	588.995	849.9	1408.0	1271.7			
		824.9 – 854.1	1211.8 – 1411.6	1228.8 – 1351.7			
		629.0±85.4[k]	512.3±52.8[k]	285.5±7.1	1.2	2.2	1.8
Si	251.611	629.4	504.6	287.3			
		543.4 – 714.3	463.7 – 568.4	277.6 – 291.6			
		82.8±1.8[l]	54.0±1.3[l]	54.5±7.0	1.5	1.5	1.0
Zn	213.857	83.0	54.0	55.3			
		81.0 – 84.6	52.7 – 55.4	47.1 – 61.0			

(*) – value represent the elemental concentration, (**) – the median value and (***) represents the range of concentration, TF – represents transfer factor for a particular element and BAF – represents bioaccumulation factor. In each column, different letters mean significant differences ($p < 0.05$) for multivariate Tukey Post Hoc Multiple comparison for observed means.

5.6.3 Yields of distilled hexane extracts and dichloromethane/methanol extracts

Extraction was carried out using distilled hexane, dichloromethane and methanol. Calculated using Equation 5.1, the yields from the hexane extract produced 5.50 % (dry biomass) of the powdered pileus part of the mushroom. This amount was consistent with non-polar solvent extraction recorded for other related studies of Basidiomycetes (Wong & Chye, 2009). The combined yields from the dichloromethane/methanol and methanol was 28.8 % dry biomass. This yield was similar to that obtained from *T. clypeatus* (Mitra *et al.*, 2017) and higher than that of *T. albuminosus* (Mau *et al.*, 2004).

5.6.4 Antioxidant activity studies of methalonic extracts from T. sagittiformis

For organisms to protect themselves against reactive O, N-species, non-enzymatic defences are employed to stop spontaneous reactions that are otherwise harmful to human health (Berker *et al.*, 2007). Antioxidants from natural food or plant material extracts are used to alleviate the problems associated with free radicals in the human body. For this reason, antioxidant assays were conducted on methanolic extracts from *T. sagittiformis* in order to determine the efficacy of the mushroom species. This was carried out using DPPH and Frap analysis which are both described in Section 5.3.7.

5.6.4.1 DPPH free radical scavenging activity of T. sagittiformis

Termitomyces sagittiformis species showed appreciable scavenging activities of DPPH free radicles at concentrations above 4000 μg mL^{-1} as shown in Figure 5.1. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry at 517 nm.

The curve was based on the range of concentrations from 62.5 to 25000 µg mL^{-1}. Our results were however very low compared to the scavenging capabilities shown by other wild edible species of mushrooms in the literature (Gebreyohannes *et al.*, 2019; Mau *et al*., 2004; Shameem *et al.*, 2015; Tsai *et al*., 2007; Wong & Chye, 2009). The effective concentration at which 50% of DPPH radicals are scavenged (EC_{50}) for the mushroom species was 27760 ± 2.51 µg mL^{-1} while that of the control (vitamin C) was 269 ± 0.39 µg mL^{-1} showing that *T. sagittiformis's* potency in inhibiting DPPH was 103 times less than that of vitamin C. The EC_{50} value for *T. sagittiformis* was much higher than those reported for *Agaricus,* other *Termitomyces* and *Auricularia* species (Barros *et al*., 2008; Gebreyohannes *et al*., 2019; Loganathan *et al.*, 2010; Mitra *et al*., 2017).

5.6.4.2 Ferric-reducing antioxidant power (FRAP) of T. sagittiformis

The reducing power of methanolic extract from the mushroom species increased with an increase in the concentrations as shown in Figure 5.2. After 50 µg mL^{-1}, the reducing power of the crude extract and fractions were in the order: Fraction C (ethyl acetate partition) > Fraction D (ethyl acetate/methanol partition) > crude extract (methanol extract from dry sample). This shows that Fraction C had secondary metabolites that could stabilize free radicals and possibly terminate radical chain reaction. The chemistry taking place can be summed up using Equation 5.4.

$$Fe(CN)_6^{3-} + antioxidant \leftrightarrows Fe(CN)_6^{4-} + oxidizedantioxidant,$$

$$Fe(CN)_6^{4-}\ Fe^{3+} \leftrightarrows Fe[Fe(CN)_6]^{-} \quad \text{.....Equation 5.4}$$

Using the best line fit from the absorbance vs concentration curves (Figure 5.2), we were able to determine the EC_{50} for both the sample fractions and the positive standards (BHT and Vitamin C). From the IC_{50} values, it was noticed that Fraction C (5.6 µg mL^{-1}) contains antioxidant species that are 50% effective compared to both standards, BHT (2.1 µg mL^{-1}) and Vitamin C (2.6 µg mL^{-1}) while the crude extract (22.1 µg mL^{-1}) was less active compared to its fractions. Fraction D had an IC_{50} value of (9.4 µg mL^{-1})

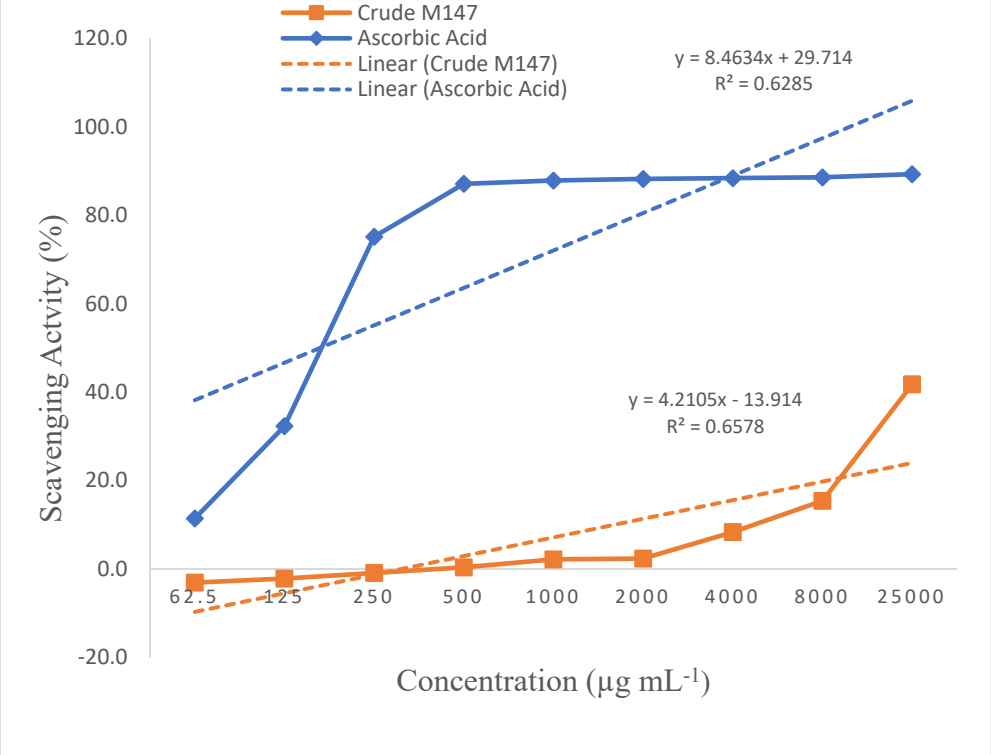


Figure 5. 1 Scavenging activity of methanolic extracts from *T. sagittiformis* on 2,2 diphenyl-1-picrylhydrazyl radicals (DPPH), mean (n = 3).

This means that *T. sagittiformis* mushroom species can be a potential source of natural antioxidants, and its consumption can provide some level of health protection against oxidative damages. The reducing power of *T. sagittiformis* was higher than that of *T. medius* (Mitra *et*

al., 2014) and much lower than that of some *Agaricus* species reported in the literature (Barros *et al.*, 2008).

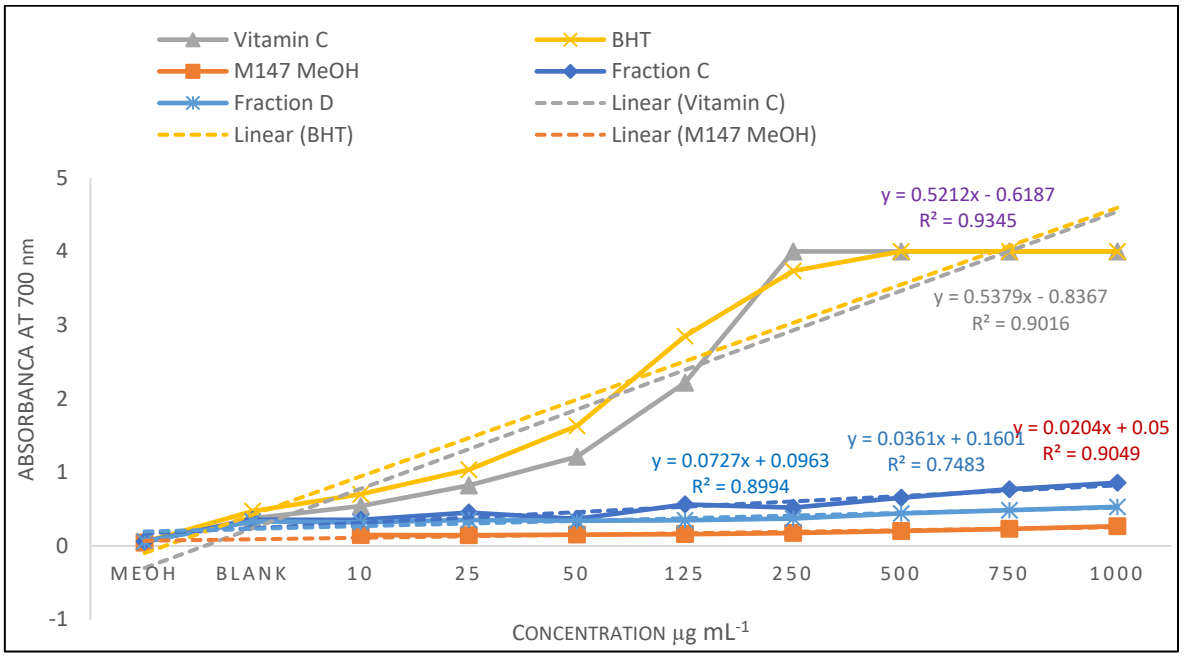


Figure 5. 2 Reducing power of methanolic extracts from *T. sagittiformis.* Each value is expressed as mean (n = 3).

5.6.5 Total Phenolic Content (TPC) of the methalonic extracts from T. sagittiformis

Phenolic compounds are secondary metabolites, some of them are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron (Song *et al*., 2010). It is widely known and accepted that the TPC in the matrix extract of plant material is enhanced by the presence of other interfering compounds like ascorbic acid and reducing sugars (Sánchez-Rangel *et al.*, 2013). In such instances pre-treatment of the extract is carried before conducting F – C assays through the use of solid phase extraction (SPE) on the extract, subtraction of ascorbic acid value from the TPC obtained or treatment of the extract with oxidative agents like hydrogen peroxide.

Having not observed the formation of blue colour before addition of the alkali, sodium carbonate, it was assumed that the presence of interfering compounds is minimal and as such no treatment of extracts was carried out. TPC was carried out on both the pileus and stipe parts of the mushroom. The total phenol content of the methalonic extract was found to be 5.40±0.44 mgGAE/g pileus and 7.38±0.68 mgGAE/g stipe. Our results agreed with what was obtained from the methanol extract of *L. salmonicolor* and *T. tylerance*, while higher than that of *T. mummiformis* and *T. microcarpus*, also being lower than that of *T. heimii* (Athanasakis *et al*., 2013; Mau *et al*., 2004).

5.6.6 Antimicrobial Assay of T. sagittiformis extracts

Antimicrobial assay of the hexane and dichloromethane extracts showed minimal activity on selected microbial strains, while the methalonic extract showed negative response against the strains as shown in Table 5.4. Our values compare well with the study conducted on *T. microcarpus* and *T. robustus* (Jonathan *et al.*, 2008). They also showed similar pattern to the study conducted on other unspecified *Termitomyces* species that were found to show a much better degree of activity on pathogenic organisms (Gebreyohannes *et al*., 2019).

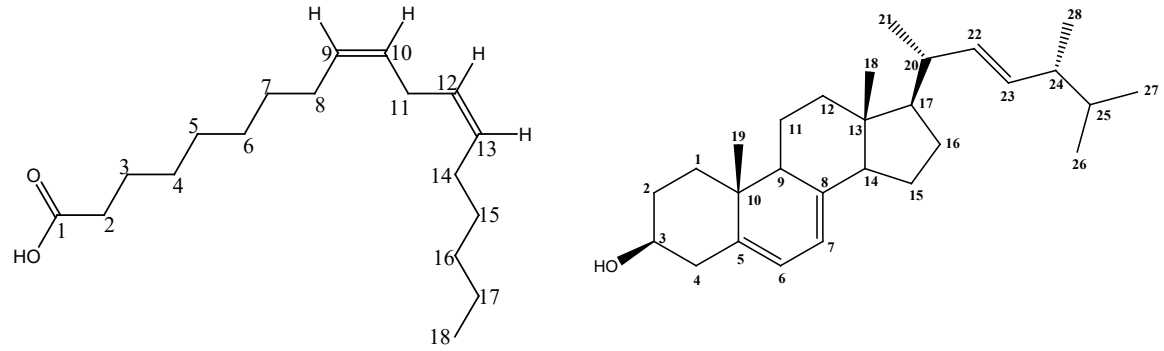
Table 5. 4 Antimicrobial activity of *T. sagittiformis* extracts against selected bacteria strains

Extraction Solvent	**MIC (mg mL^{-1})**				
	Sa	Ec	Kp+	Ms	Ma
Hexane extract	5	5	2.5	5	5
Dichloromethane extract	2.5	5	5	5	5
Methanol extract	>5	5	>5	>5	>5
Neomycin	0.12	0.98	3.9	-	-
Ciprofloxacin	-	-	-	0.03	0.95

Sa -*Staphylococcus aureus*; Ec - *Escherichia coli*; Kp+ - *Klebsiella pneumoniae* (Drug-resistant strain); Ms - *Mycobacterium smegmatis; Ma - Mycobacterium aurum A+*
MIC – minimum inhibitory concentration.

5.6.7 Mycochemical compounds extracted from T. sagittiformis

The hexane extract was partitioned with hexane/ethyl acetate solvents using Grace Reveleris^{TM} machine, shown in Figure A1.5 to give a carboxylic acid and a sterol, Compound **1** and **2**, which were identified as linoleic acid and ergosterol respectively. These compounds are widely found in higher fungi as seen from several studies conducted (Alexandre *et al*., 2017; Cai *et al.*, 2013; Yan *et al*., 2020; Yanjun Zhang *et al.*, 2002).



1 **2**

Figure 5. 3 Chemical structures of Compound **1**, linoleic acid and Compound **2**, ergosterol

The structures were elucidated using ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, GC-MS spectral techniques (Appendix A5). Compound **1**, 23.42 mg, (9Z,12Z)-octadeca-9,12-dienoic acid, a Colourless, oily liquid with a molecular formula $C_{18}H_{32}O_2$. Linoleic acid is abundant in mushroom species (Kalač, 2013) and was found to be the major fatty acid (62.4 – 63.4%) in *Lentinus squarrosulus,* a wild edible mushroom found in Africa and Asia (Lau & Abdullah, 2017). The compound contains two double bonds, one between carbons 9 and 10 and the other one between carbons 12 and 13 (Figure 5.3). The four olefinic hydrogens appear at the same position given by δ_H = 5.34 – 5.35 ppm on the ^{1}H NMR (Appendix 5) and at δ_C = 129.24 and

130.99 ppm on ^{13}C NMR. The signal of the CH_2 group at position 11, the bis-allylic group appear at position δ_H = 2.78 ppm and δ_C = 26.73 ppm. The chemical shift of the CH_2 known as the allylic group, position 8 and 14 appeared at δ_H = 2.07 ppm and δ_C = 28.35 ppm. These chemical shifts were more closely related to the study carried out on fatty acid profile by ^1H-NMR spectroscopy (Knothe & Kenar, 2004). According to the literature, several fatty acids have been isolated from mushroom species (Abubakar & Majinda, 2016; Yan *et al*., 2020).

Similar to *T. microcarpus* wherein five ergostanes derivatives were isolated (Hsieh & Ju, 2018), we were able to isolate Compound **2**, 19.5 mg, (ergosterol), molecular weight of 396 g mol^{-1} from the mushroom species, *T. sagittiformis.* The data shown in Table 5.5 were used to characterise Compound **2**. The data presented were obtained from spectra of ^1H NMR, ^{13}C NMR, COSY (^1H-^1H), HSQC (^1H-DEPT), HMBC (^1H-^{13}C) and DEPT spectra. These were closely comparable with data reported in the literature (Alexandre *et al*., 2017; Martinez, 2016; Zhao *et al.*, 2018). The results obtained from the GC-MS showed a retention time peak at 26.915 and a 93 % similarity index (SI). Ergosterol is an important sterol found in many fungal species (Elsayed *et al.*, 2014; Ragasa *et al.*, 2015; Ragasa *et al.*, 2016; Ragasa *et al*., 2018). It has shown to contain anti-*Trypanosoma cruzi* activity against trypomastigotes, hence can be used as an anti-parasite (Alexandre *et al*., 2017). It has also been established that ergosterol inhibit the secretion of inflammatory mediator, nitric oxide (NO) by at least 6.00%, while it inhibits the activity of NF-kB luciferase by 23.46%. Furthermore, it was observed that ergosterol is active against prostatic carcinoma cell line PC3 and breast carcinoma cell line MDA-MB-23 (Ma *et al.*, 2013). It is a precursor of vitamin D.

Table 5. 5 ^{13}C and ^{1}H-NMR data, and ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC correlation for ergosterol (**2**, 400 MHz, $CDCl_3$).

Carbon	Ergosterol				
	^{13}C (δ in ppm)	DEPT	^{1}H (J in Hz)	COSY	HMBC
1	39.1	CH_2	1.23		
2	32.0	CH_2	1.88	H-3	
3	70.6	CH	3.63		
4	40.9	CH_2	2.27	H-3	
5	139.8	C			C-19
6	119.7	CH	5.56		
7	116.4	CH	5.38	H-6	
8	141.4	C			
9	46.3	CH	1.96		
10	37.1	C			
11	21.2	CH_2	1.05		
12	38.5	CH_2	1.88		
13	43.0	C			
14	54.7	CH	1.90		
15	23.0	CH_2	1.34	H-16	
16	28.3	CH_2	1.75 1.33		
17	55.9	CH	1.27	H-16, 20	C-21
18	12.1	CH_3	0.62		
19	16.3	CH_3	0.92		
20	40.5	CH	2.02	H-22, 23	C-21
21	21.2	CH_3	1.59	H-20	
22	135.7	CH	5.20		C-21
23	132.1	CH	5.20		C-28
24	42.9	CH_2	1.86	H-22, 23	C-22, C-23, C-25 C-26, C-27, C-28
25	33.1	CH	1.46		C-24, C-26, C-28
26	20.0	CH_3	0.83	H-25	
27	19.7	CH_3	0.83	H-25	C-24, C-25, C-26 C-28
28	17.7	CH_3	0.91	H-24	

The (DCM/MeOH, 1:1) extract was subjected to solid phase extraction (SPE) using a DIOL SPE Cartridge (6 cc/2g) following a sequential vacuum elution described in Section 5.3.6, to afford 5 fractions. The fractions were subjected to ordinary-phase silica gel column chromatography (500 g) starting with 100% DCM that was stepped by 10% to 100% MeOH to give 18 fractions. These fractions were further partitioned while monitoring with TLC plates to give 4 compounds, compound **3** (glycosphingolipid), compound **4** (mannitol), compound **5** (oleic acid) and compound **6** (uracil) (Figure 5.2).

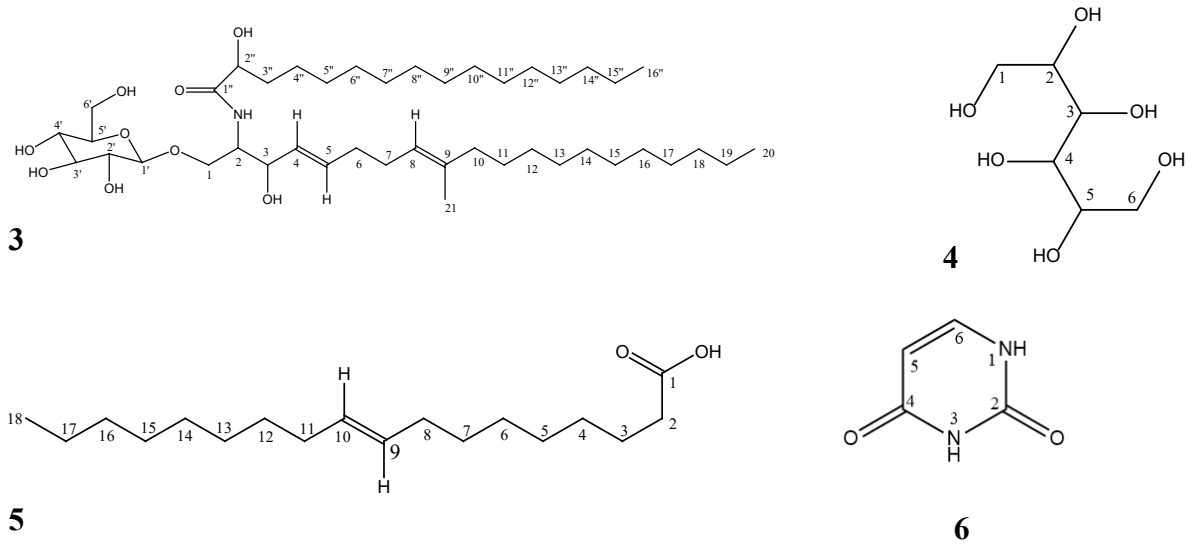


Figure 5. 4 Chemical structures of Compound **3**, glycosphingolipid, Compound **4**, Mannitol, Compound **5**, Oleic acid, and Compound **6**, Uracil.

Glycosphingolipids (GSLs) are composed of long-chain base (ceramide moiety) and fatty acid acyl chain, linked together by an amino group at C-2 and C-1, respectively, and carbohydrate sugar moiety (Müthing & Distler, 2010; Stults *et al.*, 1989).

Table 5. 6 ^{13}C and ^{1}H-NMR data, and ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC correlation for glycosphingolipid (400 MHz, CD_3OD).

Carbon	Glycosphingolipid				
	^{13}C	DEPT	^{1}H (*J* in Hz)	COSY	HMBC
1_a	69.89	CH_2	4.12 (dd, 12.0, 5.6)	H-2, H-5	C-1', C-2, C-3
1_b			3.70 (dd, 19.4, 7.4)	H-2, H-3	
2	54.81	CH	4.00 (m)	H-1_{ab}, H-3, H-21	
3	73.06	CH	4.13 (m)	H-1_b, H-2, H-5	C-2, C-4, C-5
4	134.79	CH	5.75 (dt, 15.4, 3.9)	H-5, H-6, H-7	C-3, C-6, C-7
5	131.27	CH	5.50 (dd, 7.3, 8.2)	H-3, H-4, H-6, H-7	C-3, C-6
6	33.96	CH_2	2.07 (m)	H-4, H-8	C4, C-7, C-8
7	28.84	CH_2	2.07 (m)	H-4, H-8	C-4, C-6, C-8
8	124.97	CH	5.15 (t,6.8)	H-6, H-7, H-21	C-7, C10, C-21
9	136.92	C			
10	40.94	CH_2	1.98 (t,7.1)	H-8, H-11	C-4, C-8, C-11, C-21
11	29.27	CH_2	1.41 (m)		C-21
12 – 17	30.54 – 30.99	CH_2	1.36 – 1.29 (br, s)		
18	33.24	CH_2	1.36 – 1.29 (br, s)		
19	23.91	CH_2	1.36 – 1.29 (br, s)		
20	14.61	CH_3	0.90 (t, 7.1)		C-18, C-19
21	16.31	CH_3	1.60 (s)	H-2, H-8, H-11	C-3, C-4, C-8, C-10
Glucosyl moiety					
1'	104.86	CH	4.27 (d, 7.8)	H-2'	C-1, C-5'
2'	75.15	CH	3.20 (t, 8.4)	H-1', H-3'	C-1', C-5'
3'	78.08	CH	3.36 (t, 9.3)	H-2'	C-4'
4'	71.75	CH	3.29 (m)	H-6_b'	C-5'
5'	78.15	CH	3.29 (m)	H-6_b'	
6_a'	62.85	CH_2	3.87 (br d, 11.7)	H-4, H-5, H-6_b'	C-4', C-5'
6_b'			3.69 (dd, 11, 4)	H-2', H-6_a'	
NH					
1''	177.34	C			
2''	73.27	CH	3.99 (t, 11.0)		C-3'', C-4''
3''	36.04	CH_2	1.60 (s)		
4''	26.32	CH_2	1.41 (m)		
5''	33.96	CH_2	2.07 (m)		
6''	28.84	CH_2	2.07 (m)		
7'' – 13''	30.54 – 30.99	CH_2	1.36 – 1.29 (br, s)		
14''	33.24	CH_2	1.36 – 1.29 (br, s)	H-16''	
15''	23.91	CH_2	1.36 – 1.29 (br, s)	H-16''	
16''	14.62	CH_3	0.89 (t, 7.1)	H-14'', H-15''	C-14'', C-15''

The spectral analysis of compound **3**, shows that the ceramide moiety contained 9-methyl-4,8-sphingadienine in amidic linkage to 2-hydroxyhexadecanoic acids, and a carbohydrate portion consisting of one residue of glucose which was consistent with known GSLs in the literature (Levery, 2005). ^{13}C NMR spectra, Figure A5.15, revealed four unsaturated carbons at δ_C 124.97 – 136.92 ppm corresponding to C-4 (δ_C 134.79); C-5 (δ_C 131.27), C-8 (δ_C 124.97) and C-9 (δ_C 136.92). Methyl carbons were observed in C-20 (δ_C 14.61), C-21 (δ_C 16.31) and C-16'' (δ_C 14.61). Our proposed structure was related to compounds isolated from the Basidiomycete *Polyporus ellisii*, Marine Algous Endophytic Fungus *Aspergillus niger,* filamentous soil fungus *Mortierella alpine, Clitocybe geotropa* and *Clitocybe nebularis* (Batrakov *et al*., 2002; Fogedal *et al.*, 1986; Gao *et al.*, 2001; Hsieh & Ju, 2018; Zhang *et al*., 2007). It was however different from structures of termitomycesphin isolated from the species *T. albuminosus* (Qi *et al*., 2000; Qu *et al*., 2012).

The nature of the carbohydrate sugar moiety was established using the works conducted by (Beier *et al.*, 1980), wherein they looked at the assignment of the linkage position of the anomeric carbon on different ^{13}C NMR data from several research papers. On average they found that β-D-galactopyranoside and β-D-glucopyranoside have slightly different chemical shift on the anomeric carbon, C-1 (δ_C 104.8) and (δ_C 104) ppm respectively. They however noticed a big difference in the chemical shift of C-4 which is the carbon containing constituents that differentiate these stereoisomers. Both these sugars differ only on the hydroxyl group in C-4 which is either axial or equatorial. The investigation showed that β-D-galactopyranoside contains two hydrogen bonds while β-D-glucopyranoside contains one hydrogen bond (Kotena *et al.*, 2013). This have an effect on the chemical shift observed on both, with β-D-galactopyranoside showing the carbon at 69.6 ppm and β-D-glucopyranoside at 71.0 ppm. A comparison of the ^{1}H NMR chemical shift of the anomeric H on compound **3** confirmed that it was a β-D-glucopyranoside as opposed to α-D-glucopyranoside as the chemical shifts are 4.64

and 5.24 ppm and the spin-spin couplings (J_{H-H}) are 7.0 and 2.7 Hz respectively (Drake & Brown, 1977). For our compound the ^{1}H NMR chemical shift of the anomeric H was found to be 4.27 (d,7.7).

Contrary to GSL structures (termitomycesphins) isolated from *T. albuminosus* (Hsieh & Ju, 2018) we isolated a GSL containing a C-21 that lacked a hydroxylated group on the sphingosine of the long-chain base (LCB) and a hydroxy fatty acid, a part of the amino moiety. Due to lack of a hrodroxlated group, as observed by Hsieh and Ju (2018), we expect our GSL to be inactive in inducing neuronal differentiation.

Compound **4**, mannitol was obtained as a colourless solid, molecular formula: $C_6H_{14}O_6$. Structural characterisation was established using NMR spectra, (Figures A5.18-22). ^{1}H-NMR chemical shifts (D_2O) were 3.73 (1H, d, J =5.72 Hz), 3.79 (1H, d, J =2.60 Hz), 3.81 (1H, d, J =2.74 Hz), 3.85 (1H, d, J =13.71 Hz), 3.92 (1H, dd, J =2.62, 11.65 Hz).

Mannitol is a polyol or sugar alcohol, sometimes referred to as an acyclic hexitol that is found in high abundance in mushroom species (Athanasakis *et al*., 2013; Sanmee *et al.*, 2003; Sławińska *et al*, 2020; Tan & Moore, 1994; Yang *et al.*, 2001). Literature shows that *Agaricus bisporus* contains the highest amount of mannitol, dry weight, in its fruiting body compared to other mushroom species (Kalač, 2013; Sławińska *et al*., 2020; Tan & Moore, 1994). Its role is believed to be the carbohydrate storage, a reservoir of reducing power, stress tolerance and spore dislodgement and/or disposal, quenching of reactive oxygen species and the sequestering of host derived fructose (Solomon *et al.*, 2007).

Compound **5** isolated from our study was oleic acid as supported by spectra given in Appendix A5. ^{1}H-NMR chemical shifts (MeOD) were 0.90 (1H, t, J =6.93 Hz), 1.60 (1H, d, J =13.68 Hz), 2.03 (1H, d, J =5.77 Hz), 2.27 (1H, t, J =7.45 Hz), 5.34 (1H, t, J =4.59 Hz).

Compound **6** was obtained as colourles residue. The molecular formula was established as $C_4H_4N_2O_2$ using GC-MS results with 95% similarity index, as shown in Figure A5.27. The compound was identified as uracil, a major constituent of ribonucleic acid (RNA), naturally occurring pyrimidine derivative, described in Subsection 2.6.2.5 (Ayer & Trifonov, 1994; Emel'yanenko *et al.*, 2015; Goldstein & Tarpley Jr, 1971).

In the ^{1}H-NMR spectrum, signals of two methines at δ 5.61 (1H, d, J = 7.76Hz, H-6) and δ 7.39 (1H, d, J = 7.65Hz, H-5) were observed. The ^{13}C-NMR and HMBC spectra displayed 4 carbon signals, two carboxyl carbons (at δ_c 167.7, C-4 and δ_c 153.7, C-2) and two CH carbons (at δ_c 143.8, C-6 and δ_c 101.9, C-5), In the HMBC experiment, the proton at δ 7.39 showed correlations with C-2, C-4 and C-5 while the proton at δ 5.61 showed correlations with C-6 as shown in Figures A5.28-32. This compound is common to most basidiomycetes species as illustrated by available literature (Athanasakis *et al*., 2013; Lee *et al*., 2017).

5.7 Conclusion

It was found that this mushroom species is rich in nutritional content, especially protein as compared to other *Termitomyces* species, hence it can be an excellent protein supplement. We also found that *T. sagittiformis* was a good accumulator of the elements K > Cu > Si > and Zn, with K content much higher than what is known in other *Termitomyces* species and other basidiomycetes. With regard to total phenolic content, antioxidant activities as well as biological activities of crude extracts, this was the first study conducted on *T. sagittiformis*

species. The crude extract showed appreciable scavenging activities of DPPH free radicles but the (EC_{50}) value was higher (27760 ± 2.51 µg mL^{-1}) than that of the control (vitamin C). The ethyl acetate fraction exhibited high ferric-reducing antioxidant power (FRAP) compared to the crude extract. The total phenol content of the methalonic extract was found to be 5.40±0.44 mgGAE/g pileus and 7.38±0.68 mgGAE/g stipe, comparable with studies conducted on other *Termitomyces* but lower than those of *T. heimii*. Antimicrobial assay of the hexane and dichloromethane extracts showed minimal activity on selected microbial strains compared to the methanol extract. In addition to all these findings, we were able to isolate and fully characterise six mychochemical compounds from the extracts of *T. sagittiformis*, namely, linoleic acid, ergosterol, glycosphingolipid, mannitol, oleic acid and uracil.

5.8 Acknowledgements

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5.9 Conflict of Interest

The authors declare that they have no conflict of interest.

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CHAPTER 6

Conclusions and Future Research

6.1 Introduction

It is estimated that the total number of fungal species on earth is between 2.2 and 3.8 million, yet very few species are currently known. This number is at least six times the estimated number of plants species. New species are discovered yearly at a rate of approximately 2,000 species. Fungus have found many applications in our daily lives and these includes agriculture, beverages, biofuels, bioremediation, cotton processing, food, hallucinogens, leather processing, medicines, paper manufacturing, plastics and biomaterials, vitamins and washing detergents. Of the known fungal species, only 350 are consumed as food and it is important to know the nutritional and medicinal benefits derived from their consumption. Since fungi live in competitive environments for their survival, similar to plant species, they produce secondary metabolites that are important to combat certain disease that attacks humans and animals.

6.2 Revisiting the aims and objectives

The focus of this study was to determine the elemental intake and distribution in six species of wild growing mushrooms, namely *Amanita pantherina* (inedible); *Boletus edulis* (edible); *Boletus mirabilis* (edible); *Lactarius deliciosus* (edible); *Russula sardonia* (inedible) and *Termitomyces sagittiformis* (edible) as well as isolation and characterisation of mychochemical compounds in *Termitomyces sagittiformis* (edible).

6.3 Contributions to new knowledge

The bioaccumulation of elements of the wild growing mushrooms under study were in the following order: *Amanita pantherina* (K > Na > Zn > Cu > Mg); *Boletus edulis* (K > Cu > Zn > Se > Na > Mg); *Boletus mirabilis* (K > Cu > Zn > Na > Mg); *Lactarius deliciosus* (K > Zn > Mg); *Russula sardonia* (K > Na > Zn > Cu > Mg) and *Termitomyces sagittiformis* (K > Cu > Si > Zn). All mushroom species were bioexcluders of As, Be, Ca, Cd, Co, Cr, Fe, Mn, and Ni. The results showed elemental concentrations to be species and habitat dependent but independent of the edibility of the mushroom. Correlation analysis showed accumulation of elements by the edible mushrooms to be affected by soil parameters more than inedible ones, with As and Cd accumulation being inhibited by available Cu in the soil substrate, indicating the self-preservation tendency of wild mushrooms. These findings validate the ethno-medicinal use and consumption of wild growing mushrooms due to low accumulation of toxic metals therefore, their promotion as food supplements should be encouraged to overcome nutritional deficiencies in low income markets. The edible mushroom species, *B. edulis,* was found to be rich in Se and could contribute over 100 % towards the RDA.

The nutritional content of the four edible mushrooms (*B. edulis, B. mirabilis, L. deliciosus, T. sagittiformis*) contained proteins (21.5 – 55 %), carbohydrates (34 – 72 %), ash (3 - 10 %) and lipids (0.8 - 5.5 %). The estimated energy from consuming 100 g, dry biomass, ranges between 383 and 410 kcal.

The crude extract from *T. sagittiformis* showed appreciable scavenging activities of DPPH free radicles but the EC_{50} value was higher than that of vitamin C. The ethyl acetate fraction exhibited high ferric-reducing antioxidant power (FRAP) compared to the crude extract. In addition to all these findings, we were, for the first time able to isolate and fully characterise six mychochemical compounds from the extracts of *T. sagittiformis*, namely, linoleic acid, ergosterol, glycosphingolipid, mannitol, oleic acid, and uracil.

6.4 Challenges

The greatest challenge in this project was the availability of mushroom species that form symbiotic relationship with termites. These mushrooms are less distributed and, at some instances, competition emerged between local people who needed them to supplement their daily food needs with us as researchers. Hence, small amounts of the sample could be collected making it difficult while carrying out mycochemical analysis.

6.5 Future possibilities

To carry out biological assays on isolated compounds as the crude extract and fractions showed positive antioxidant activities. To extract, isolate, characterise mycochemical compounds from other species of *Termitomyces* which are consumed yearly during rainy seasons.

6.6 Final comments and summary conclusions

Mushrooms, like plant species have over centuries proven to contain bioactive compounds that can be beneficial to certain ailments. Nutritional value deduced from edible species obtainable in our environment encourages us to continue with conducting similar studies on other unknown species. Isolated and characterised mycochemical compounds from *Termitomysis sagittiformis* paved a way to conduct more studies in future with the hope of finding safer compounds that can be used as substitutes to dangerous methods like chemotherapy.

List of Figures and Tables in the Appendices

APPENDIX 1: Samples Collection, Treatment and Preservation

Samples of mushrooms handpicked from their natural environments, either in the plantations (pine or eucalyptus), nature reserves or in the vicinity of residential places. Soil and plants debris were immediately cleaned with a paint brush or plastic knife. Cleaned samples were separated according species. Soil substrate were collected from five different in the vicinity where mushrooms were collected, mixed and kept in plastic bottle.

a.  **b.**  **c.** 

Figure A1. 1 Mushrooms and soil samples collection (a) cleaned mushrooms separated on site before taken to the laboratory. (b) collecting soil substrates, 18 cm sampled using a hand augur. (c) Pine plantations where samples were collected.

On arrival at the laboratory, mushroom samples were separated into caps and stipes, placed on porcelain dishes or crucibles, weighed and then dried in an oven set at 40^{o}C as shown in Figure A1.2 (c)

A.  B.  C. 

Figure A1. 2 Preparation of mushroom samples for drying A. cleaned mushrooms pileus/caps, B. cleaned mushroom stipes/stems and C. drying in a digital oven.

Mass measurements of dried samples were taken at 24 hours interval until constant mass. The dried samples were agitated using a ceramic hand mortar, while sieving to get the finest powdered grains as in Figure A1.3. Dried powdered samples were stored in High Density Polyethylene plastic bottles until further use.

A.  B. 

Figure A1. 3 Mushrooms pulverization A. mortar and pestle used to grind dried mushroom samples, B. fine dried powdered samples after sieving.

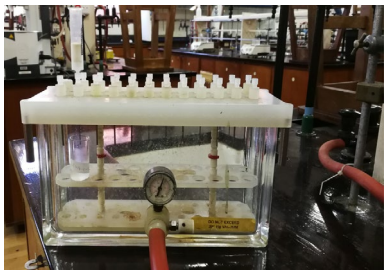
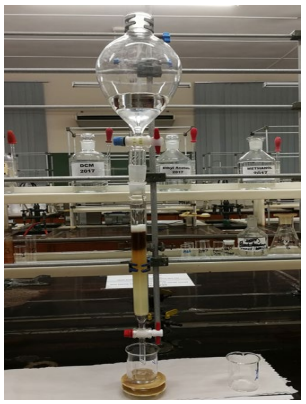
A.  **B.**  **C.** 

Figure A1. 4 Separation techniques employed during mycochemical isolation (A) DIOL SPE used to fractionate the crude extract. (B) silica gel packed column used to separate mycochemical compounds while monitoring with TLC plates. (C) Grace Reveleris machine used to separate ultra-violate active compounds.

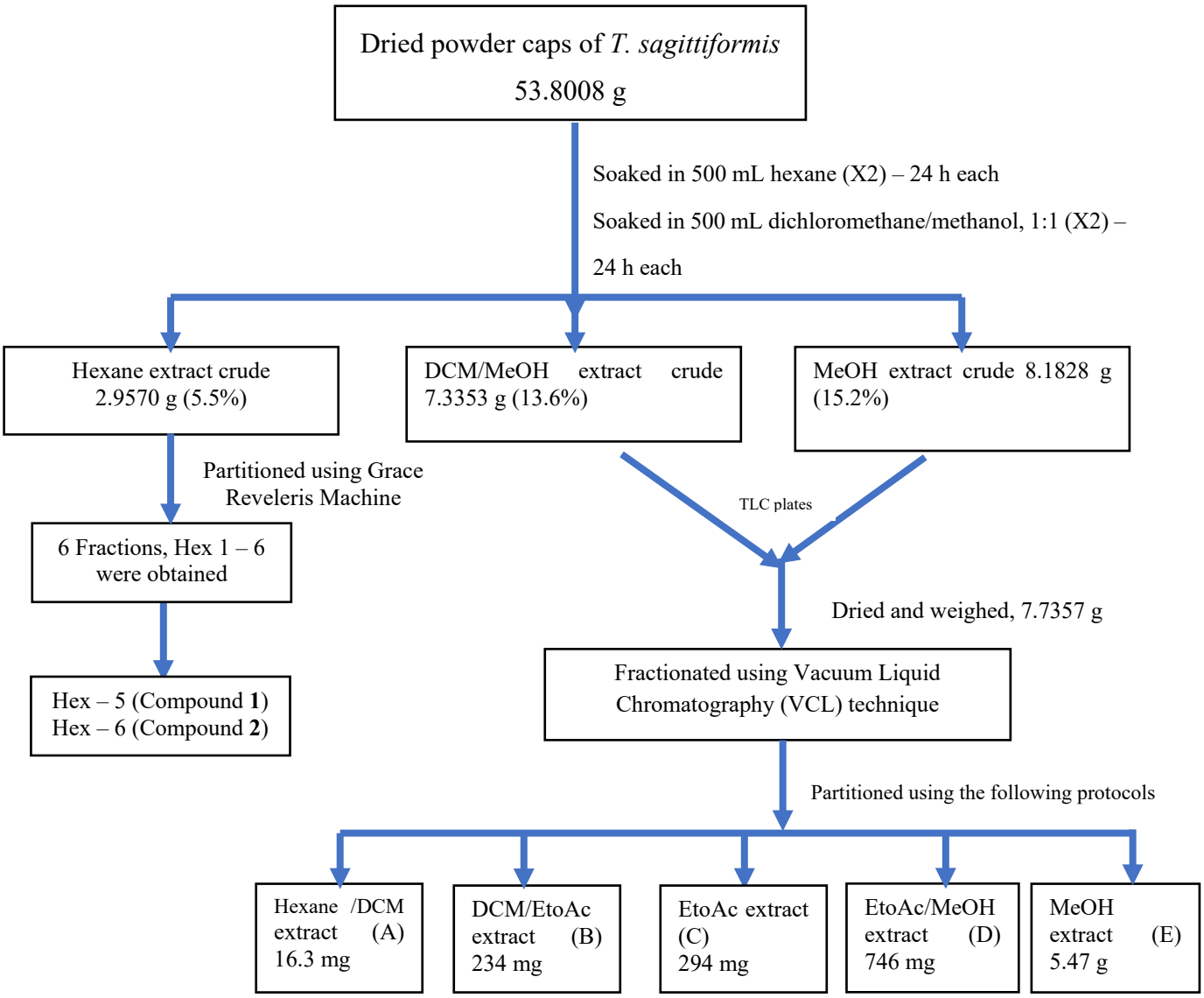


Figure A1. 5 Outline of the partition protocols and purification process carried out during mycochemical isolation.

APPENDIX 2: Bioaccumulation factors for the different species of mushrooms

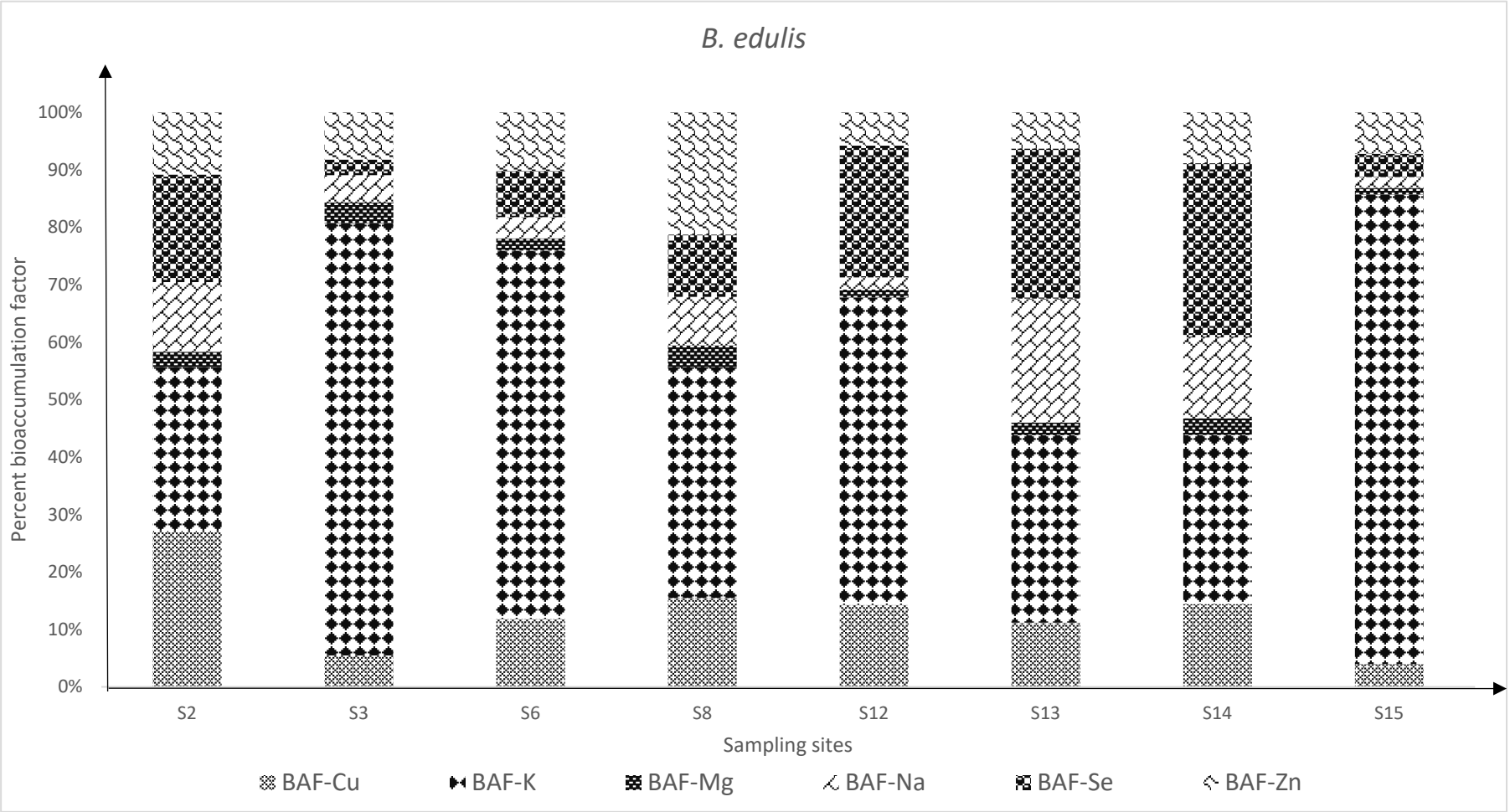


Figure A2. 1 Percent bioaccumulation factor of accumulated minerals by *B. edulis* mushrooms collected from different geographical areas, where sampling sites are: S2 – Bulwer, S3 – Cidara, S6 – Donnybrook, S8 – Greytown, S12 – Singisi, S13 – Stepmore A, S14 – Stepmore B and S15 – Twasamhlobo.

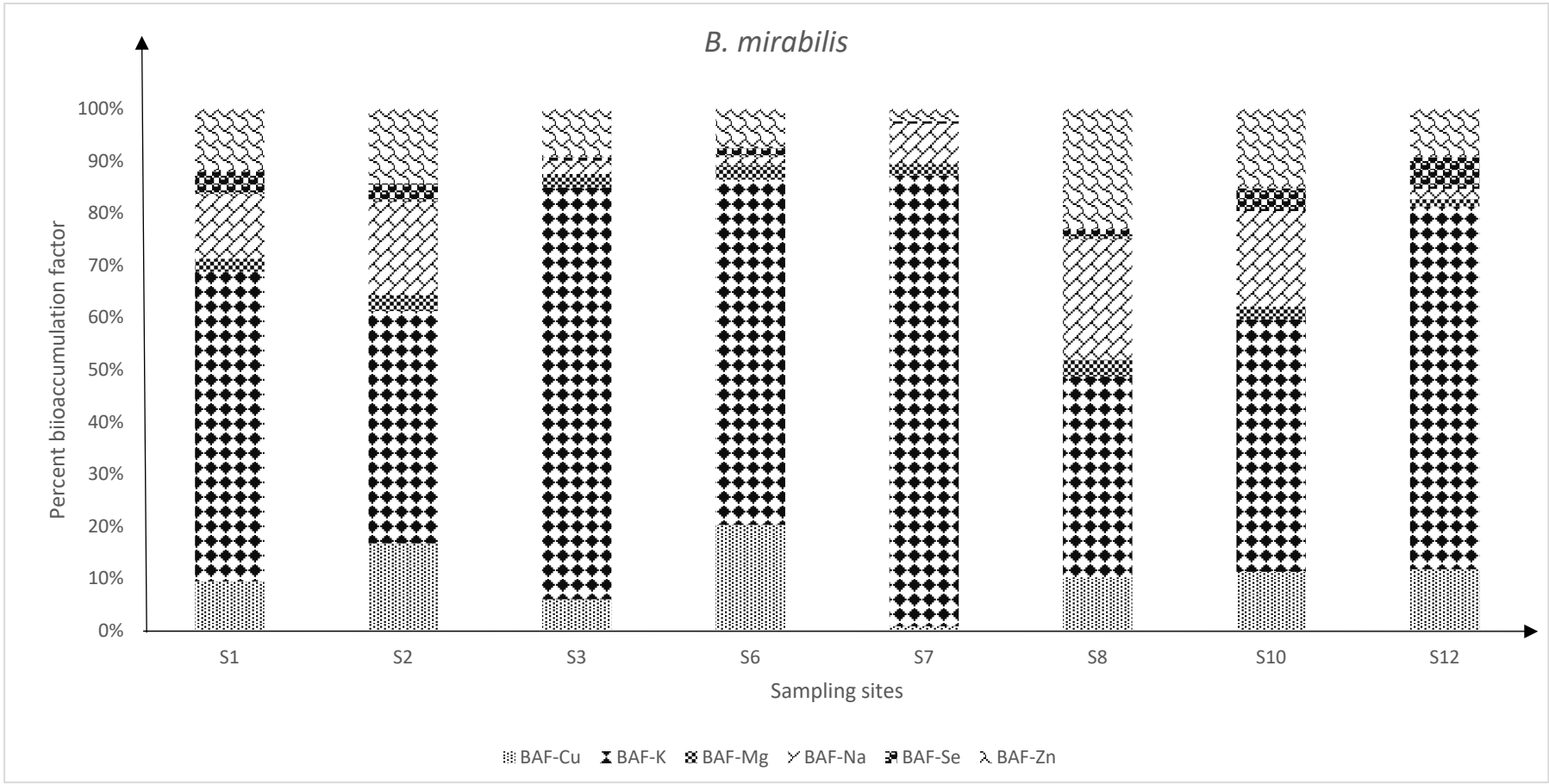


Figure A2. 2 Percent bioaccumulation factor of accumulated minerals by *B. mirabilis* mushrooms collected from different geographical areas, where sampling sites are: S1 -Boston, S2 – Bulwer, S3 – Cidara, S6 – Donnybrook, S7 – Gilboa farm, S8 – Greytown, S10 – Mafakathini and S12 – Singisi.

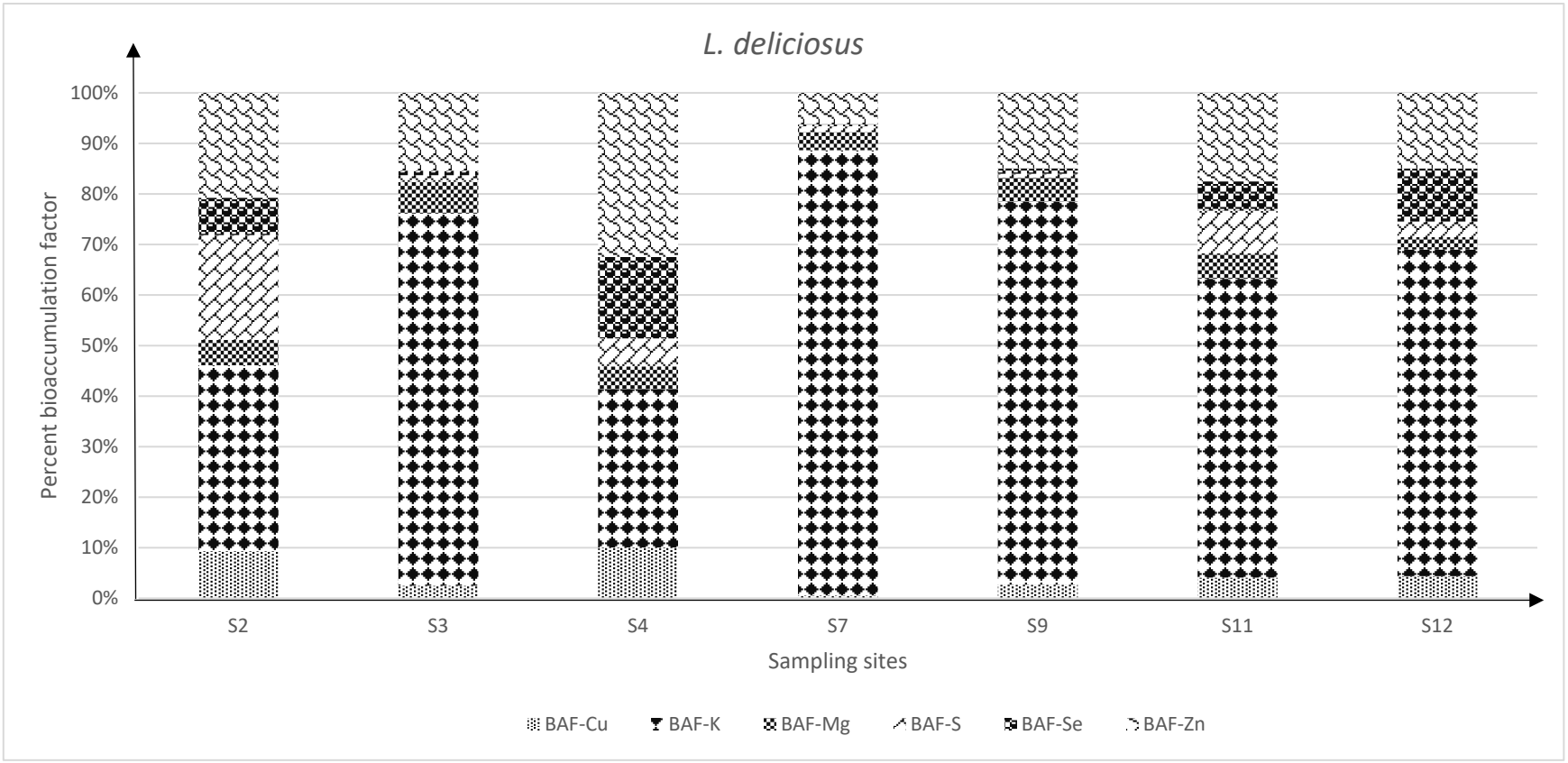


Figure A2. 3 Percent bioaccumulation factor of accumulated minerals by *L. deliciosus* mushrooms collected from different geographical areas, where sampling sites are: S2 – Bulwer, S3 – Cidara, S4 – Clemont, S7 – Gilboa farm, S9 – Karkloof, S11 – Richmond and S12 – Singisi.

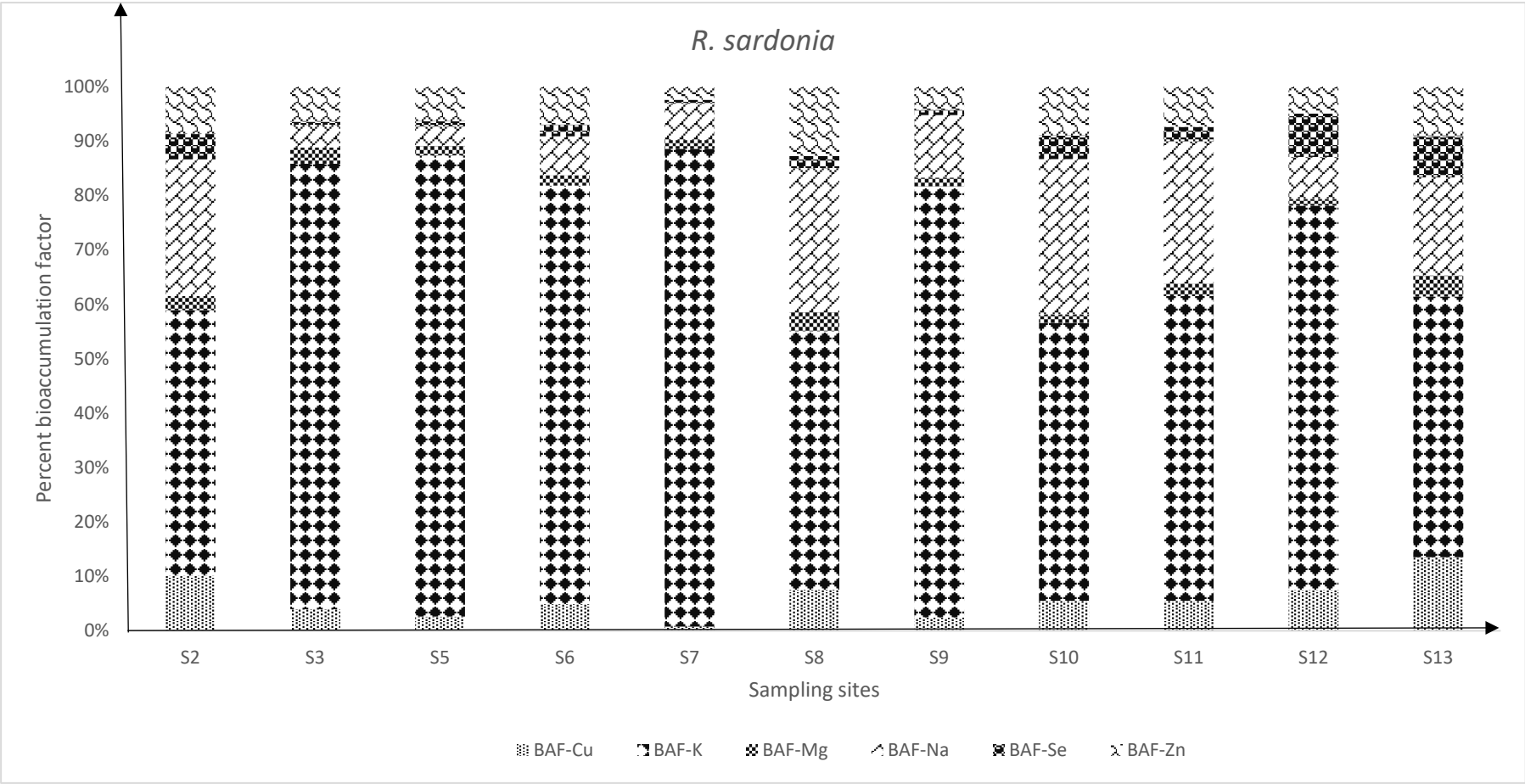


Figure A2. 4 Percent bioaccumulation factor of accumulated minerals by *R. sardonia* mushrooms collected from different geographical areas, where sampling sites are: S2 – Bulwer, S3 – Cidara, S5 – Currypost, S6 – Donnybrook, S7 – Gilboa farm, S8 – Greytown, S9 – Karkloof, S10 – Mafakathini, S11 – Richmond, S12 – Singisi and S13 – Stepmore A.

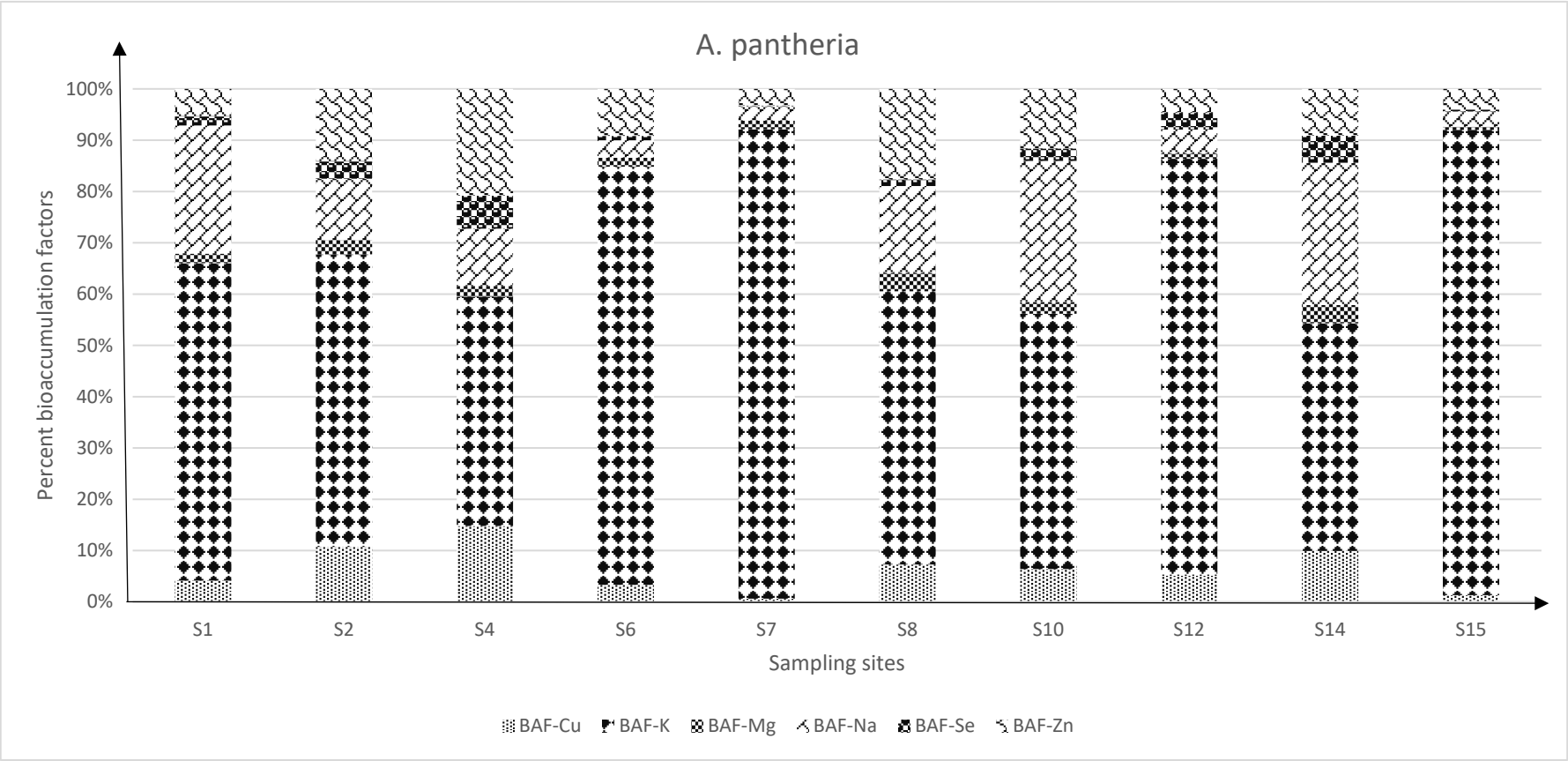


Figure A2. 5 Percent bioaccumulation factor of accumulated minerals by *A. pantherina* mushrooms collected from different geographical areas, where sampling sites are: S1 – Boston, S2 – Bulwer, S4 – Clermont, S6 – Donnybrook, S7 – Gilboa farm, S8 – Greytown, S10 – Mafakathini, S12 – Singisi, S14 – Stepmore B and S15 – Twasamhlobo.

APPENDIX 3: Correlation matrices

Table A3. 1 Correlations matrix between minerals concentration in the mushroom species, *A. pantherina* and soil parameters (pH, SOM) as well as altitude.

	As-M***	As-S****	Ca-M	Ca-S	Cd-M	Cd-S	Co-M	Co-S	Cr-M	Cr-S	Cu-M	Cu-S	Fe-M	Fe-S	K-M	K-S	Mg-M	Mg-S	Mn-M	Mn-S	Na-M	Na-S	Ni-M	Ni-S	Pb-M	Pb-S	Se-M	Se-S	Si-M	Si-S	Zn-M	Zn-S	pH	SOM
As-S	-0,13																																	
Ca-M	0.72**	-0,29																																
Ca-S	0,36	0,01	0,21																															
Cd-M	**0.96****	-0,05	0.73**	0,37																														
Cd-S	-0,07	0,50	-0,09	-0,04	-0,09																													
Co-M	-0,01	-0,06	0,17	-0.58*	-0,15	0,48																												
Co-S	0,11	0,26	-0,02	-0,11	0,07	0.67*	0,35																											
Cr-M	0.59*	-0,16	0,38	0.66*	0.68*	-0,49	-0.77**	-0,26																										
Cr-S	-0,36	0,49	-0,26	-0,08	-0,35	**0.91****	0,41	0.66*	-0.57*																									
Cu-M	0.60*	-0,07	0,27	0,19	0.59*	0,06	-0,08	-0,10	0,43	-0,20																								
Cu-S	0,15	0,28	0,11	-0,03	0,14	0.76**	0,41	**0.90****	-0,27	0.71**	0,11																							
Fe-M	0,28	0.56*	0,09	0,48	0,39	0,11	-0,47	-0,14	0,55	0,00	0,32	-0,10																						
Fe-S	-0,13	0,50	-0,13	-0,08	-0,15	**1.00****	0,49	0.66*	-0,53	**0.92****	0,04	0.74**	0,06																					
K-M	0.70**	-0,34	0,48	0.56*	0.73**	-0,49	-0.56*	-0,33	**0.91****	-0.67*	0,46	-0,38	0,45	-0,53																				
K-S	0,43	0,33	0,19	-0,10	0,42	0,32	0,34	0,19	-0,20	0,14	0,14	0,23	-0,08	0,29	-0,09																			
Mg-M	0.83**	-0,32	0.61*	0,17	0.84**	-0,35	-0,13	-0,19	0.66*	-0.62*	0.60*	-0,19	0,34	-0,41	0.81**	0,12																		
Mg-S	0,53	0,30	0,22	0,08	0,44	0,38	0,33	0,25	-0,11	0,20	0,28	0,27	-0,02	0,36	-0,01	**0.91****	0,12																	
Mn-M	0,54	0,04	0,55	-0,27	0.59*	0,09	0,46	-0,03	-0,02	-0,06	0,38	0,10	0,09	0,04	0,09	0.61*	0,55	0,50																
Mn-S	0,12	-0,06	0,02	0,32	0,05	0,51	0,12	0.78**	0,02	0.56*	0,07	0.76**	-0,08	0,50	-0,09	-0,16	-0,18	0,07	-0,26															
Na-M	-0,51	0,18	-0,40	-0,24	-0,54	0,15	0,34	-0,20	-0,47	0,25	-0,09	-0,05	0,11	0,15	-0,47	-0,32	-0,35	-0,35	-0,08	-0,08														
Na-S	-0,38	0,00	-0,18	-0,01	-0,23	-0,51	-0,44	-0,48	0,15	-0,24	-0,51	-0.63*	0,07	-0,50	0,02	-0,13	-0,15	-0,28	-0,03	-0,49	-0,08													
Ni-M	0.60*	-0.58*	0.65*	0,45	0.60*	-0,23	-0,24	-0,08	0.64*	-0,36	0.61*	0,07	0,01	-0,25	0.64*	-0,19	0.56*	-0,03	0,13	0,29	-0,46	-0,28												
Ni-S	0,29	0.55*	0,14	0,01	0,24	**0.87****	0,53	0.65*	-0,36	0.71**	0,12	0.72**	0,15	0.84**	-0,32	0.66*	-0,11	0.72**	0,37	0,40	0,00	-0,51	-0,22											
Pb-M	0.80**	-0,37	0.71**	0.56*	0.80**	-0,24	-0,32	0,10	0.79**	-0,39	0,51	0,14	0,19	-0,27	0.77**	-0,05	0.67*	0,11	0,14	0,35	-0.61*	-0,28	**0.89****	-0,07									
Pb-S	0,12	0.62*	0,05	0,01	0,12	**0.90****	0,45	0,46	-0,39	0.74**	0,22	0.63*	0,22	**0.89****	-0,37	0.59*	-0,22	0.62*	0,30	0,25	0,11	-0,50	-0,21	**0.93****	-0,18								
Se-M	-0.59*	-0,34	-0.64*	0,02	-0.63*	-0,32	-0,26	-0,49	-0,12	-0,20	-0,26	-0.60*	-0,14	-0,29	-0,03	-0,49	-0,25	-0,51	-0,55	-0,25	0,34	0,35	-0,25	-0.59*	-0,45	-0,48								
Se-S	-0,49	0,40	-0,38	-0,22	-0,43	0.74**	0,30	0.70**	-0.57*	**0.91****	-0,35	0.70**	-0,16	0.75**	-0.73**	0,08	-0.69**	0,04	-0,15	0,53	0,19	-0,07	-0,42	0,52	-0,44	0,52	-0,15						
Si-M	0,38	0.60*	0,02	0,08	0,41	-0,09	-0,29	-0,01	0,38	-0,24	0,13	-0,15	0.70**	-0,13	0,36	0,18	0,42	0,17	0,17	-0,29	-0,11	0,04	-0,27	0,14	0,09	0,06	-0,26	-0,32					
Si-S	-0,09	0,13	-0,25	-0,27	-0,05	0,28	0,28	0,44	-0,32	0,28	0,08	0.61*	-0,24	0,25	-0,43	0,19	-0,15	0,00	0,12	0,27	0,31	-0,36	-0,18	0,28	-0,19	0,24	-0,18	0,46	-0,15				
Zn-M	0,24	0,11	-0,12	0,23	0,29	-0,24	-0.56*	-0,18	0,48	-0,30	0,48	-0,22	0,21	-0,22	0,34	0,13	0,26	0,23	0,03	-0,17	-0,55	0,15	0,31	-0,20	0,30	-0,13	0,01	-0,30	0,28	-0,24			
Zn-S	0,48	0,37	0,17	-0,03	0,40	0.58*	0,48	0,48	-0,26	0,38	0,22	0,52	-0,04	0,55	-0,14	**0.90****	0,06	**0.93****	0,48	0,21	-0,21	-0,46	-0,14	**0.87****	0,03	0.75**	-0.57*	0,25	0,16	0,26	-0,02		
pH	0,27	-0,16	0,03	0,52	0,18	0,13	-0,12	0,38	0,36	0,17	0,15	0,30	0,24	0,09	0,32	-0,33	0,13	-0,06	-0,27	0.78**	0,09	-0,30	0,29	0,13	0,43	-0,05	-0,01	0,10	-0,02	0,02	-0,20	0,02	
SOM	-0,33	0,09	-0,17	-0,17	-0,27	0,37	0,06	0.56*	-0,22	0,49	0,02	0.69**	-0,25	0,40	-0,47	-0,30	-0,43	-0,32	-0,28	0,55	0,08	-0,32	0,13	0,10	0,00	0,17	-0,23	0.65*	-0,39	0.57*	-0,05	-0,14	0,08	
Altitude	0,37	-0,13	0,41	-0,29	0,23	0,25	0.68*	0,55	-0,26	0,25	0,05	0,50	-0,39	0,25	-0,20	0,32	0,08	0,48	0,44	0,45	-0,12	-0,37	0,14	0,47	0,23	0,22	-0.61*	0,19	-0,15	0,12	-0,25	0,52	0,27	0,11

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Where As-M*** and As-S**** represent element, As, in mushroom and soil substrate respectively.

Table A3. 2 Correlations matrix between minerals concentration in the mushroom species, *B. edulis* and soil parameters (pH, SOM) as well as altitude.

	As-M*****	As- S*****	Ca-M	Ca-S	Cd-M	Cd-S	Co-M	Co-S	Cr-M	Cr- S	Cu-M	Cu-S	Fe-M	Fe- S	K-M	K-S	Mg-M	Mg-S	Mn-M	Mn-S	Na-M	Na-S	Ni-M	Ni-S	Pb-M	Pb- S	Se-M	Se-S	Si-M	Si- S	Zn-M	Zn-S	pH	SOM
As-S	-0,48																																
Ca-M	-0,65	0,24																															
Ca-S	-0,12	0,12	-0,28																														
Cd-M	**0.95****	-0,39	-0,60	-0,35																													
Cd-S	-0,43	**0.90****	0,01	0,26	-0,39																												
Co-M	-0,58	0,25	0,24	0.76'	-0.77'	0,31																											
Co-S	-0,64	0.74'	0,53	0,31	-0,69	0,69	0,70																										
Cr-M	0,44	0,27	-0,46	-0,04	0,41	0,48	-0,23	0,14																									
Cr- S	-0,44	0.77'	0,02	0,38	-0,48	**0.95****	0,47	0.74'	0,52																								
Cu-M	-0,04	0,04	-0,02	0,10	0,02	-0,19	0,23	0,03	-0,46	-0,29																							
Cu-S	**-0.85****	0.78'	0,43	0,43	**-0.85****	0.72'	0,70	0.81'	-0,19	0,69	0,11																						
Fe-M	-0.80'	0,50	0,29	0,05	-0.71'	0,55	0,22	0,34	-0,17	0,50	-0,33	0,70																					
Fe-S	-0,34	**0.89****	-0,04	0,29	-0,31	**0.99****	0,29	0,66	0,53	**0.95****	-0,15	0,67	0,45																				
K-M	-0.76'	0,22	**0.92****	-0,03	-0.78'	0,05	0,50	0,64	-0,46	0,12	-0,04	0,55	0,38	-0,03																			
K-S	-0,66	0,70	0,64	-0,23	-0,50	0,39	0,12	0,55	-0,29	0,22	0,17	0,67	0,56	0,32	0,62																		
Mg-M	-0,58	0,03	0.72'	0,04	-0,63	-0,05	0,63	0,59	-0,40	0,09	0,32	0,38	-0,01	-0,07	0.77'	0,24																	
Mg-S	-0.73'	0,67	0.79'	-0,01	-0,66	0,39	0,44	0.76'	-0,39	0,30	0,33	0.77'	0,38	0,35	0.76'	**0.87****	0,60																
Mn-M	-0,66	0,17	**0.91****	-0,25	-0,63	0,08	0,16	0,40	-0,39	0,12	-0,34	0,39	0,47	0,01	0,82'	0,48	0,56	0,58															
Mn-S	-0,36	0,11	0,04	0,63	-0,55	0,32	**0.87****	0,61	0,12	0,55	0,11	0,43	0,03	0,33	0,27	-0,21	0,61	0,14	0,01														
Na-M	0,31	0,00	0,29	-0,34	0,29	-0,06	-0,46	-0,02	0,32	-0,06	-0,67	-0,29	-0,14	-0,06	0,14	0,04	-0,22	-0,05	0,38	-0,45													
Na-S	-0,31	0,36	0,09	0,12	-0,37	0,61	0,15	0,42	0,54	0.72'	-0.85''	0,34	0,57	0,55	0,20	0,05	-0,09	-0,03	0,35	0,27	0,38												
Ni-M	-0.78'	0,20	0,18	0,48	-0.83'	0,37	0.76'	0,44	-0,31	0,49	0,11	0,68	0,59	0,32	0,36	0,10	0,50	0,30	0,28	0.719'	-0,65	0,26											
Ni-S	-0.79'	**0.88****	0,60	0,14	-0.73'	0.72'	0,49	**0.86****	-0,11	0,64	0,09	**0.93****	0,62	0,67	0,64	**0.85****	0,41	**0.90****	0,50	0,25	-0,06	0,31	0,43										
Pb-M	0,16	0,02	-0.79'	0,66	0,05	0,30	0,18	-0,19	0,30	0,34	-0,18	0,09	0,21	0,32	-0,59	-0,43	-0,59	-0,52	-0,58	0,22	-0,35	0,28	0,28	-0,21									
Pb-S	-0,59	**0.94****	0,19	0,09	-0,44	**0.85****	0,20	0,57	0,06	0,68	0,12	0.81'	0,66	0,82'	0,15	0.71'	-0,03	0,64	0,17	0,02	-0,20	0,26	0,34	0,84''	0,11								
Se-M	0,46	**-0.85****	0,01	-0,13	0,36	-0.82'	-0,12	-0,47	-0,26	-0,67	0,16	-0,70	-0,74'	-0,76'	-0,07	-0,64	0,29	-0,40	-0,02	0,05	0,08	-0,48	-0,23	-0,71	-0,34	**-0.87****							
Se-S	-0,43	0.76'	-0,01	0,21	-0,42	**0.93****	0,36	0,70	0,59	**0.95****	-0,30	0,63	0,54	**0.90****	0,10	0,29	0,04	0,27	0,06	0,47	-0,10	0.73'	0,43	0,62	0,30	0,67	-0.74'						
Si-M	0,05	-0,37	0,40	-0.81'	0,23	-0,53	-0,49	-0,34	-0,35	-0,58	0,24	-0,44	-0,27	-0,52	0,14	0,05	0,33	0,08	0,33	-0,39	0,08	-0,47	-0,25	-0,23	-0.77'	-0,29	0,54	-0,51					
Si-S	-0,45	0,30	0,70	-0,40	-0,35	0,09	-0,21	0,18	-0,30	-0,02	-0,41	0,30	0,56	0,00	0,60	0,71	0,03	0,51	0.76'	-0,50	0,56	0,31	-0,13	0,48	-0,45	0,31	-0,35	0,00	0,17				
Zn-M	-0,45	0,09	-0,08	0,50	-0,54	0,28	0.78'	0,43	-0,05	0,44	0,34	0,44	0,16	0,28	0,14	-0,14	0,54	0,12	-0,12	**0.89****	-0.79'	0,06	0.83'	0,22	0,28	0,14	-0,03	0,42	-0,24	-0,57			
Zn-S	-0,70	0,70	0.81'	-0,16	-0,59	0,41	0,29	0.72'	-0,31	0,29	0,22	0.72'	0,43	0,35	0.75'	**0.93****	0,51	**0.98****	0,61	0,00	0,08	0,04	0,18	**0.90****	-0,57	0,66	-0,47	0,29	0,12	0,63	-0,02		
pH	-0,30	-0,23	0,39	0,25	-0,46	-0,08	0,64	0,44	-0,07	0,19	-0,03	0,12	-0,15	-0,08	0,51	-0,26	0.82'	0,13	0,37	0.80'	-0,11	0,18	0,49	0,05	-0,25	-0,34	0,47	0,10	0,09	-0,26	0,62	0,03	
SOM	-0,12	0,34	-0,54	0,53	-0,12	0,57	0,22	0,01	0,17	0,54	0,04	0,36	0,35	0,60	-0,50	-0,20	-0,36	-0,17	-0,34	0,25	-0,49	0,19	0,49	0,11	0.79'	0,50	-0,44	0,43	-0,52	-0,38	0,40	-0,24	-0,22
Altitude	-0,69	-0,03	0.73'	0,14	-0.74'	-0,15	0,68	0,49	-0,65	-0,03	0,37	0,46	0,15	-0,19	0.82'	0,33	**0.94****	0,63	0,58	0,52	-0,32	-0,17	0,59	0,42	-0,49	-0,01	0,24	-0,09	0,26	0,14	0,51	0,53	0,70	-0,31

**: Correlation is significant at the 0.01 level (2-tailed).

*: Correlation is significant at the 0.05 level (2-tailed).

Where As-M***** and As- S***** represent element, As , in mushroom and soil substrate respectively.

Table A3. 3 Correlations matrix between minerals concentration in the mushroom species, *B. mirabilis* and soil parameters (pH, SOM) as well as altitude.

	As-M*****	As-S*****	Ca-M	Ca-S	Cd-M	Cd-S	Co-M	Co-S	Cr-M	Cr-S	Cu-M	Cu-S	Fe-M	Fe-S	K-M	K-S	Mg-M	Mg-S	Mn-M	Mn-S	Na-M	Na-S	Ni-M	Ni-S	Pb-M	Pb-S	Se-M	Se-S	Si-M	Si-S	Zn-M	Zn-S	pH	SOM
As-S	0,21																																	
Ca-M	0,65	-0,27																																
Ca-S	0,01	0,10	-0,39																															
Cd-M	**0,97****	0,22	0,62	-0,04																														
Cd-S	-0,02	0,62	-0,41	0,56	-0,17																													
Co-M	0,35	0,78'	0,03	-0,42	0,40	0,15																												
Co-S	0,36	0,34	0,02	0,09	0,20	0,60	0,13																											
Cr-M	0,36	-0,42	0,44	0,30	0,41	-0,41	-0,28	-0,59																										
Cr-S	-0,18	0,53	-0,46	0,46	-0,34	**0,96****	0,10	0,63	-0,54																									
Cu-M	-0,01	-0,26	-0,12	0,04	0,19	-0,68	-0,08	-0,66	0,50	-0,69																								
Cu-S	0,49	0,29	0,11	0,33	0,30	0,68	0,07	**0,91****	-0,28	0,67	-0,63																							
Fe-M	0,28	-0,22	0,18	-0,24	0,15	0,09	-0,06	0,75'	-0,39	0,20	-0,40	0,67																						
Fe-S	-0,06	0,63	-0,42	0,50	-0,21	**1,00****	0,17	0,62	-0,47	**0,96****	-0,71	0,67	0,10																					
K-M	-0,14	0,41	0,08	-0,14	-0,20	0,43	0,17	0,21	-0,42	0,34	-0,68	0,06	-0,22	0,46																				
K-S	0,31	0,67	0,23	0,22	0,28	0,47	0,33	0,25	-0,08	0,37	-0,34	0,27	-0,37	0,46	0,60																			
Mg-M	0,36	0,21	0,60	-0,33	0,32	0,05	0,25	0,12	-0,02	-0,10	-0,49	0,05	-0,12	0,08	0,80'	0,52																		
Mg-S	0,40	0,66	0,23	0,29	0,36	0,50	0,30	0,24	0,00	0,32	-0,36	0,28	-0,38	0,50	0,67	**0,94****	0,66																	
Mn-M	0,38	-0,14	0,34	-0,27	0,22	0,14	0,01	0,79'	-0,36	0,24	-0,53	0,74'	**0,97****	0,15	-0,10	-0,18	0,04	-0,22																
Mn-S	0,28	-0,14	-0,04	0,48	0,11	0,50	-0,38	0,76'	-0,14	0,49	-0,45	0,83'	0,71'	0,48	-0,12	-0,13	-0,13	-0,03	0,67															
Na-M	0,22	0,17	0,06	-0,19	0,05	0,44	0,24	0,82'	-0,55	0,56	-0,66	0,80'	**0,86****	0,46	-0,01	-0,07	-0,05	-0,14	**0,90****	0,61														
Na-S	-0,48	-0,12	-0,17	-0,31	-0,34	-0,51	0,06	-0,62	0,05	-0,34	0,57	-0,68	-0,42	-0,50	-0,33	-0,12	-0,44	-0,38	-0,43	-0,75'	-0,38													
Ni-M	0,47	-0,56	0,48	0,02	0,35	-0,17	-0,34	0,33	0,33	-0,12	-0,09	0,50	0,73'	-0,20	-0,53	-0,42	-0,18	-0,41	0,73'	0,64	0,51	-0,34												
Ni-S	0,34	0,75'	-0,08	0,51	0,23	**0,87****	0,30	0,56	-0,21	0,73'	-0,57	0,65	-0,08	**0,86****	0,52	0,77'	0,36	0,84''	0,04	0,36	0,24	-0,57	-0,22											
Pb-M	0,71	-0,31	0,57	0,31	0,60	-0,01	-0,23	0,27	0,58	-0,06	-0,05	0,56	0,43	-0,06	-0,45	-0,05	-0,04	-0,01	0,49	0,58	0,30	-0,43	**0,87****	0,11										
Pb-S	0,22	0,74'	-0,16	0,58	0,12	**0,88****	0,29	0,39	-0,11	0,75'	-0,51	0,53	-0,23	**0,86****	0,48	0,75'	0,27	0,80'	-0,12	0,24	0,15	-0,44	-0,29	**0,97****	0,07									
Se-M	-0,63	-0,43	-0,58	0,04	-0,67	0,07	-0,46	0,07	-0,37	0,17	-0,12	-0,05	0,36	0,10	-0,11	-0,75'	-0,40	-0,66	0,17	0,37	0,20	-0,13	0,11	-0,36	-0,25	-0,35								
Se-S	-0,18	0,24	-0,32	0,19	-0,33	0,63	-0,04	0,72'	-0,64	0,81'	-0,55	0,69	0,54	0,64	0,03	0,15	-0,34	-0,04	0,57	0,53	0,75'	-0,08	0,18	0,36	0,04	0,33	0,22							
Si-M	0,31	0,81'	-0,10	-0,32	0,35	0,27	**0,85****	0,35	-0,52	0,17	-0,20	0,16	0,02	0,31	0,41	0,37	0,40	0,44	0,04	-0,17	0,21	-0,22	-0,46	0,44	-0,39	0,34	-0,26	-0,02						
Si-S	0,43	0,02	0,07	0,21	0,26	0,44	-0,05	**0,85****	-0,25	0,49	-0,46	**0,92****	**0,87****	0,43	-0,24	-0,10	-0,19	-0,09	**0,86****	**0,89****	**0,85****	-0,59	0,71'	0,32	0,62	0,20	0,18	0,66	0,01				
Zn-M	-0,26	0,21	0,10	-0,16	-0,26	0,11	0,24	-0,47	0,13	0,03	-0,29	-0,41	-0,63	0,13	0,64	0,32	0,56	0,38	-0,51	-0,56	-0,35	0,07	-0,53	0,16	-0,38	0,30	-0,19	-0,38	0,11	-0,63				
Zn-S	0,45	0,64	0,18	0,39	0,37	0,64	0,22	0,48	-0,11	0,47	-0,48	0,52	-0,15	0,63	0,63	**0,88****	0,59	**0,96****	-0,01	0,24	0,06	-0,56	-0,24	**0,92****	0,11	0,84''	-0,53	0,15	0,43	0,17	0,18			
pH	0,18	-0,21	-0,08	0,54	0,04	0,43	-0,46	0,40	0,09	0,29	-0,36	0,50	0,34	0,40	0,09	-0,19	0,10	0,08	0,27	0,82'	0,19	**-0,87****	0,40	0,33	0,42	0,26	0,46	0,06	-0,16	0,52	-0,17	0,27		
SOM	0,25	-0,12	0,19	-0,11	0,07	0,29	-0,03	0,75'	-0,34	0,42	-0,57	0,78'	**0,92****	0,30	-0,17	-0,20	-0,13	-0,27	**0,95****	,708'	**0,95****	-0,38	0,74'	0,09	0,52	0,00	0,24	0,70	-0,08	**0,90****	-0,46	-0,05	0,29	
Altitude	**0,89****	0,09	0,83'	-0,19	0,83'	-0,05	0,23	0,41	0,23	-0,19	-0,29	0,46	0,34	-0,06	0,20	0,36	0,68	0,47	0,48	0,28	0,26	-0,59	0,45	0,32	0,61	0,16	-0,55	-0,19	0,27	0,37	-0,06	0,51	0,23	0,30

**: Correlation is significant at the 0.01 level (2-tailed).

*: Correlation is significant at the 0.05 level (2-tailed).

Where As-M***** and As-S***** represent element, As, in mushroom and soil substrate respectively.

Table A3. 4 Correlations matrix between minerals concentration in the mushroom species, *L. deliciosus* and soil parameters (pH, SOM) as well as altitude.

	As-M****	As-S*****	Ca-M	Ca-S	Cd-M	Cd-S	Co-M	Co-S	Cr-M	Cr-S	Cu-M	Cu-S	Fe-M	Fe-S	K-M	K-S	Mg-M	Mg-S	Mn-M	Mn-S	Na-M	Na-S	Ni-M	Ni-S	Pb-M	Pb-S	Se-M	Se-S	Si-M	Si-S	Zn-M	Zn-S	pH	SOM
As-S	0,44																																	
Ca-M	-0,04	-0,45																																
Ca-S	-0,27	0,11	0,21																															
Cd-M	**0.94****	0,30	-0,13	-0,43																														
Cd-S	-0,09	0,69	-0,08	0,33	-0,20																													
Co-M	0,05	-0,03	-0,32	-0,72	0,09	0,01																												
Co-S	-0,07	0,52	-0,05	0,05	-0,26	0,80'	0,48																											
Cr-M	-0,86'	-0,27	0,25	0,67	**-0.94****	0,21	-0,41	0,11																										
Cr-S	-0,34	0,55	-0,19	0,47	-0,44	**0.95****	-0,05	0,72	0,45																									
Cu-M	-0,17	-0,07	0,14	-0,27	-0,34	-0,22	0,29	0,22	0,17	-0,24																								
Cu-S	-0,11	0,33	0,18	0,14	-0,34	0,68	0,42	**0.96****	0,21	0,61	0,31																							
Fe-M	0,09	-0,71	0,21	-0,15	0,10	**-0.92****	0,04	-0,60	-0,12	**-0.86***	0,26	-0,41																						
Fe-S	-0,15	0,71	-0,14	0,33	-0,27	**0.99****	0,00	0,79'	0,27	**0.95****	-0,14	0,66	**-0.94****																					
K-M	0,12	-0,47	0,55	-0,56	0,09	-0,35	0,55	0,13	-0,26	-0,50	0,52	0,30	0,45	-0,39																				
K-S	0,58	0,67	0,16	0,12	0,37	0,26	-0,37	0,15	-0,24	0,04	0,26	0,13	-0,28	0,28	-0,08																			
Mg-M	0,08	-0,43	0,68	-0,42	0,10	-0,44	-0,05	-0,30	-0,11	-0,59	0,48	-0,17	0,33	-0,44	0,70	0,25																		
Mg-S	0,63	0,56	0,36	0,18	0,42	0,24	-0,43	0,11	-0,26	0,00	0,15	0,13	-0,22	0,22	0,01	**0.97****	0,32																	
Mn-M	-0,17	0,04	0,27	-0,48	-0,23	0,35	0,62	0,67	0,00	0,22	0,53	0,68	-0,32	0,36	0,65	0,04	0,42	0,02																
Mn-S	-0,43	-0,04	0,14	-0,14	-0,58	0,32	0,60	0,76'	0,34	0,33	0,65	0,83'	-0,14	0,35	0,51	-0,14	0,08	-0,20	0,82'															
Na-M	0,84'	0,36	-0,41	-0,56	**0.89****	-0,11	0,47	0,01	**-0.96****	-0,30	-0,24	-0,10	0,07	-0,16	0,14	0,16	-0,15	0,15	-0,07	-0,29														
Na-S	-0,30	-0,12	-0,44	0,27	-0,13	-0,30	-0,54	-0,69	0,26	-0,09	-0,34	-0,78'	0,08	-0,24	-0,74	-0,22	-0,29	-0,30	-0,75	-0,64	-0,25													
Ni-M	**-0.94****	-0,30	0,25	0,41	**-0.93****	0,27	-0,25	0,15	**0.93****	0,47	0,17	0,21	-0,27	0,33	-0,15	-0,32	0,04	-0,34	0,24	0,41	**-0.94****	0,21												
Ni-S	0,33	0,79'	0,09	0,49	0,07	0,81'	-0,22	0,66	0,04	0,67	-0,11	0,62	-0,65	0,78'	-0,28	0,66	-0,32	0,67	0,12	0,13	0,05	-0,40	-0,08											
Pb-M	-0,78'	0,01	-0,04	0,49	-0,71	0,54	-0,33	0,18	0,76'	0,73	-0,29	0,11	-0,59	0,57	-0,54	-0,31	-0,33	-0,33	0,02	0,12	-0,71	0,39	0,85'	0,11										
Pb-S	0,29	0,80'	0,08	0,54	0,06	0,80'	-0,37	0,56	0,09	0,68	-0,16	0,50	-0,69	0,79'	-0,40	0,70	-0,31	0,70	0,03	0,02	-0,02	-0,25	-0,01	**0.98****	0,21									
Se-M	-0,36	-0,07	0,46	0,28	-0,30	0,18	-0,68	-0,24	0,47	0,18	-0,05	-0,23	-0,36	0,21	-0,22	0,26	0,44	0,30	0,04	-0,20	-0,65	0,31	0,60	0,06	0,58	0,21								
Se-S	-0,57	0,36	-0,38	0,18	-0,64	0,72	0,33	0,76'	0,48	0,84'	0,13	0,65	-0,64	0,77'	-0,28	-0,20	-0,54	-0,33	0,42	0,65	-0,32	-0,11	0,55	0,34	0,63	0,31	-0,06							
Si-M	0,67	-0,22	0,21	-0,26	0,77'	-0,35	0,13	-0,31	-0,70	-0,49	-0,50	-0,26	0,40	-0,46	0,32	-0,11	0,10	0,07	-0,25	-0,46	0,68	-0,24	-0,73	-0,13	-0,61	-0,20	-0,39	-0,69						
Si-S	-0,27	-0,10	0,25	-0,40	-0,38	0,17	0,66	0,64	0,12	0,10	0,73	0,71	-0,06	0,19	0,72	-0,05	0,37	-0,08	**0.93****	**0.94****	-0,15	-0,73	0,26	0,02	-0,08	-0,10	-0,14	0,43	-0,32					
Zn-M	-0,08	-0,45	0,51	-0,49	-0,01	-0,50	-0,02	-0,36	-0,04	-0,60	0,56	-0,26	0,33	-0,46	0,60	0,16	**0.96****	0,18	0,40	0,10	-0,23	-0,12	0,14	-0,47	-0,23	-0,43	0,47	-0,44	-0,07	0,37				
Zn-S	0,61	0,72	0,20	0,11	0,36	0,40	-0,19	0,39	-0,28	0,16	0,27	0,38	-0,34	0,40	0,04	**0.96****	0,18	**0.95****	0,21	0,05	0,23	-0,46	-0,35	0,78'	-0,34	0,77'	0,11	-0,08	-0,04	0,12	0,05			
pH	-0,58	-0,34	0,05	0,67	-0,71	-0,03	-0,15	0,10	0,73	0,23	0,08	0,24	0,29	-0,02	-0,17	-0,43	-0,43	-0,42	-0,26	0,30	-0,56	0,15	0,49	-0,02	0,33	-0,05	-0,19	0,34	-0,30	0,02	-0,41	-0,38		
SOM	-0,41	-0,25	0,09	-0,43	-0,44	0,11	,785'	0,59	0,15	0,12	0,58	0,65	0,01	0,13	0,66	-0,37	0,19	-0,41	0,85'	**0.93****	-0,13	-0,62	0,31	-0,16	0,01	-0,29	-0,29	0,51	-0,26	**0.94****	0,22	-0,19	0,16	
Altitude	0,47	0,03	0,14	-0,78'	0,42	-0,28	0,58	0,15	-0,61	-0,51	0,62	0,17	0,25	-0,28	0,78'	0,32	0,62	0,30	0,60	0,34	0,48	-0,63	-0,48	-0,12	-0,74	-0,22	-0,29	-0,28	0,22	0,61	0,59	0,39	-0,56	0,46

**: Correlation is significant at the 0.01 level (2-tailed).

*: Correlation is significant at the 0.05 level (2-tailed).

Where As-M**** and As-S***** represent element, As, in mushroom and soil substrate respectively.

Table A3. 5 Correlations matrix between minerals concentration in the mushroom species, *R. sardonia* and soil parameters (pH, SOM) as well as altitude.

	As-M****	As-S*****	Ca-M	Ca-S	Cd-M	Cd-S	Co-M	Co-S	Cr-M	Cr-S	Cu-M	Cu-S	Fe-M	Fe-S	K-M	K-S	Mg-M	Mg-S	Mn-M	Mn-S	Na-M	Na-S	Ni-M	Ni-S	Pb-M	Pb-S	Se-M	Se-S	Si-M	Si-S	Zn-M	Zn-S	pH	SOM
As-S	-0,24																																	
Ca-M	0,25	0,13																																
Ca-S	0,05	0,11	0,19																															
Cd-M	**0.97****	-0,14	0,23	0,06																														
Cd-S	0,04	0,59	0,40	0,42	0,00																													
Co-M	0,59	-0,54	0,25	-0,11	0,53	-0,29																												
Co-S	0,12	0,21	0,24	0,16	-0,03	0.74''	0,02																											
Cr-M	0,14	-0,03	0,54	-0,01	0,16	0,44	0,08	0,12																										
Cr-S	-0,19	0.61'	0,10	0,48	-0,19	**0.91****	-0,52	0.64'	0,31																									
Cu-M	-0,10	-0,36	0,25	0,06	-0,16	0,08	-0,05	-0,18	0.61'	-0,03																								
Cu-S	0,17	0,24	0,11	0,27	0,03	0.71'	-0,05	**0.92****	0,01	0.65'	-0,20																							
Fe-M	-0,06	-0,04	0,56	-0,08	-0,03	0,01	0,45	0,06	0,30	-0,11	-0,10	-0,07																						
Fe-S	0,02	0.62'	0,34	0,42	-0,01	**0.99****	-0,31	0.72'	0,41	**0.92****	0,05	0.70'	-0,06																					
K-M	0,03	0,44	0.80''	-0,07	0,08	0,24	-0,08	-0,11	0,43	0,00	0,20	-0,15	0,37	0,21																				
K-S	-0,04	0,31	0.80''	0,02	-0,05	0,37	0,02	0,37	0,35	0,20	-0,09	0,28	0,58	0,32	0.74''																			
Mg-M	0,15	-0,30	0.72'	0,08	0,16	0,03	0,12	-0,18	0.72'	-0,13	0.63'	-0,24	0,39	-0,04	0,60	0,50																		
Mg-S	0,06	0,37	**0.87****	0,18	0,03	0,45	-0,02	0,40	0,22	0,22	-0,08	0,31	0,49	0,39	0.76''	**0.91****	0,46																	
Mn-M	0,13	0,04	0.67'	-0,35	0,18	0,12	0,26	-0,09	0.74''	-0,10	0,35	-0,17	0.67'	0,04	0.71'	0,58	0.72'	0,45																
Mn-S	0,26	0,07	-0,37	-0,07	0,14	0,39	-0,03	0.73'	-0,17	0,43	-0,35	0.80''	-0,37	0,41	-0,53	-0,16	-0,55	-0,18	-0,40															
Na-M	0.65'	0,17	-0,24	0,15	0.73'	0,01	0,12	-0,27	-0,09	-0,02	-0,17	-0,06	-0,39	0,04	-0,10	-0,48	-0,25	-0,35	-0,15	0,16														
Na-S	-0.67'	-0,02	-0,10	-0,02	-0,55	-0,19	-0,39	-0,46	0,34	0,05	0,29	-0,52	0,21	-0,18	0,10	0,07	0,35	-0,16	0,25	-0,54	-0,42													
Ni-M	-0,13	0,06	0,20	-0,13	-0,09	0,37	-0,26	0,01	**0.89****	0,38	0.63'	-0,05	0,02	0,37	0,27	0,14	0,54	-0,05	0,56	-0,09	-0,10	0,54												
Ni-S	-0,03	0,51	0.69'	0,41	-0,09	0.83''	-0,11	0.74''	0,29	0.68'	-0,05	0.69'	0,37	0.79''	0,46	0.73'	0,19	0.82''	0,25	0,18	-0,30	-0,21	0,09											
Pb-M	0,35	-0,34	0,32	0,00	0,25	0,35	0,39	0,35	0.76''	0,17	0.61'	0,28	0,01	0,35	0,05	0,10	0,44	-0,02	0,38	0,21	-0,04	-0,06	0.63'	0,17										
Pb-S	-0,08	0.69'	0,59	0,47	-0,09	**0.90****	-0,34	0,59	0,34	0.78''	0,06	0.61'	0,14	**0.88****	0,53	0.61'	0,17	0.71'	0,21	0,12	-0,10	-0,12	0,24	**0.93****	0,16									
Se-M	-0,35	-0,25	-0.72'	-0,04	-0,46	-0,22	-0,07	0,07	-0,51	-0,07	0,04	0,11	-0,55	-0,15	-0.72'	-0.65'	-0.66'	-0.65'	-0.73'	0,37	-0,11	-0,11	-0,28	-0,38	-0,02	-0,36								
Se-S	-0,32	0,43	-0,17	0,16	-0,34	0.71'	-0,57	0.66'	0,22	**0.88****	-0,10	0.65'	-0,19	0.73'	-0,22	0,12	-0,24	0,02	-0,17	0.62'	-0,20	0,15	0,42	0,46	0,16	0,51	0,13							
Si-M	0,30	-0,19	0,38	-0,43	0,33	-0,41	0,30	-0,21	-0,07	-0,55	-0,23	-0,31	0,51	-0,49	0,35	0,34	0,35	0,41	0,46	-0,30	-0,09	-0,14	-0,26	-0,07	-0,32	-0,26	-0,59	-0,52						
Si-S	-0,23	-0,06	-0,46	-0,42	-0,32	-0,05	0,06	0,43	-0,30	0,06	-0,40	0,49	0,08	-0,05	-0,47	-0,02	-0,52	-0,21	-0,16	0.66'	-0,29	-0,12	-0,16	-0,01	-0,04	-0,16	0,43	0,41	-0,09					
Zn-M	-0,44	0,58	0,17	-0,22	-0,40	0,40	-0,44	-0,06	0,41	0,34	0,43	-0,11	-0,03	0,41	0,50	0,11	0,08	0,11	0,38	-0,25	-0,08	0,29	0,56	0,22	0,13	0,44	-0,05	0,22	-0,29	-0,18				
Zn-S	0,07	0,40	0.77''	0,06	0,02	0,54	0,02	0.61'	0,22	0,33	-0,22	0,51	0,49	0,49	0.62'	**0.92****	0,30	**0.95****	0,41	0,09	-0,39	-0,23	-0,02	**0.86****	0,06	0.71'	-0,55	0,22	0,34	0,06	0,07			
pH	0,16	0,06	-0,15	0,36	0,12	0,34	0,07	0,60	-0,31	0,43	-0,51	0,59	0,13	0,32	-0,54	-0,12	-0,45	0,03	-0,41	0,59	0,06	-0,41	-0,41	0,34	-0,20	0,17	0,10	0,40	0,00	0,35	-0,47	0,17		
SOM	-0,04	-0,05	-0.64'	-0,27	-0,15	0,10	-0,16	0,41	-0,24	0,21	-0,13	0,57	-0,49	0,13	-0,60	-0,36	-0,57	-0,47	-0,41	0.84''	0,07	-0,28	0,00	-0,14	0,16	-0,12	0.62'	0,52	-0,43	0.78''	-0,08	-0,23	0,25	
Altitude	0,01	-0,11	0,09	-0.79''	-0,09	-0,28	0,24	0,13	-0,13	-0,46	-0,12	0,11	0,18	-0,31	0,23	0,33	-0,02	0,22	0,32	0,17	-0,33	-0,26	-0,14	-0,03	0,03	-0,18	0,03	-0,20	0,45	0,55	0,05	0,33	-0,27	0,32

**: Correlation is significant at the 0.01 level (2-tailed).

*: Correlation is significant at the 0.05 level (2-tailed).

Where As-M**** and As-S***** represent element, As, in mushroom and soil substrate respectively.

Table A3. 6 Summary of correlations matrix between minerals concentration: extracts from Tables A2.1 through Table A2.5

	As	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Se	Si	Zn
A. pantherina																
B. edulis	Cu_s = -0.848		Cu_s = -0.847	Mn_s = 0.873		Na_s = -0.851			Alt = 0.937					As_s = -0.847 Pb_s = -0.874		Mn_s = 0.891
B. mirabilis	Alt = 0.893						Si_s = 0.866 SOM = 0.922			Si_s = 0.863 SOM = 0.953	Si_s = 0.850 SOM = 0.945					
L. deliciosus							Fe_s = -0.936 Cd_s = -0.923 Cr_s = -0.858			Si_s = 0.929						
R. sardonia		Mg_s = 0.874														

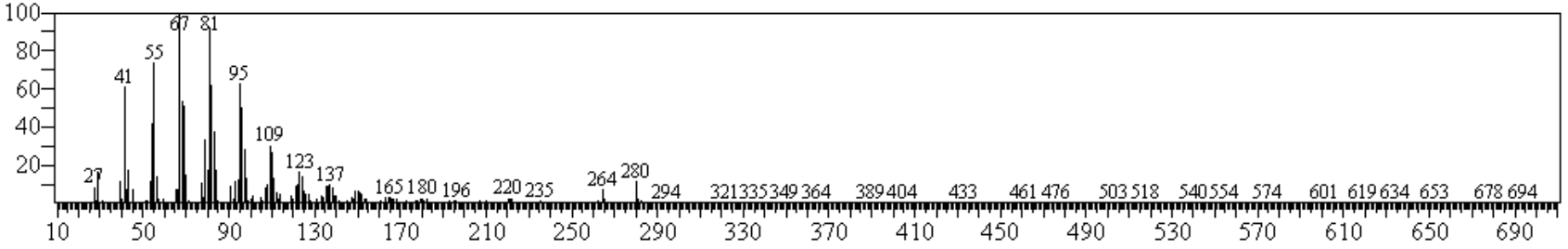
APPENDIX 4: Conference certificates

Figure A4. 1 Certificate of Excellence, Paper Presented at the 10th International Conference of African Material Research Society (AMRS 2019).

Figure A4. 2 Certificate of Appreciation, Paper Presented at the 6th International Conference on Food Chemistry & Technology (FCT- 2020) – A Virtual Conference.

APPENDIX 5: Spectral data (NMR, IR and GC-MS) data of isolated compounds

<< Target >>
Line#:26 R.Time:19.025(Scan#:3206) MassPeaks:412
RawMode:Averaged 19.020-19.030(3205-3207) BasePeak:67.10(3037827)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



Hit#:1 Entry:102816 Library:NIST11.lib
SI:92 Formula:C18H32O2 CAS:60-33-3 MolWeight:280 RetIndex:2183
CompName:9,12-Octadecadienoic acid (Z,Z)- $$ cis-9,cis-12-Octadecadienoic acid $$ cis,cis-Linoleic acid $$ Grape seed oil $$ Linoleic $$ Linoleic acid $$

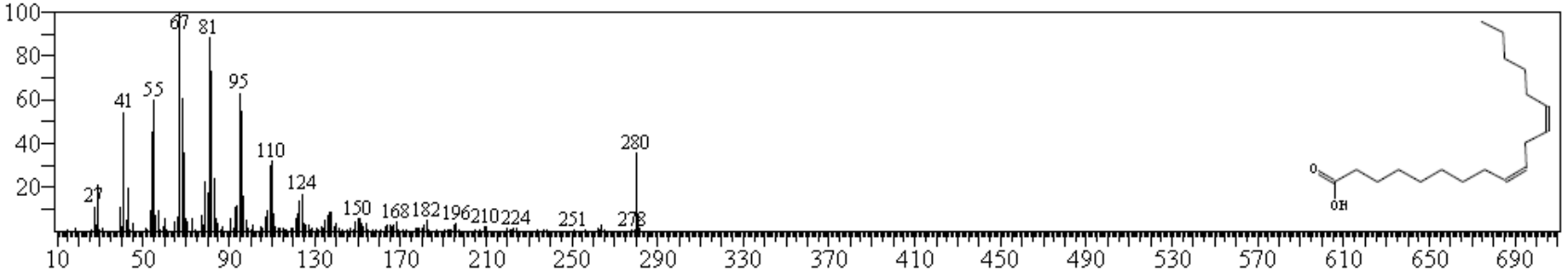


Figure A5. 1 GC-MS spectrum of linoleic acid in $CDCl_3$

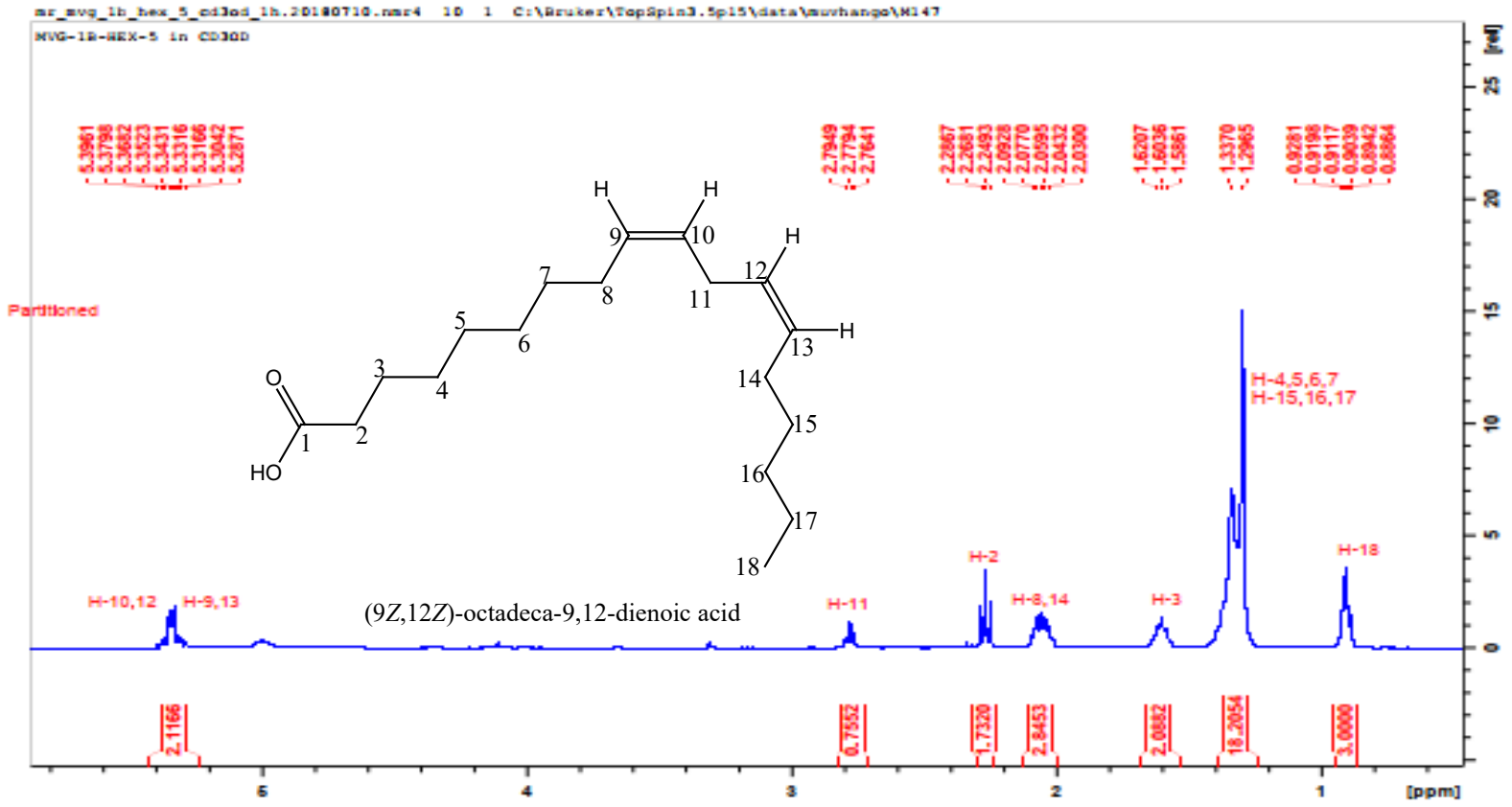


Figure A5. 2 The ^{1}H NMR spectrum of linoleic acid in CD_3OD.

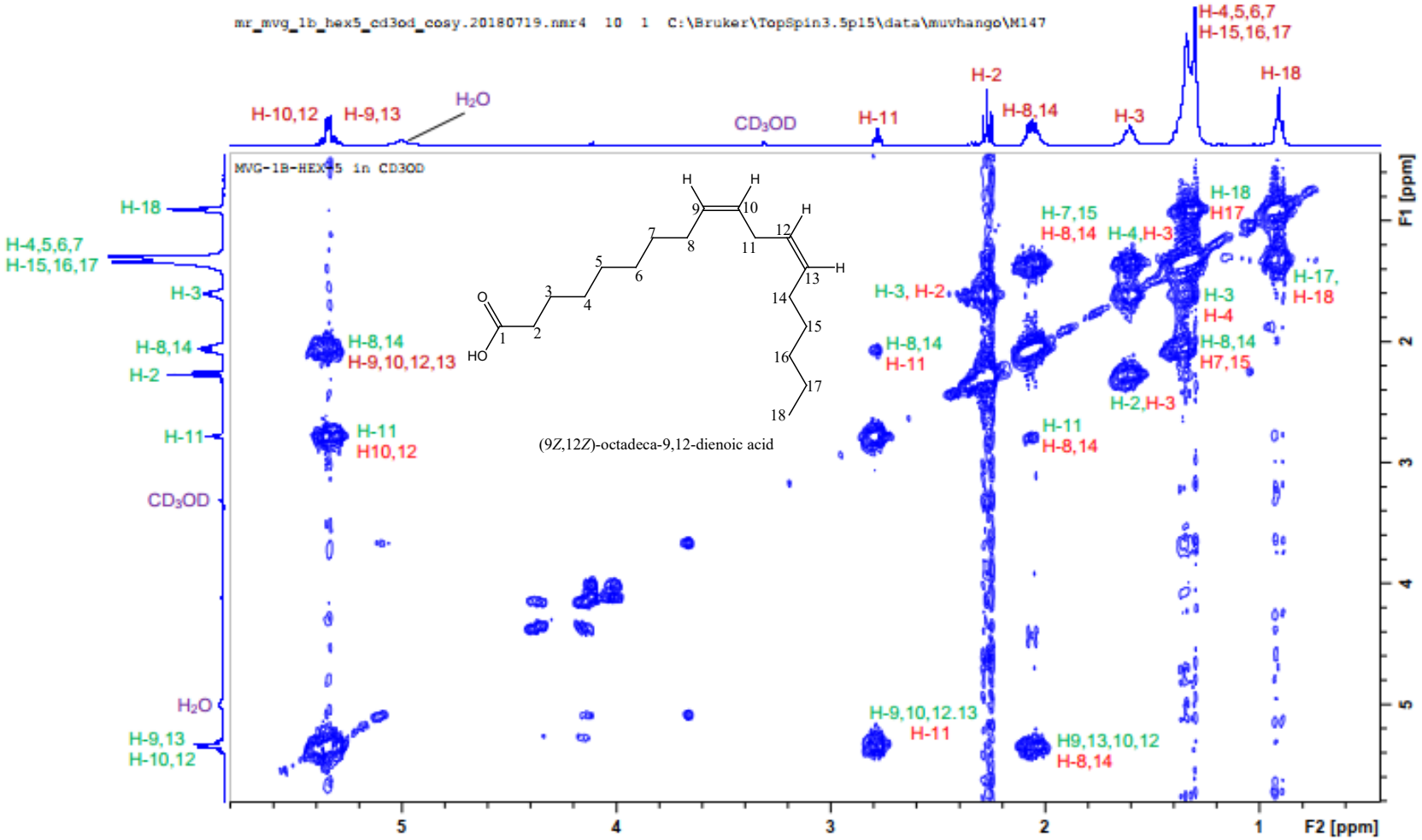


Figure A5. 3 The homonuclear correlation (COSY) spectrum of linoleic acid in CD_3OD.

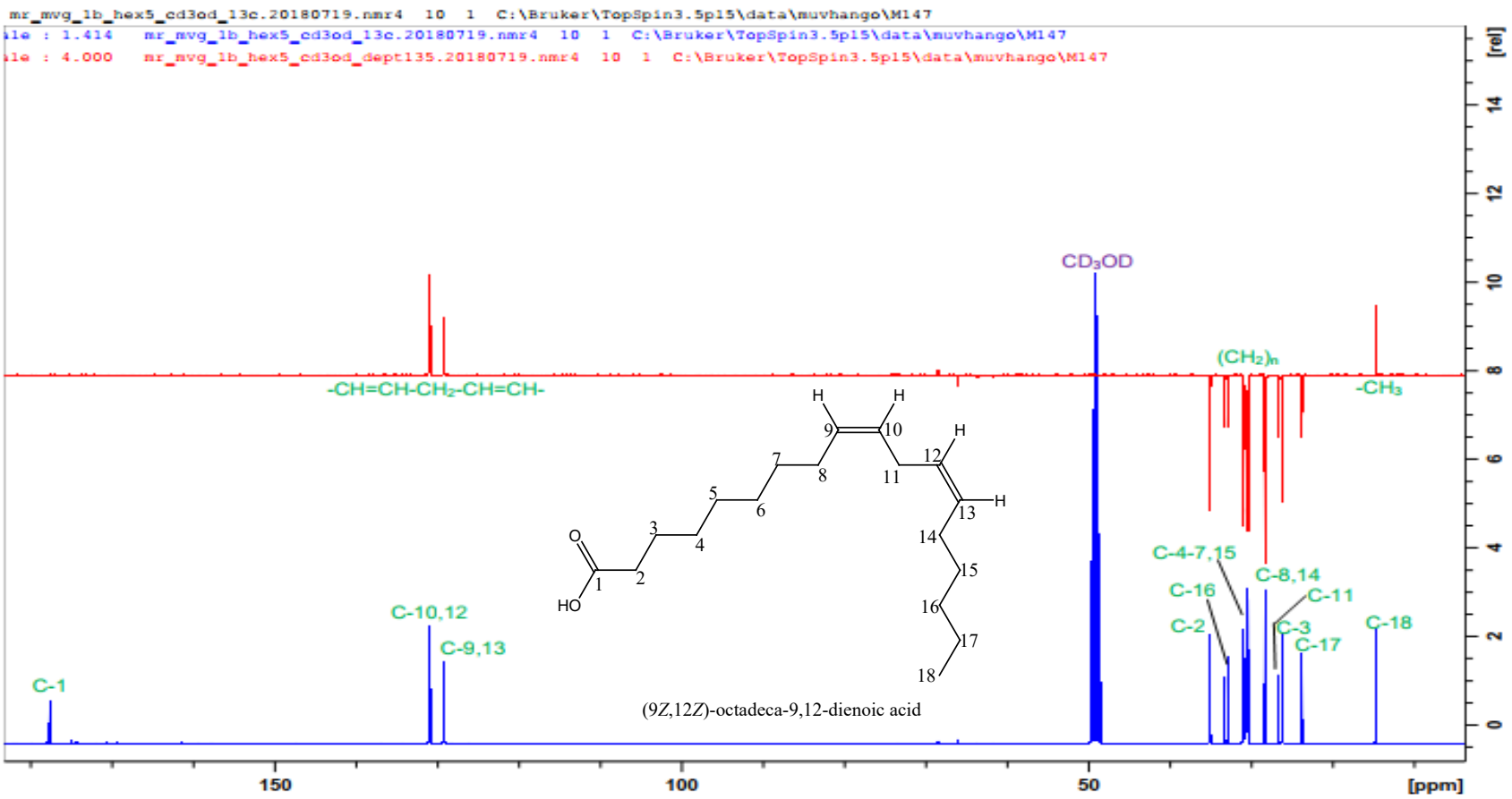


Figure A5. 4 The 13C NMR and distortionless enhancement by polarization transfer (DEPT) spectrum of linoleic acid in CD3OD.

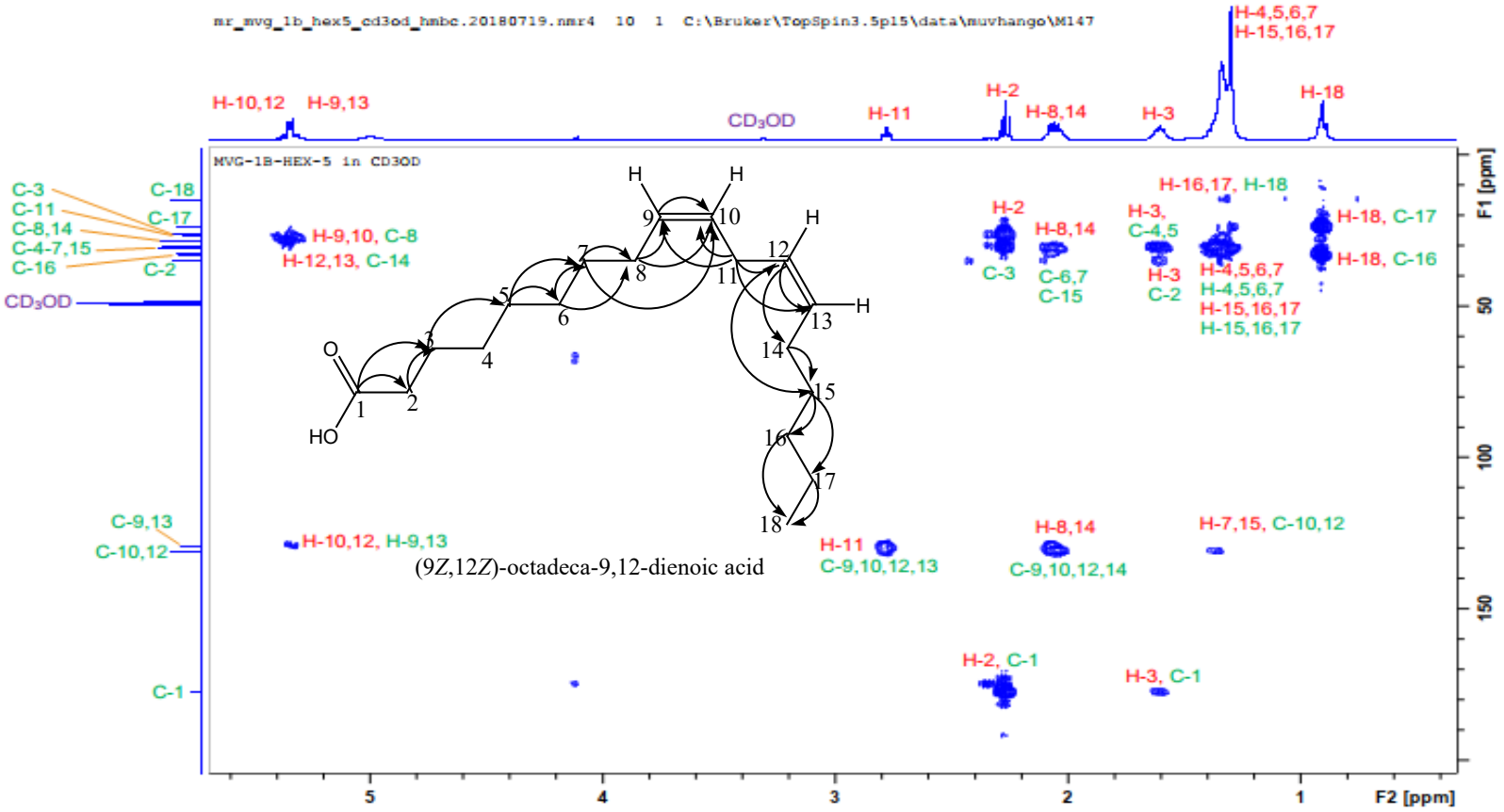


Figure A5. 5 The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of linoleic acid in CD_3OD

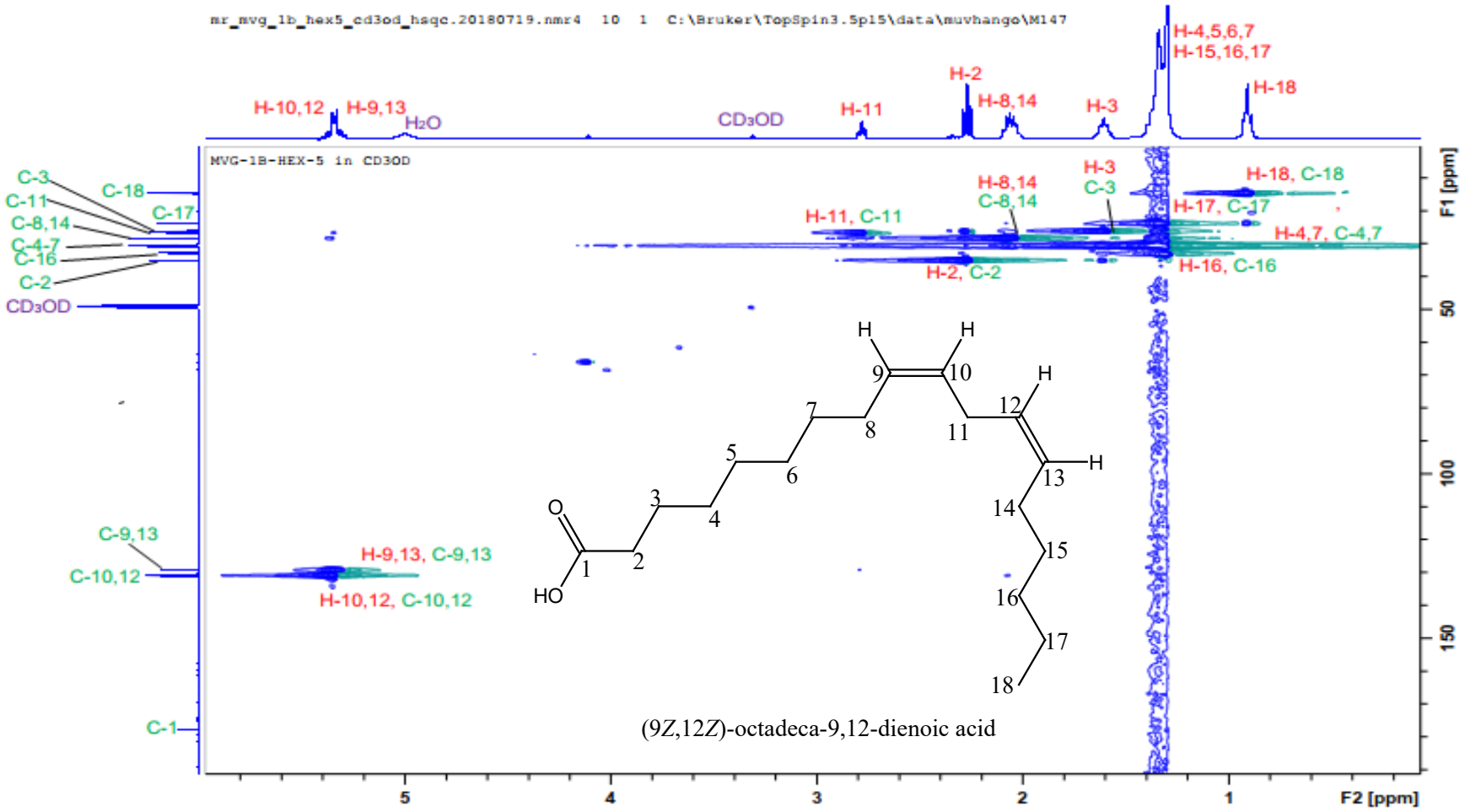


Figure A5. 6 The heteronuclear correlation (HSQC) spectrum of linoleic acid in CD_3OD.

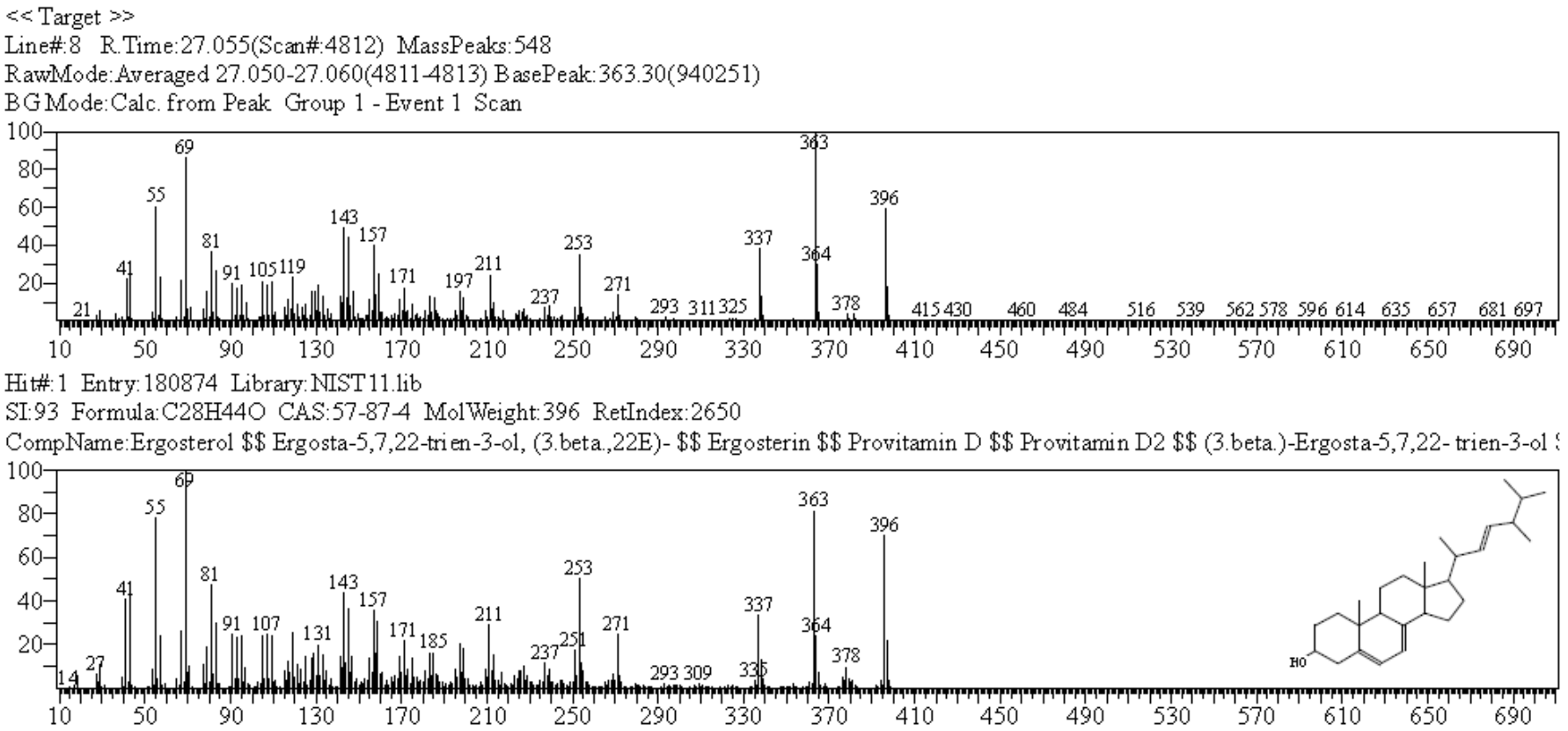


Figure A5. 7 GC-MS spectrum of ergosterol in DCM.

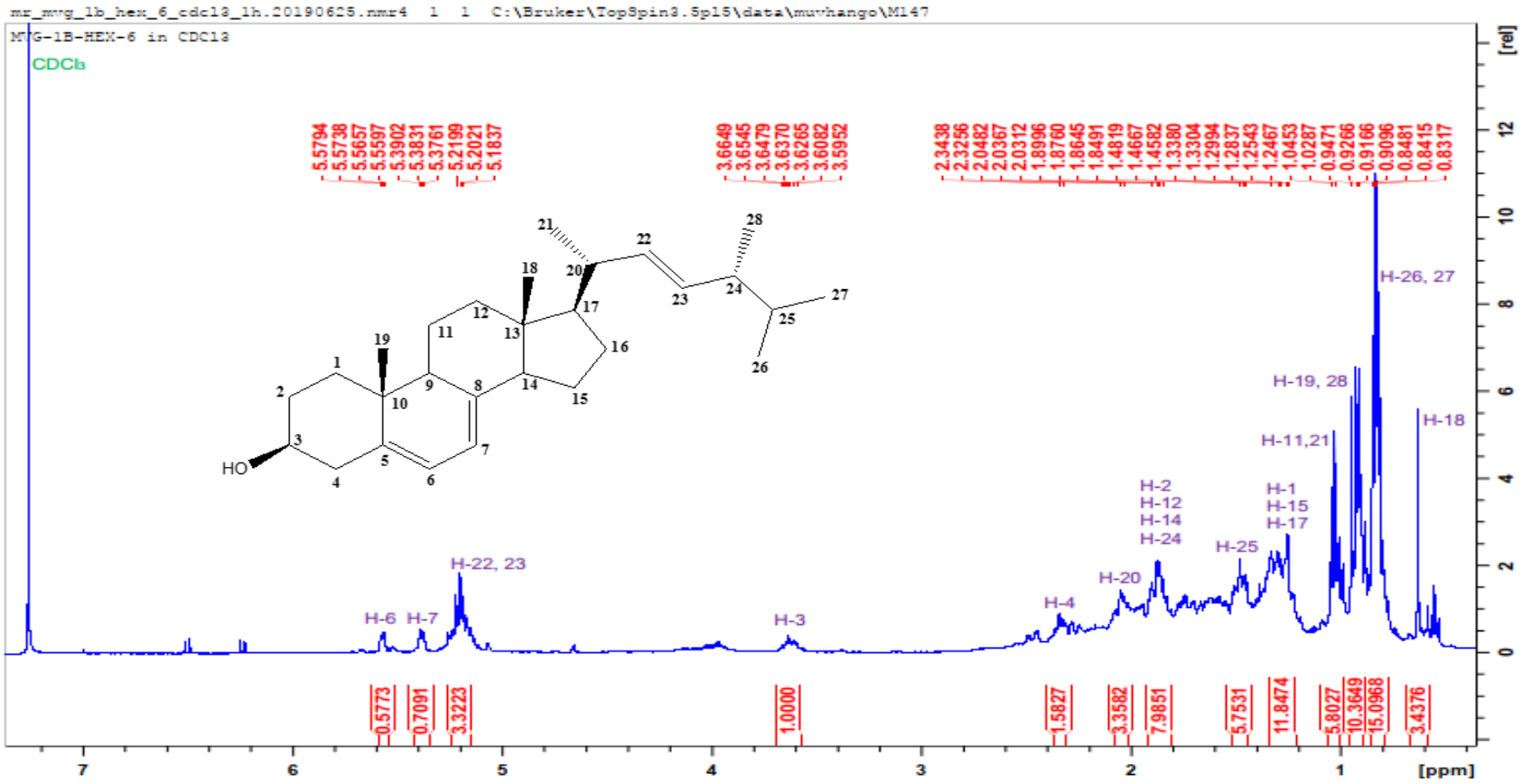


Figure A5. 8 The ^{1}H NMR spectrum of ergosterol in $CDCl_3$.

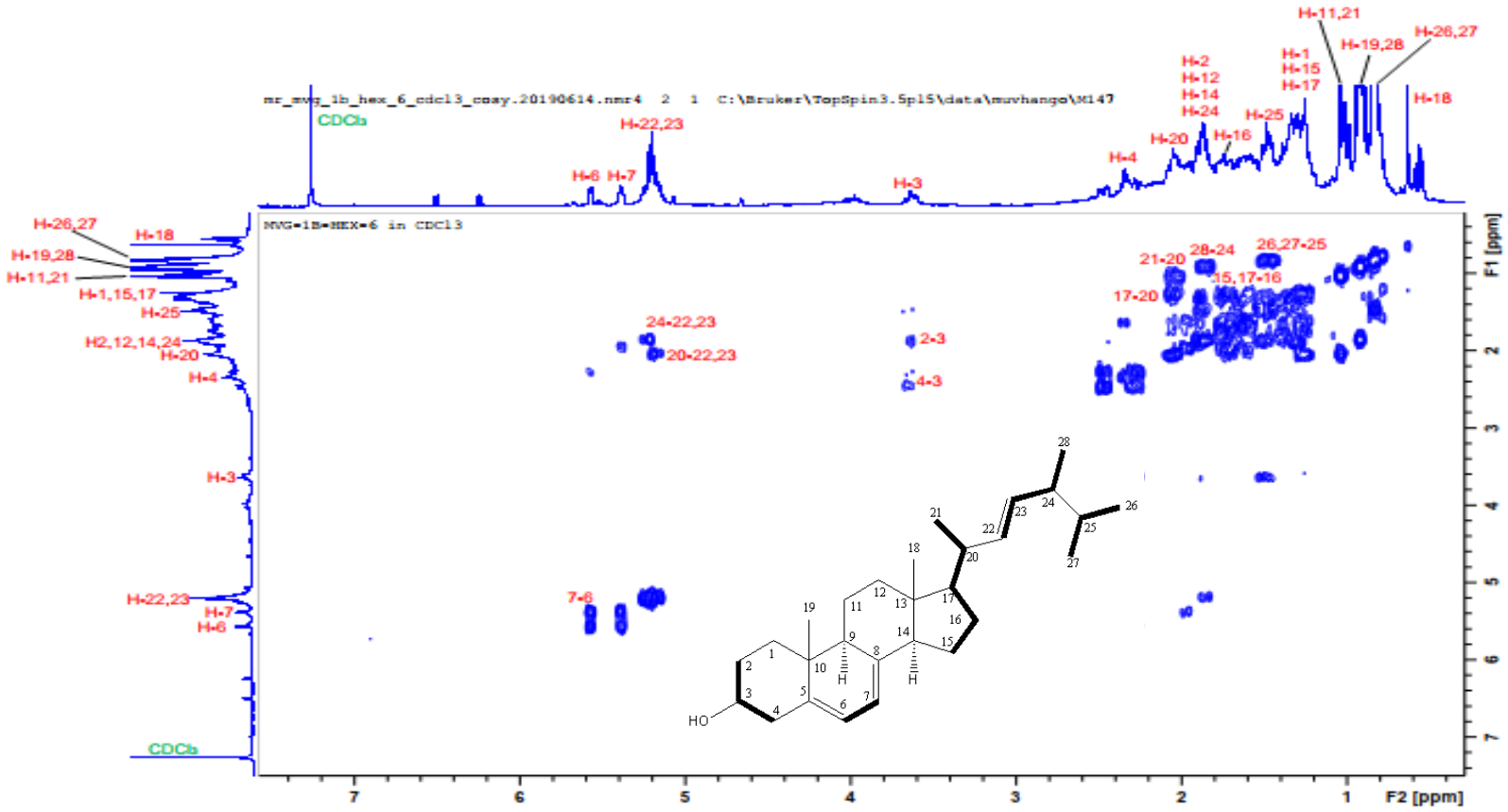


Figure A5. 9 The homonuclear correlation (COSY) spectrum of ergosterol in $CDCl_3$.

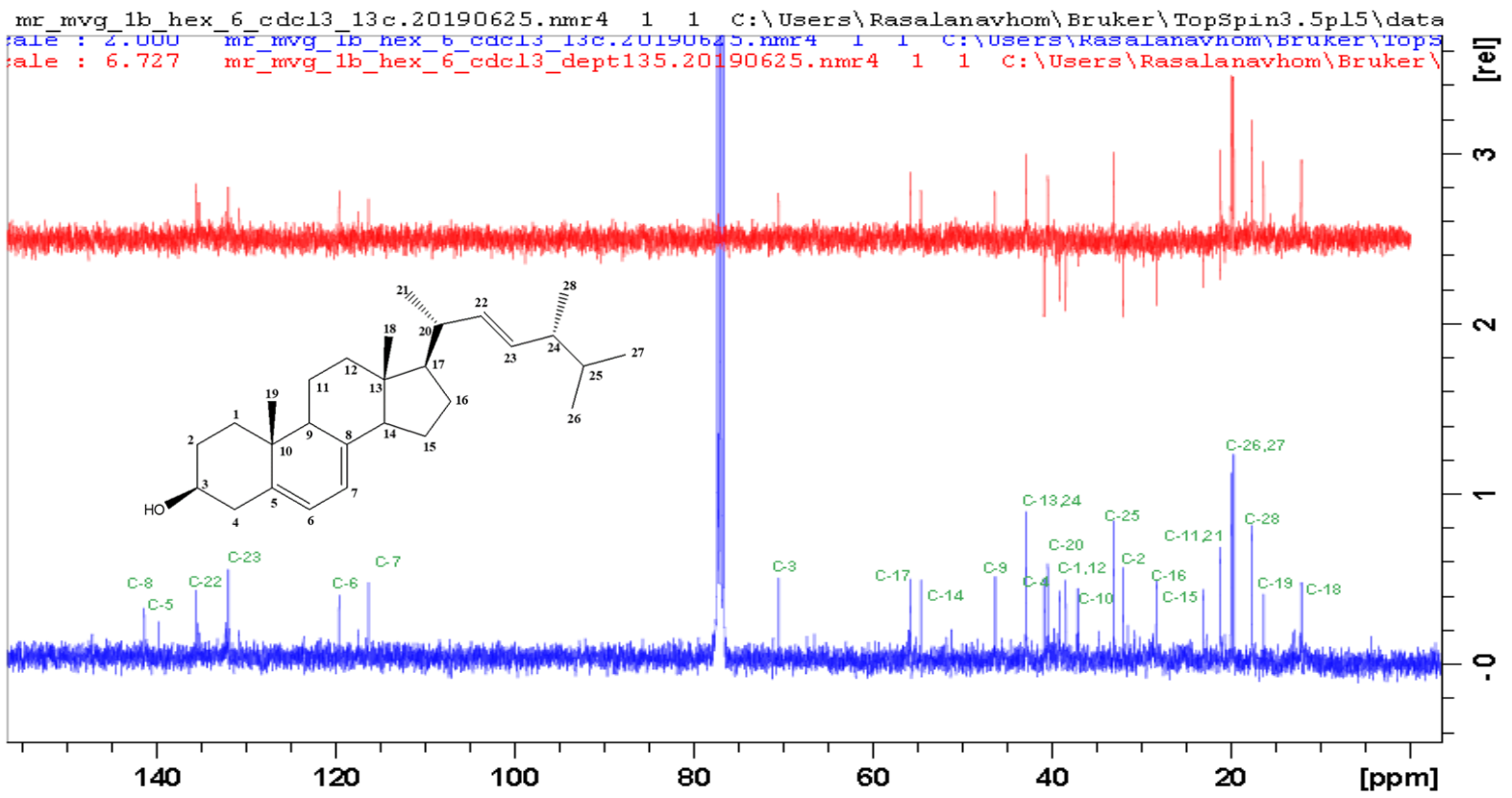


Figure A5. 10 The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra of ergosterol in $CDCl_3$.

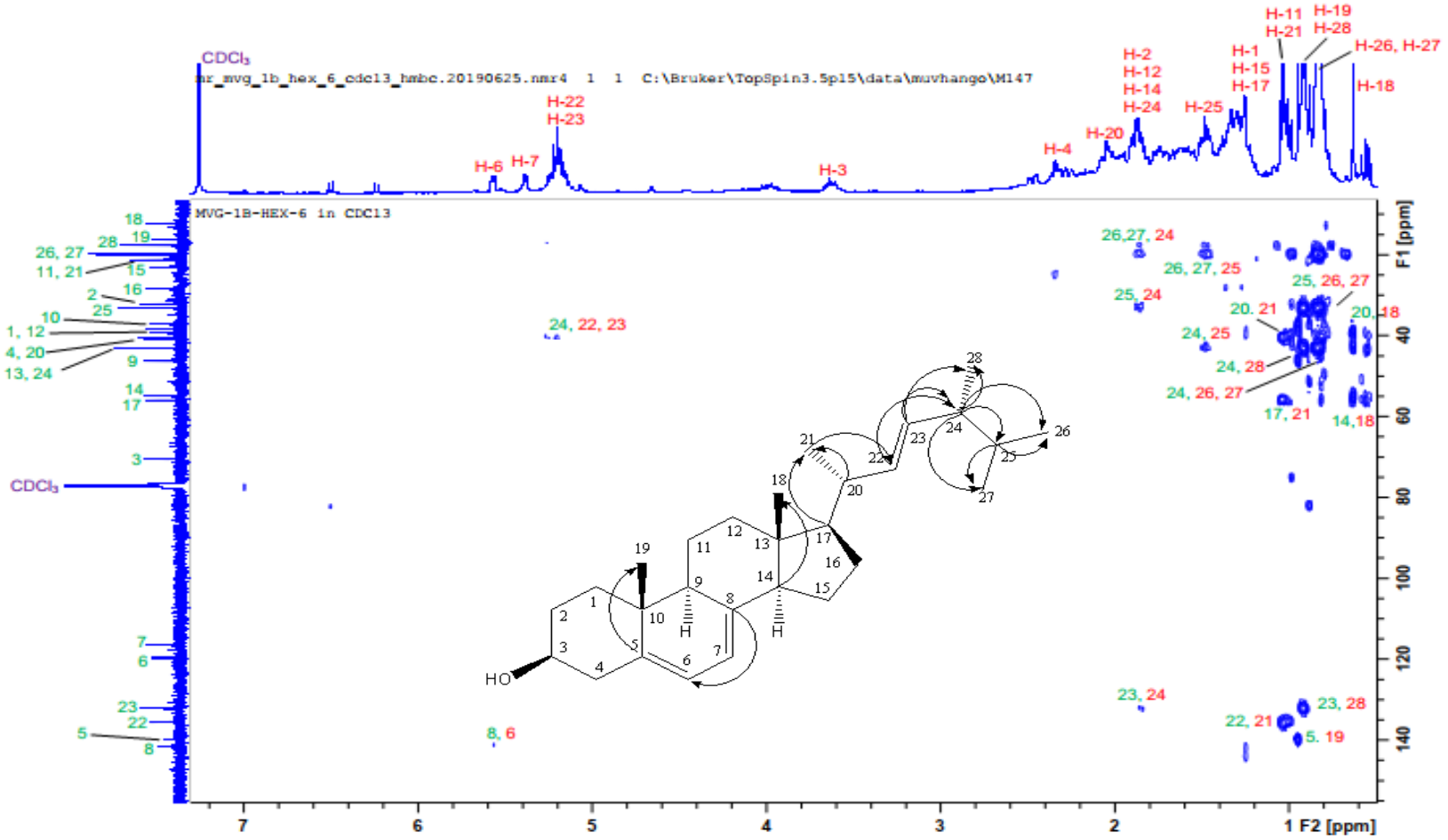


Figure A5. 11 The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of ergosterol in CD3OD.

Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
2449 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-10 O: 0-50
MVG-1-8D-24J-16 3 (0.068) Cm (1:61)
TOF MS ES-

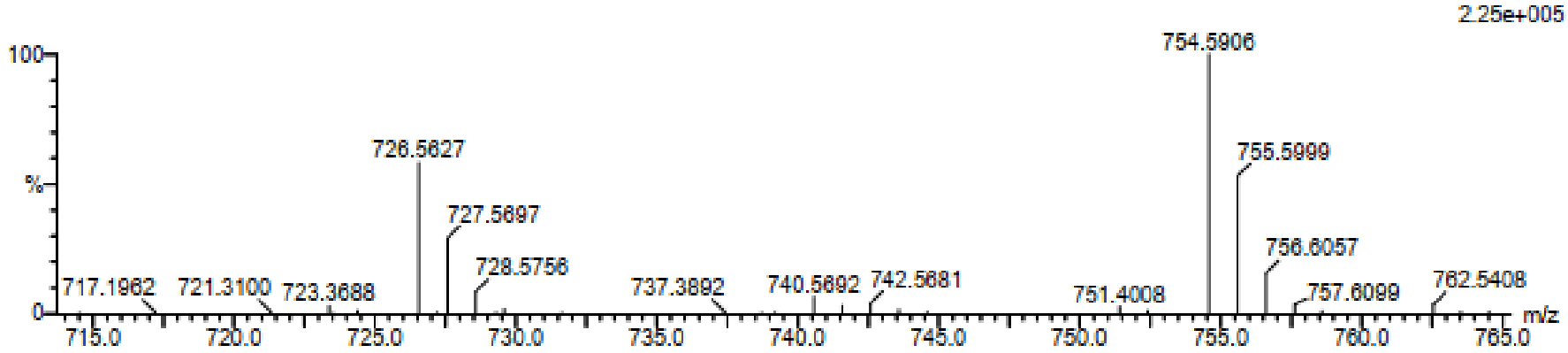


Figure A5. 12 Mass spectrum of the glycosphingolipid

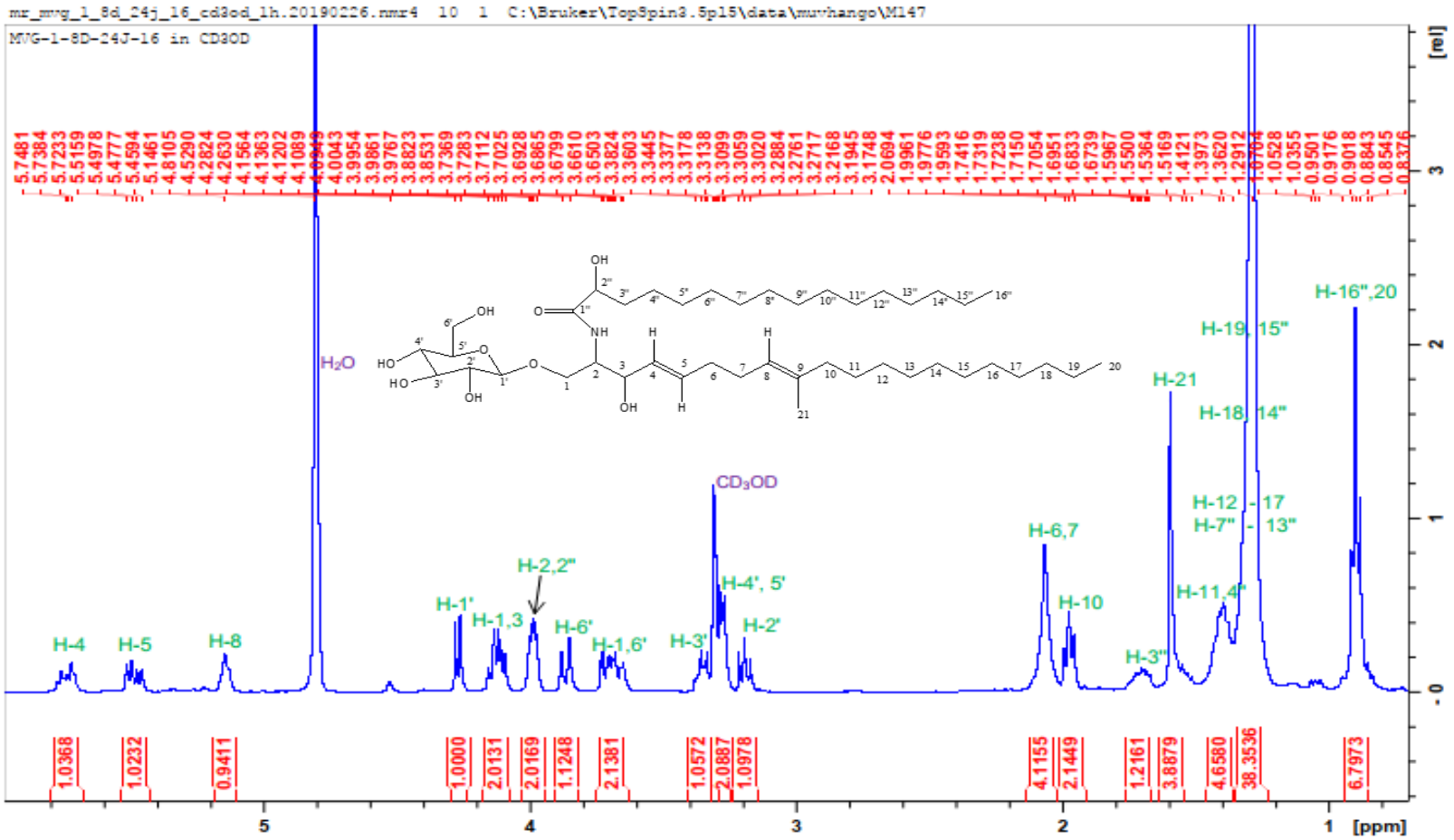


Figure A5. 13 The ^{1}H NMR spectrum of the glycosphingolipid in CD_3OD.

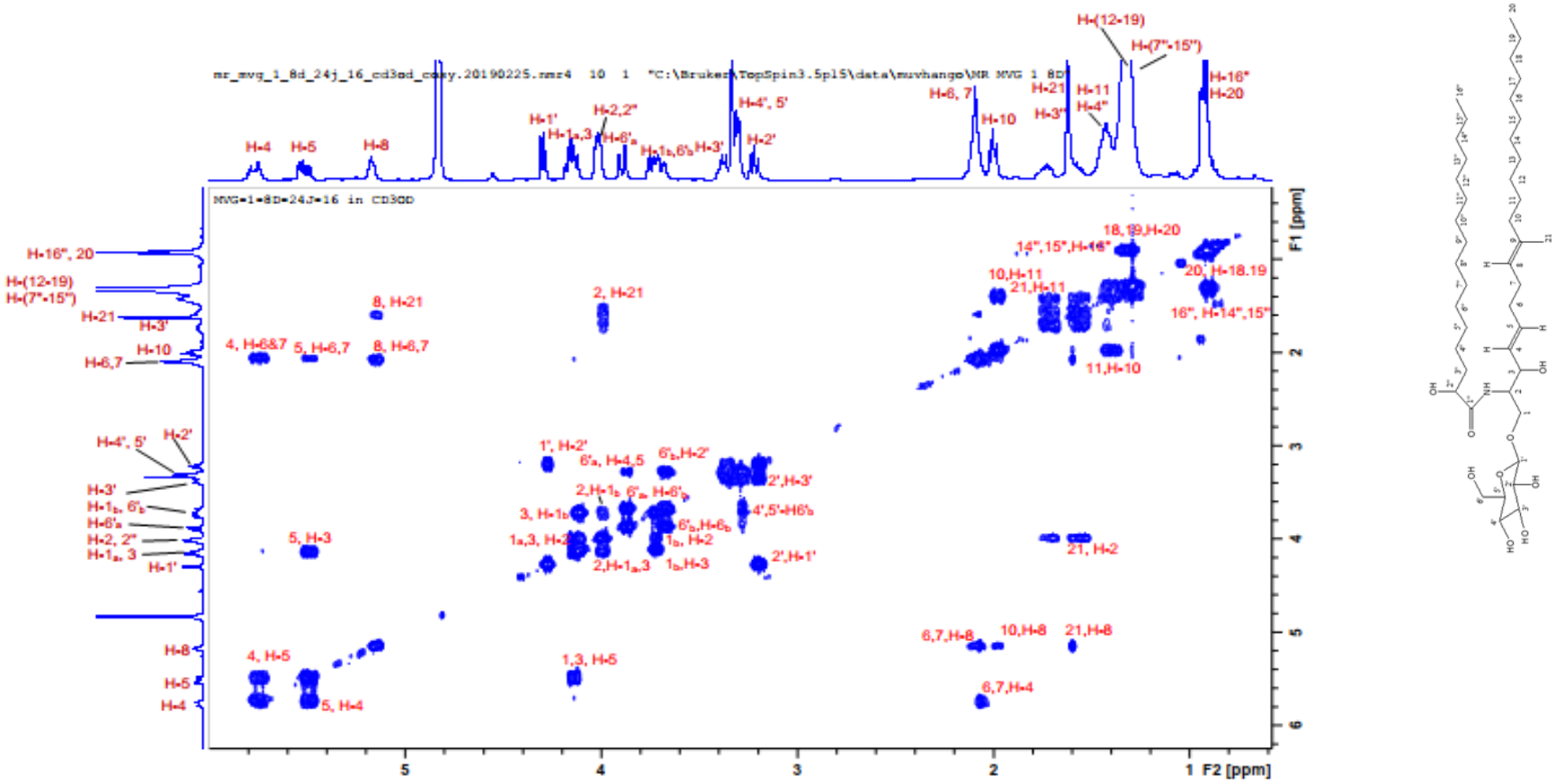


Figure A5. 14 The homonuclear correlation (COSY) NMR spectrum of the glycosphingolipid in CD_3OD.

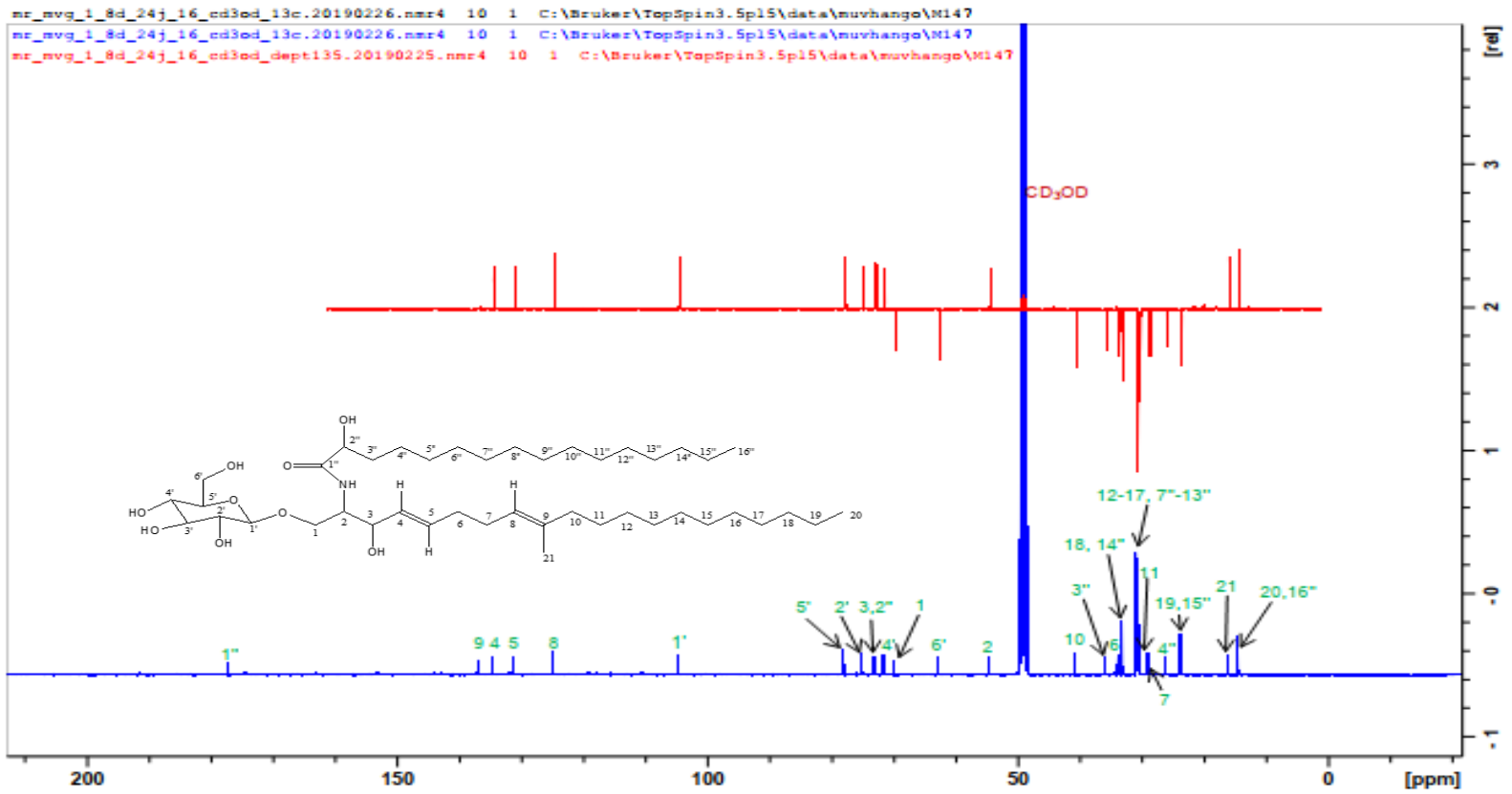


Figure A5. 15 The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectrum of the glycosphingolipid in CD_3OD.

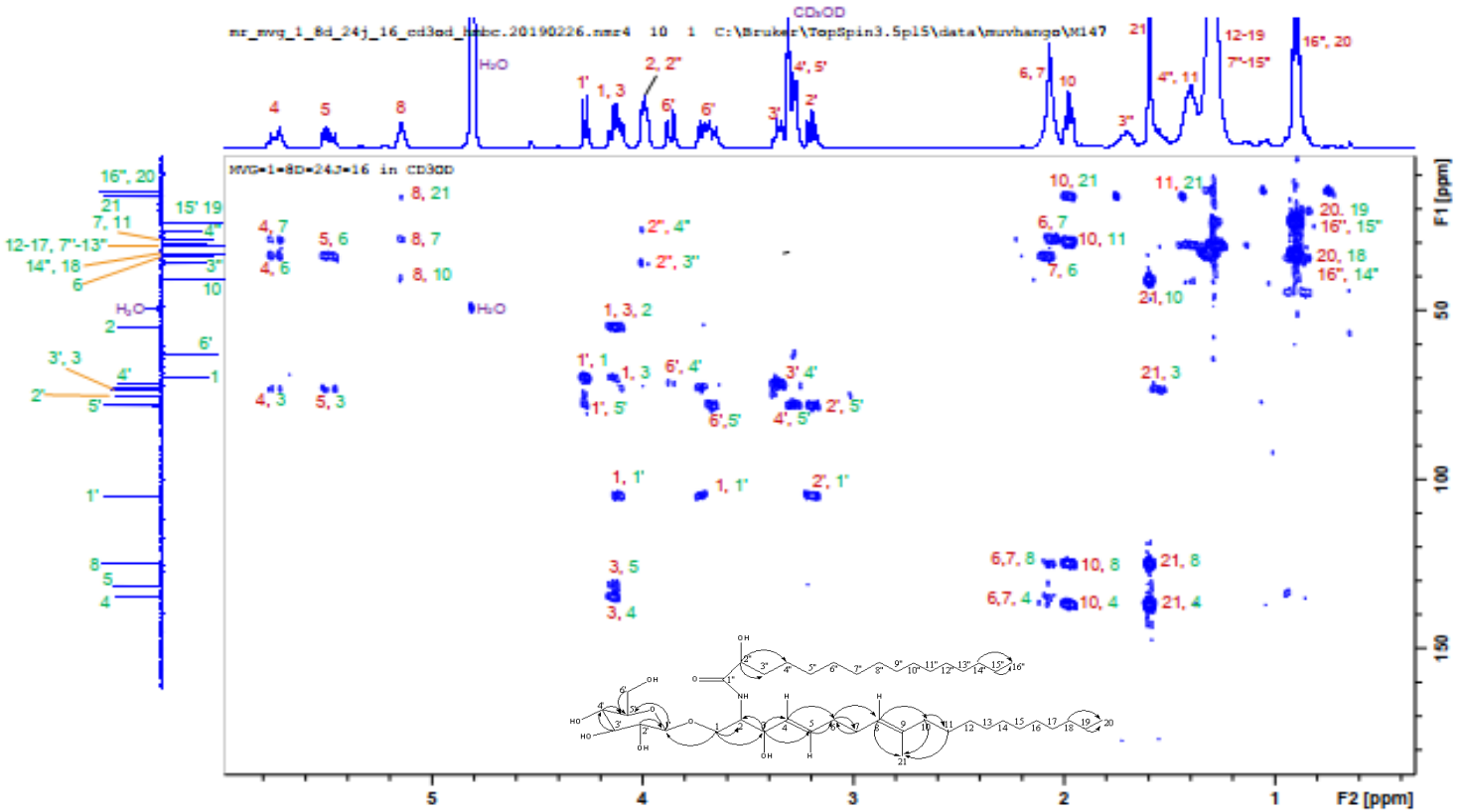


Figure A5. 16 The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of glycosphingolipid in CD_3OD.

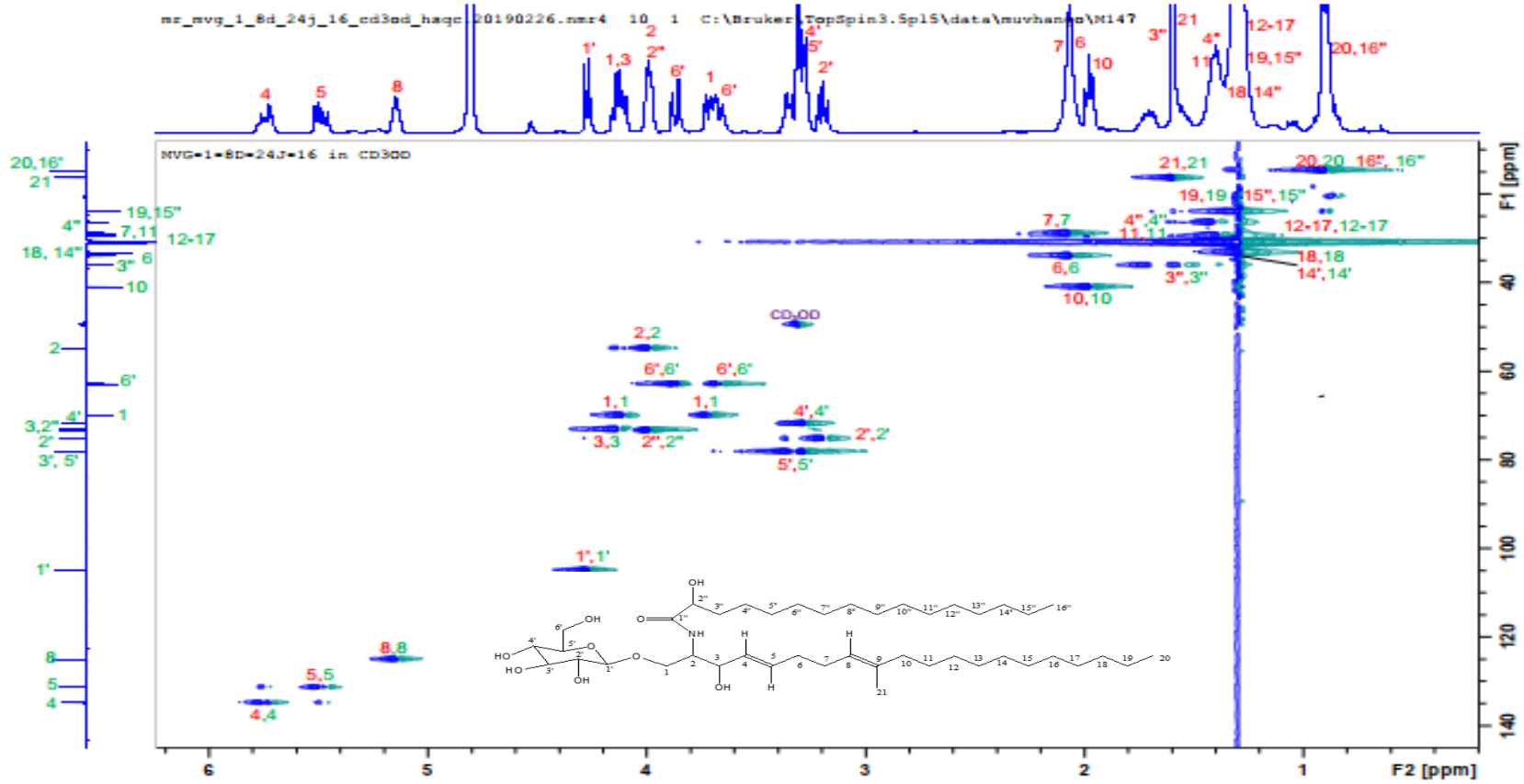


Figure A5. 17 The heteronuclear correlation (HSQC) spectrum of the glycosphingolipid in CD_3OD.

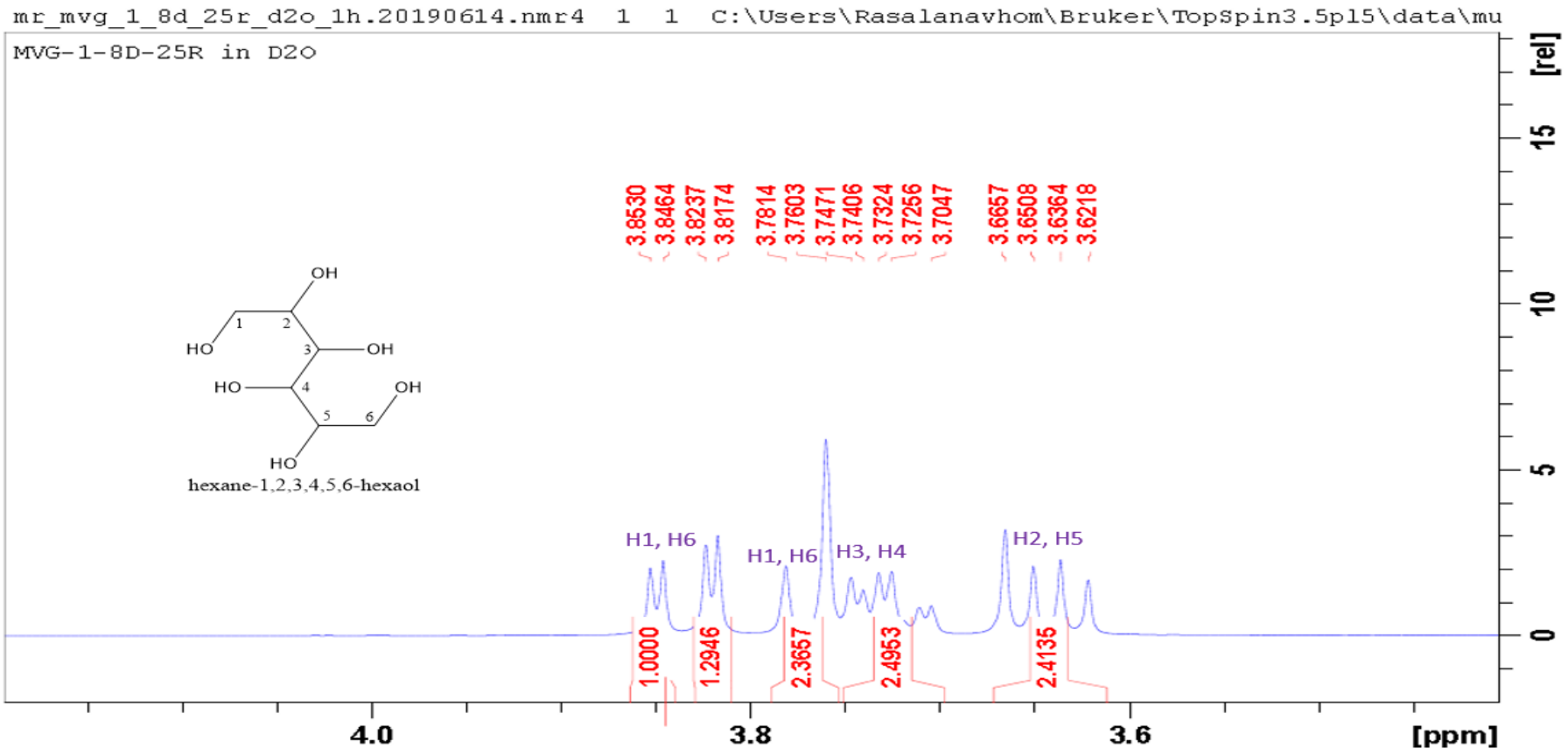


Figure A5. 18 The ^{1}H NMR spectrum of mannitol in D_2O.

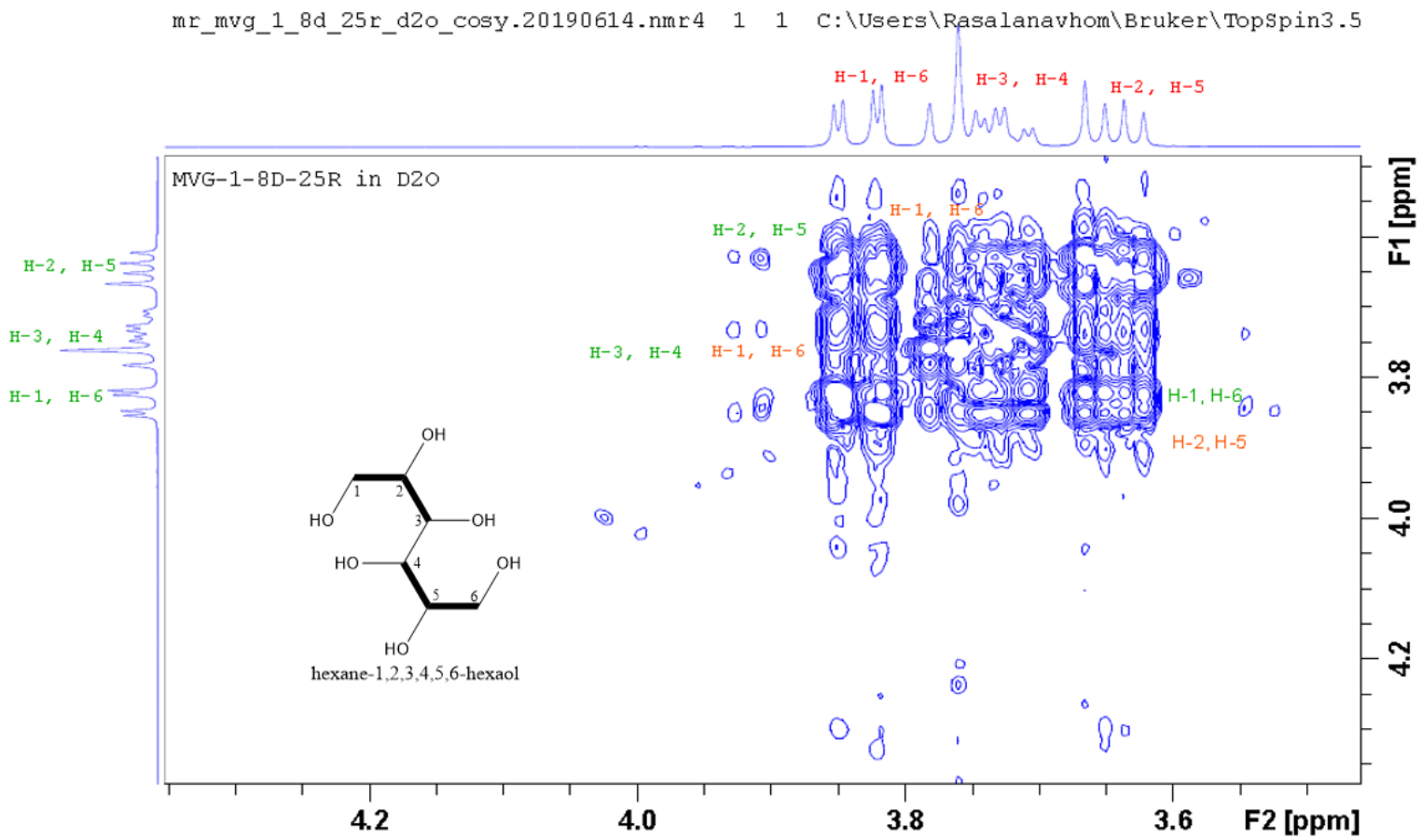


Figure A5. 19 The homonuclear correlation (COSY) NMR spectrum of mannitol in D_2O.

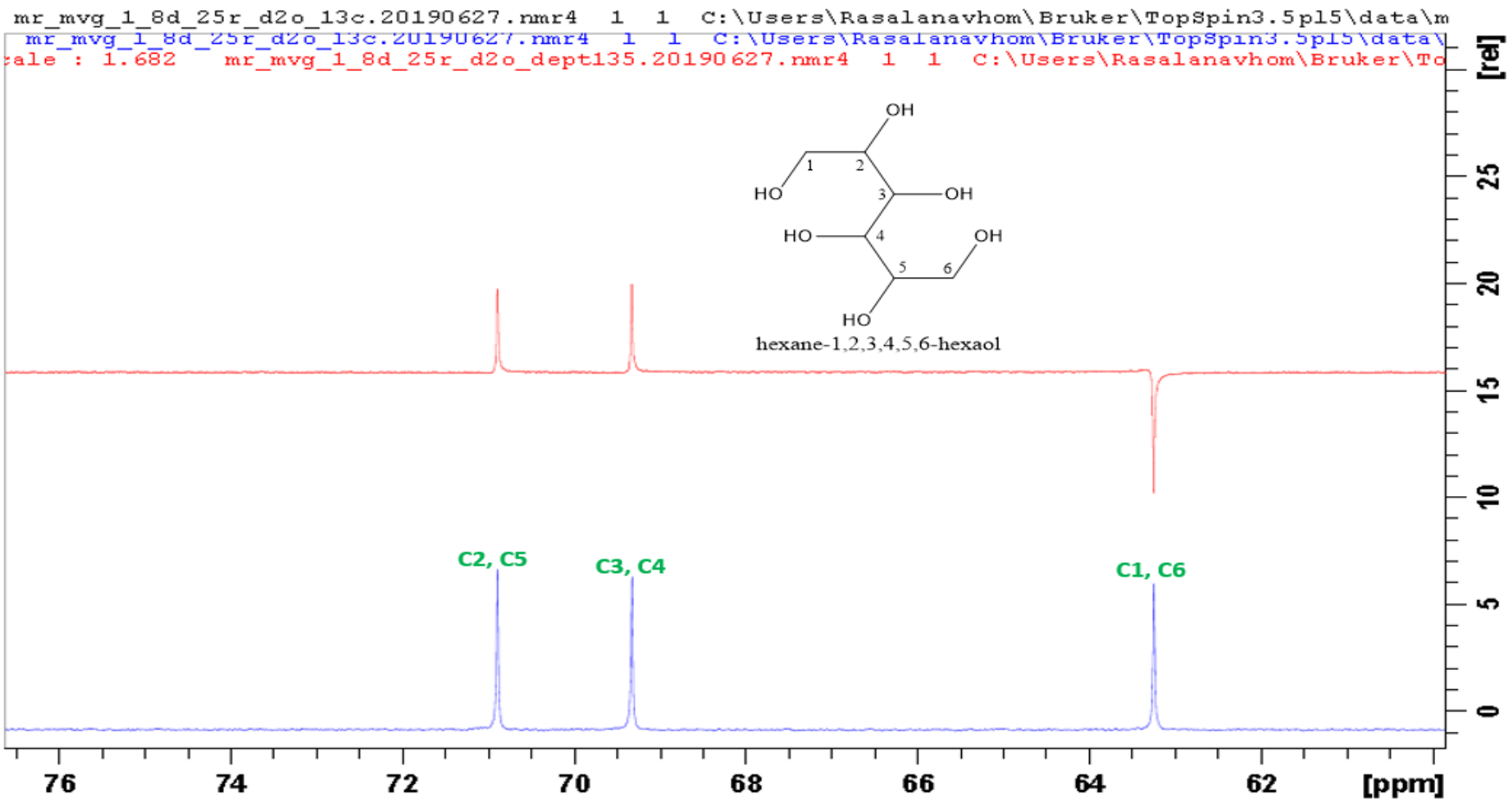


Figure A5. 20 The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectrum of mannitol in D_2O.

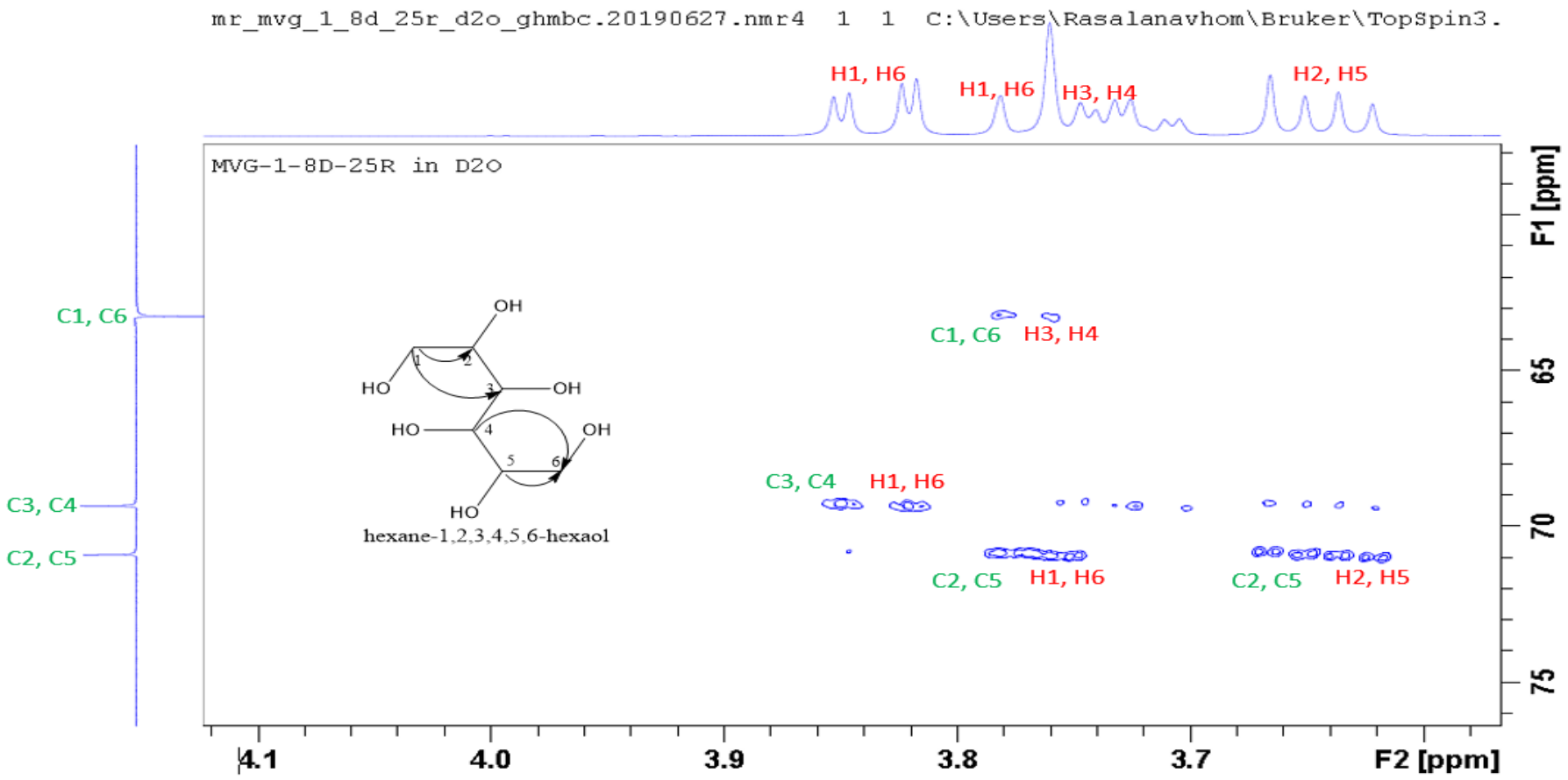


Figure A5. 21 The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of mannitol in D_2O.

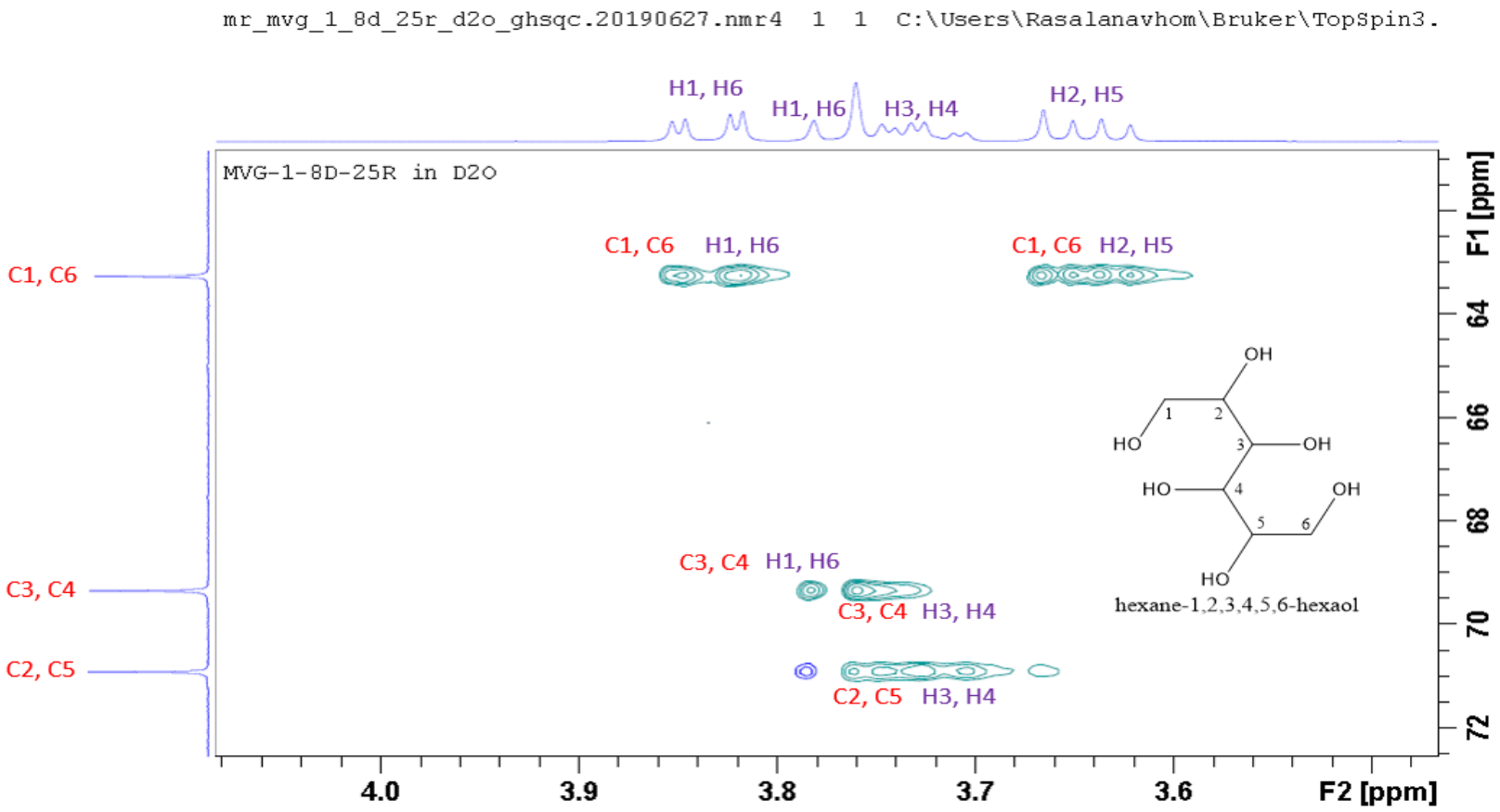


Figure A5. 22 The heteronuclear correlation (HSQC) spectrum of mannitol in D_2O.

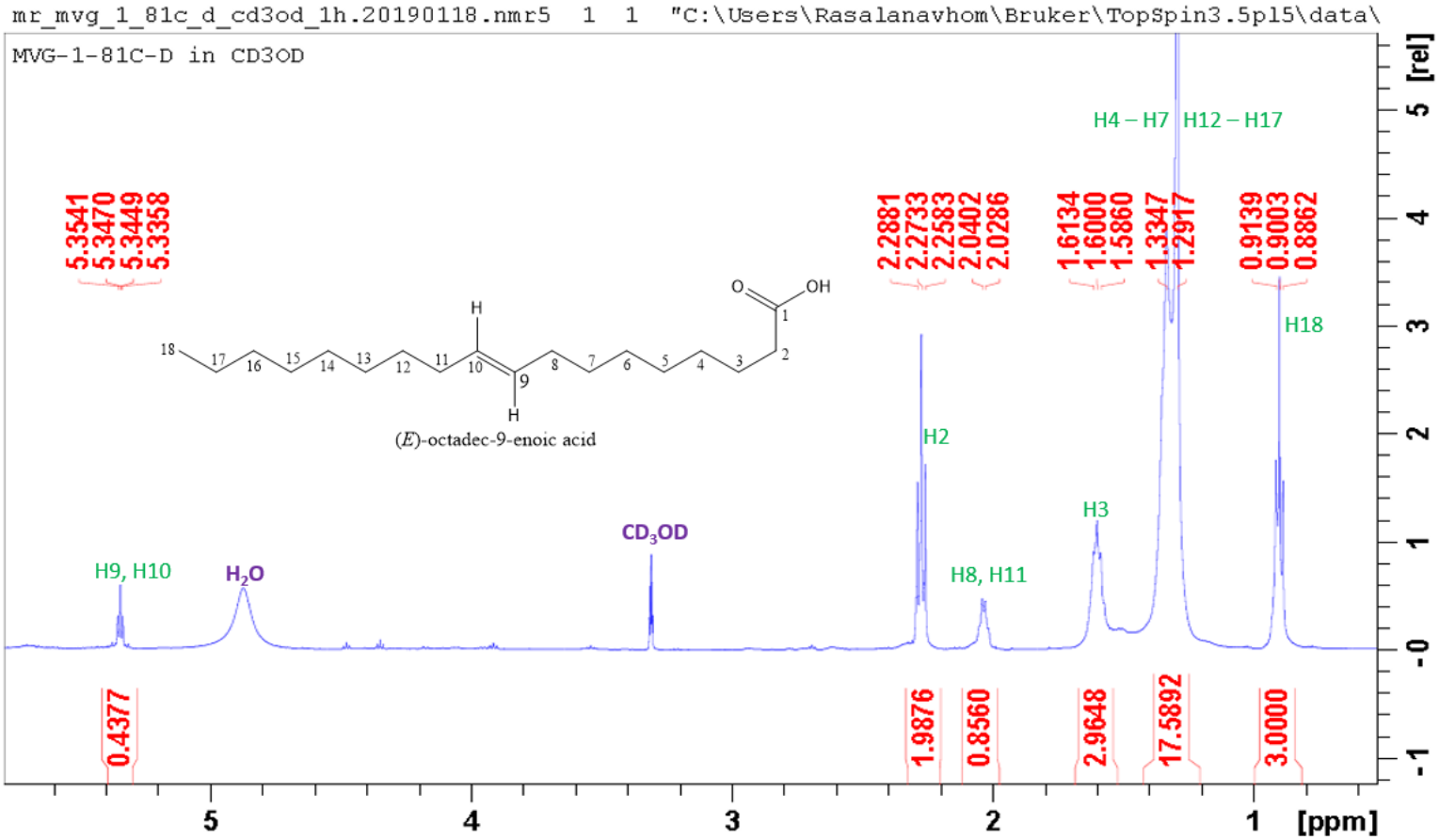


Figure A5. 23 The ^{1}H NMR spectrum of oleic acid in CD_3OD.

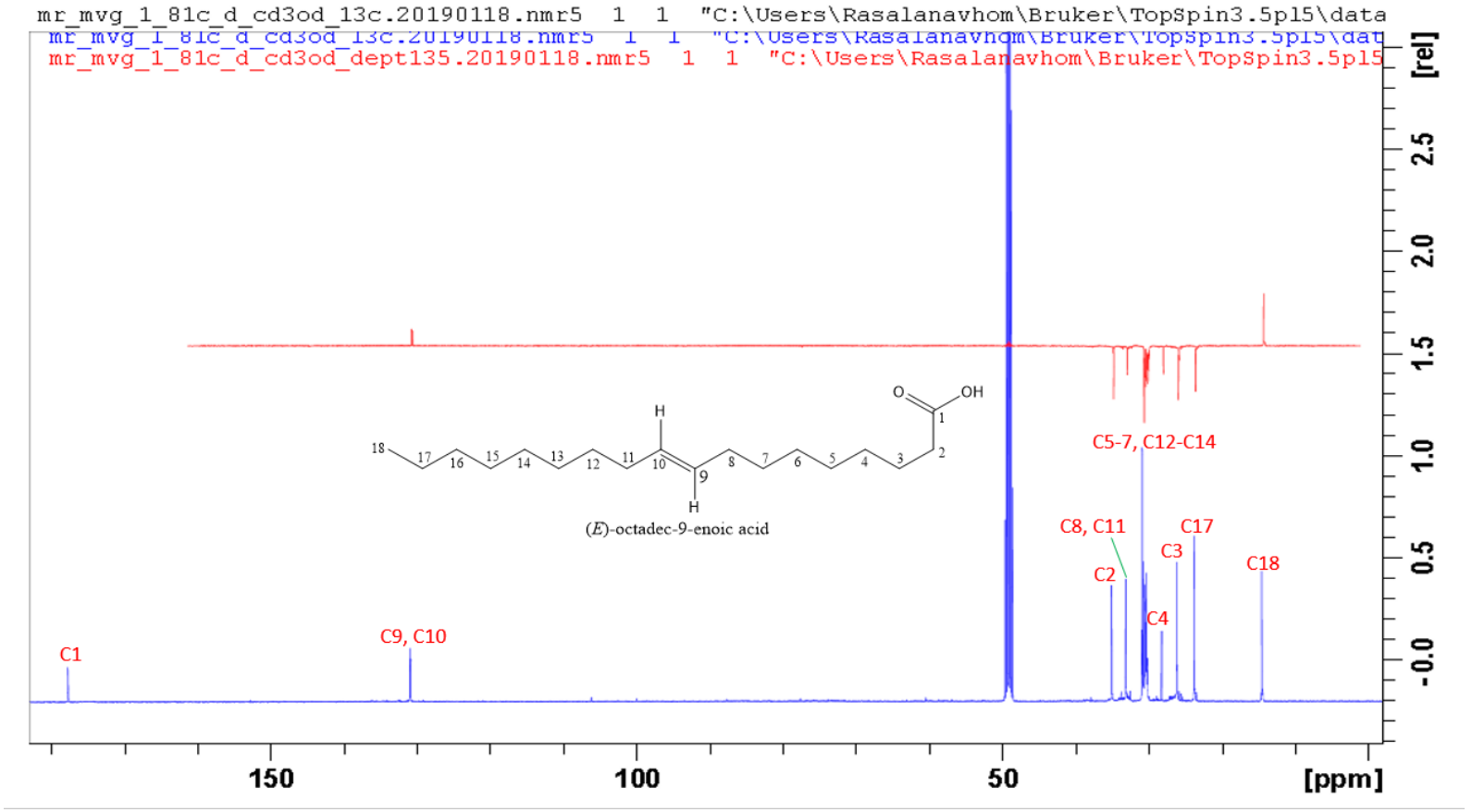


Figure A5. 24 The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectrum of oleic acid in CD_3OD.

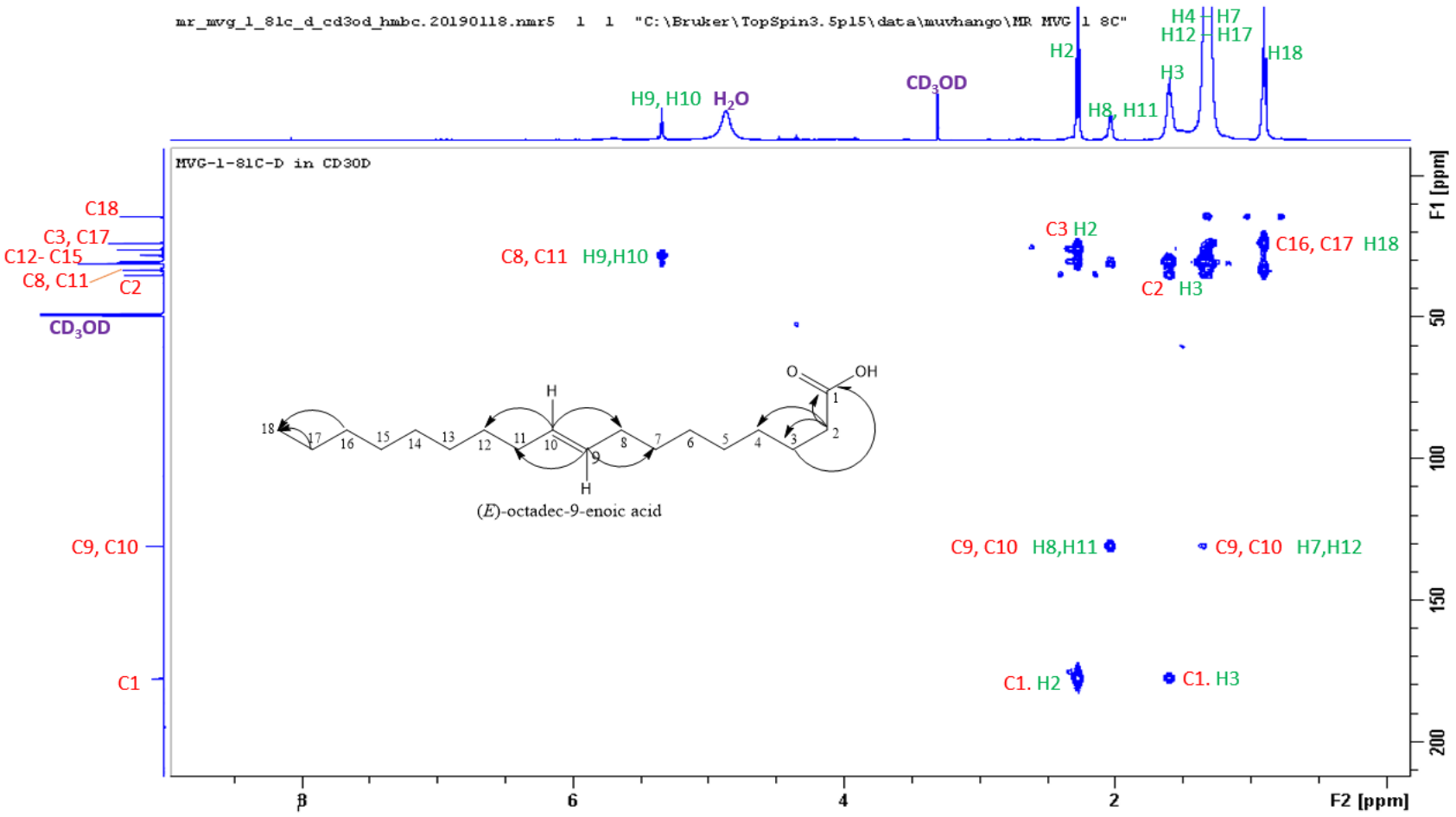


Figure A5. 25 The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of oleic acid in CD_3OD.

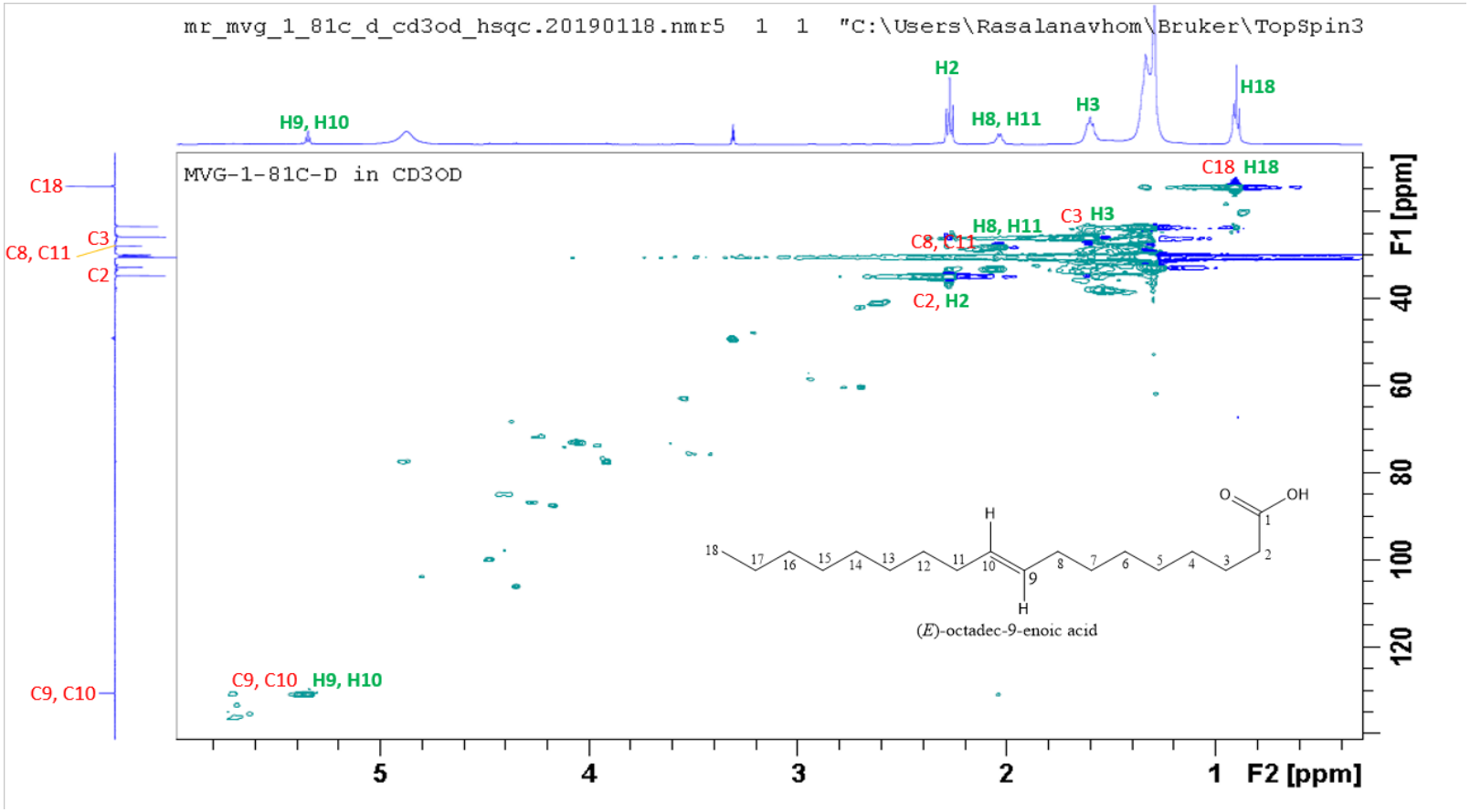


Figure A5. 26 The heteronuclear correlation (HSQC) spectrum of oleic acid in CD_3OD.

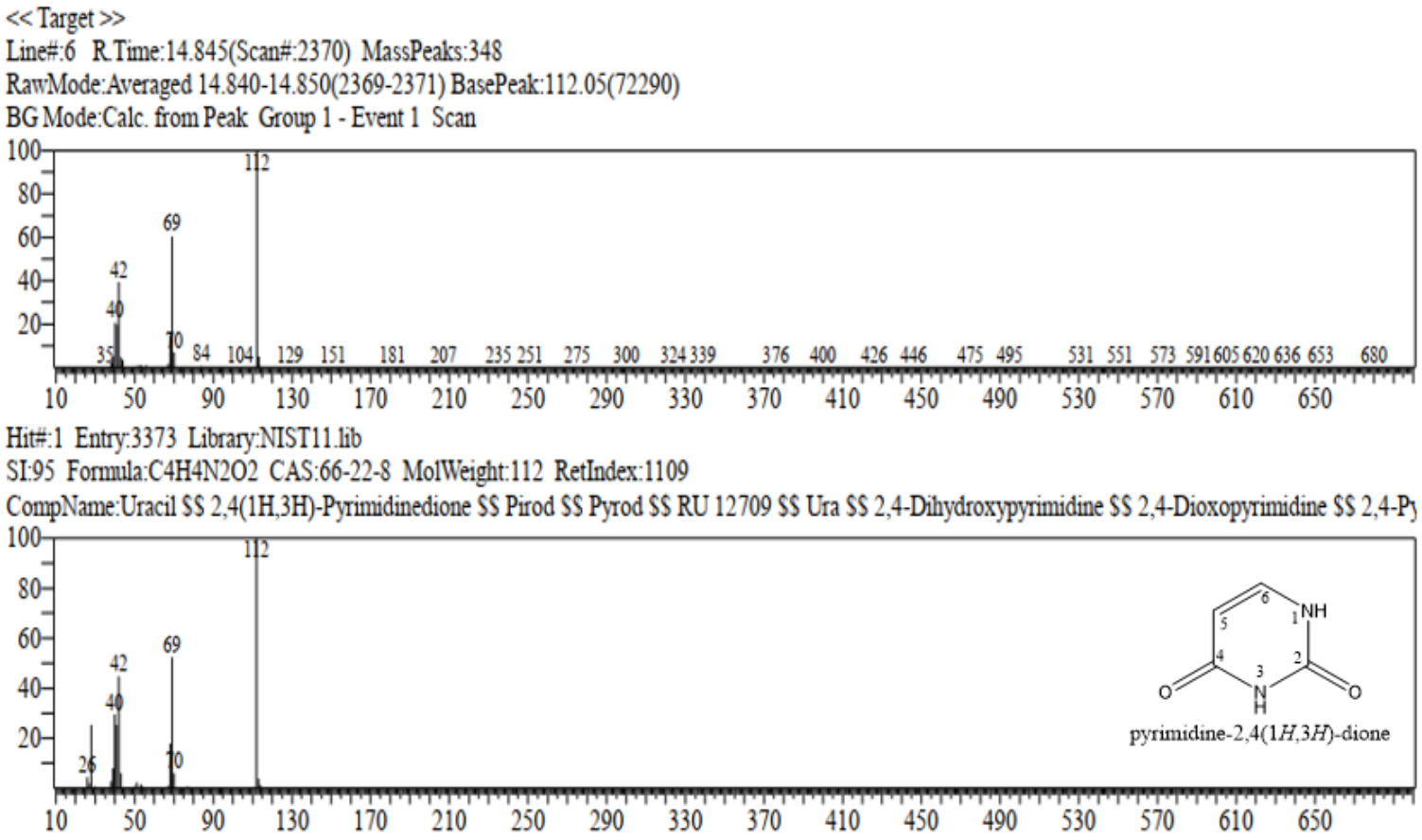


Figure A5. 27 The GC-MS spectrum of uracil showing 95% similarity index to the target compound.

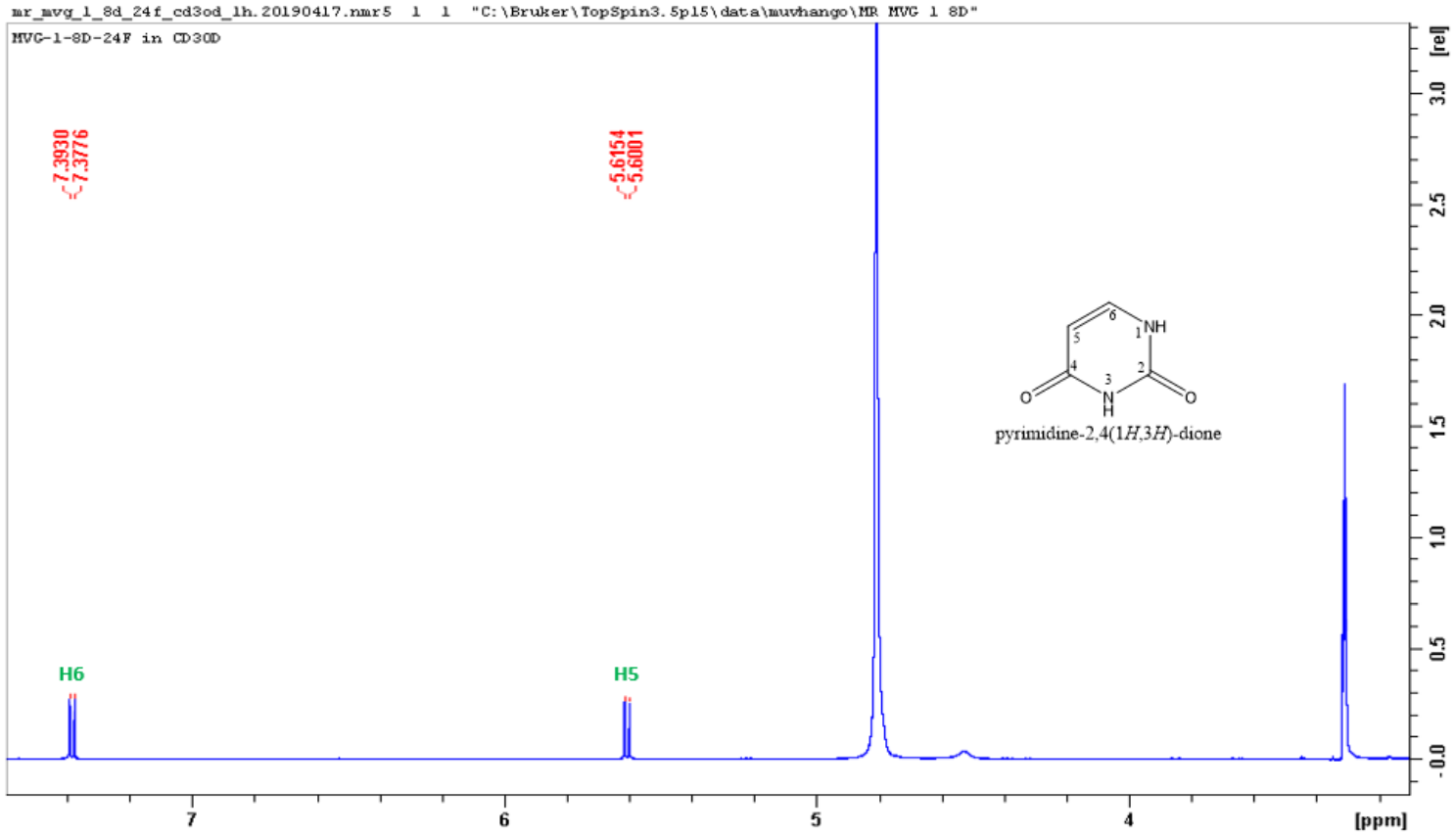


Figure A5. 28 The ^{1}H NMR spectrum of uracil in CD_3OD.

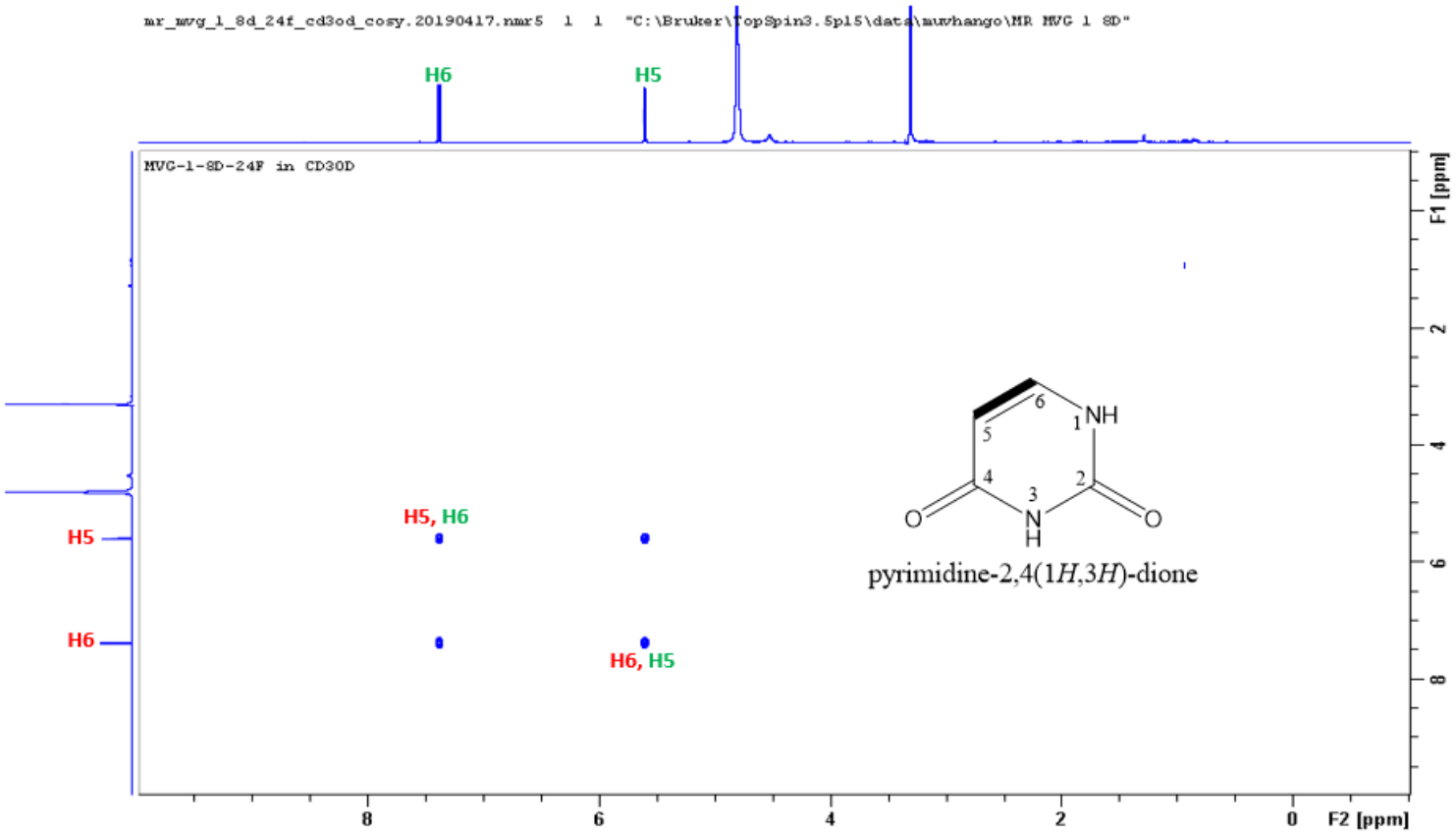


Figure A5. 29 The homonuclear correlation (COSY) NMR spectrum of uracil in CD_3OD.

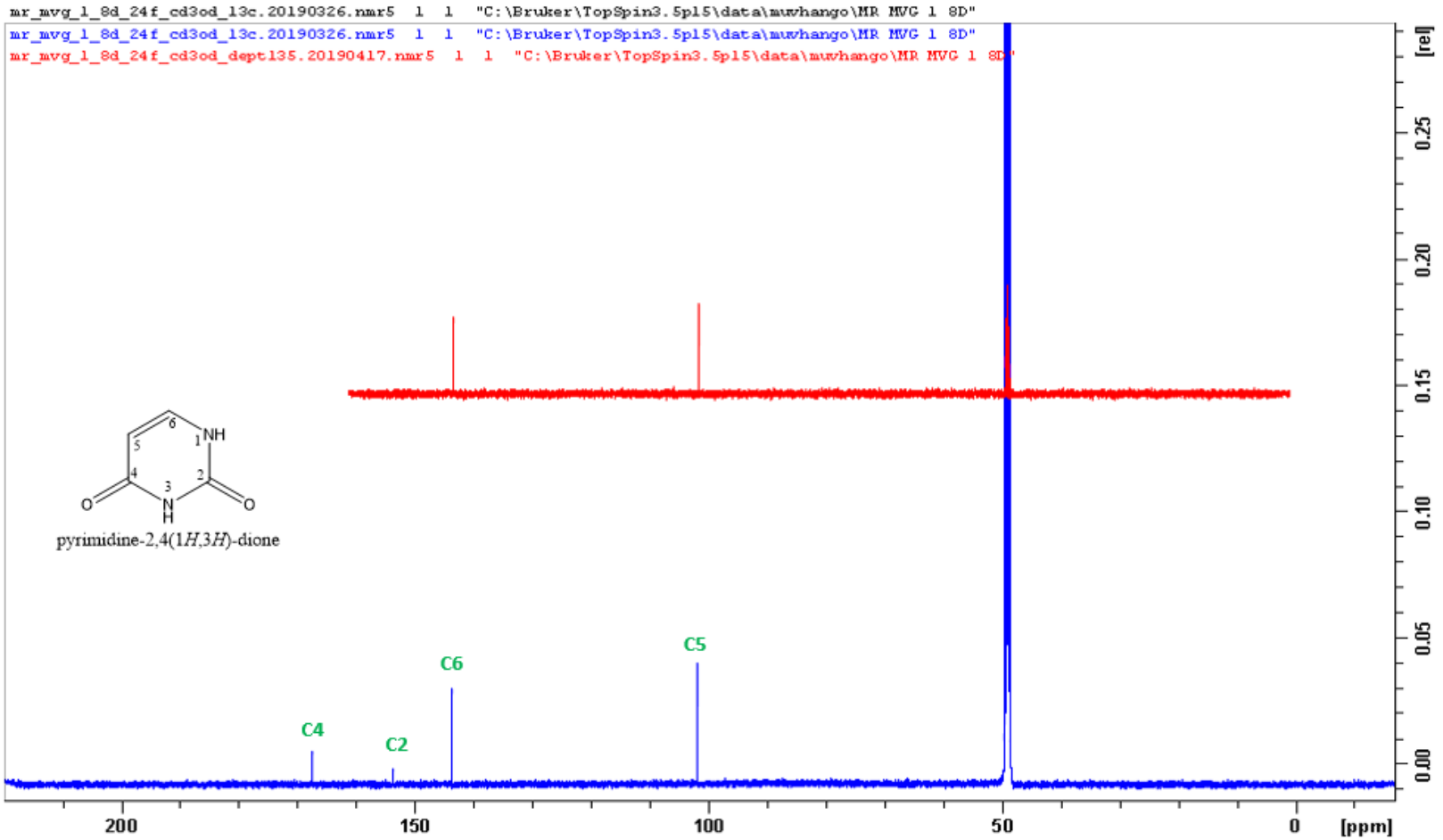


Figure A5. 30 The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectrum of uracil in CD_3OD.

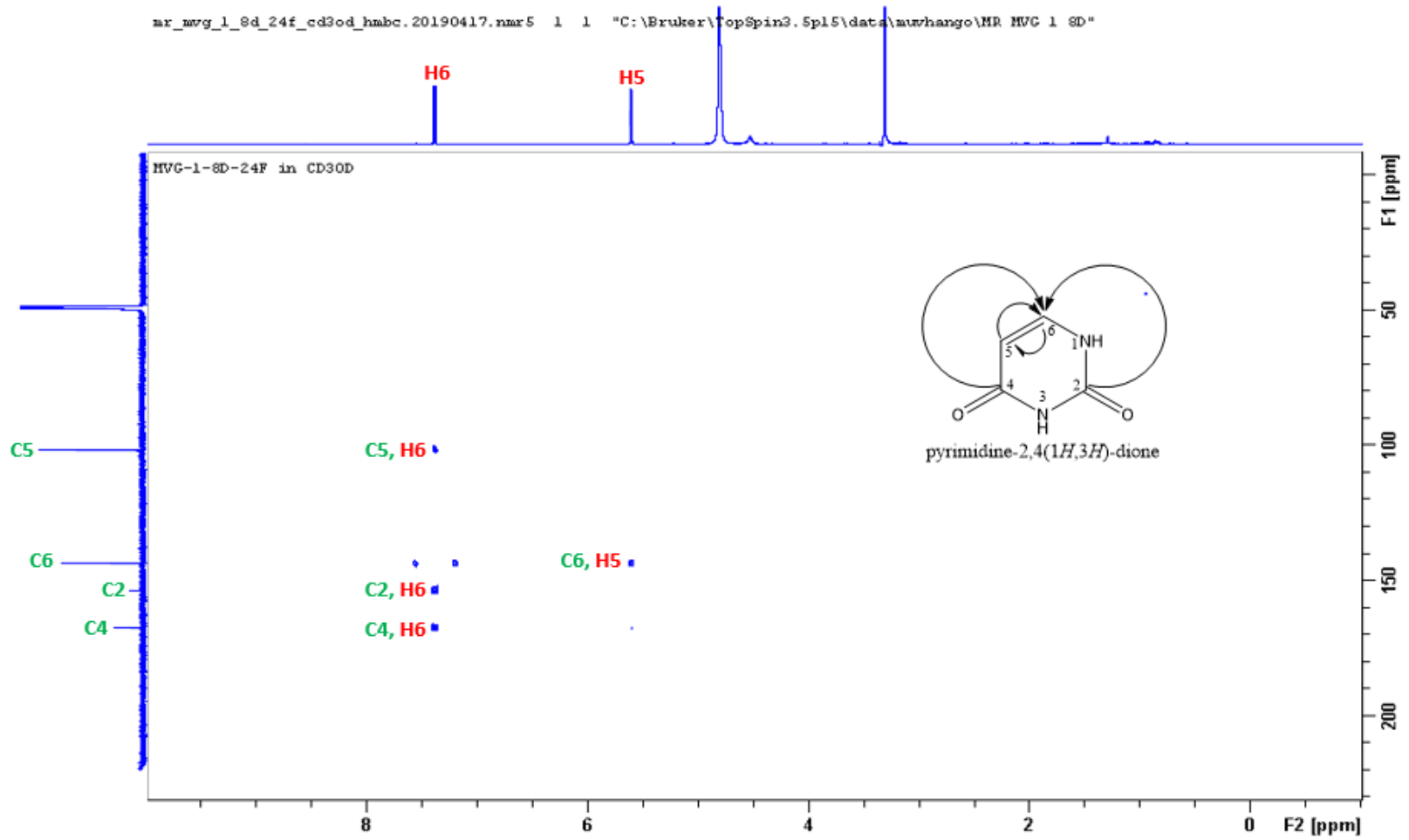


Figure A5. 31 The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of uracil in CD_3OD.

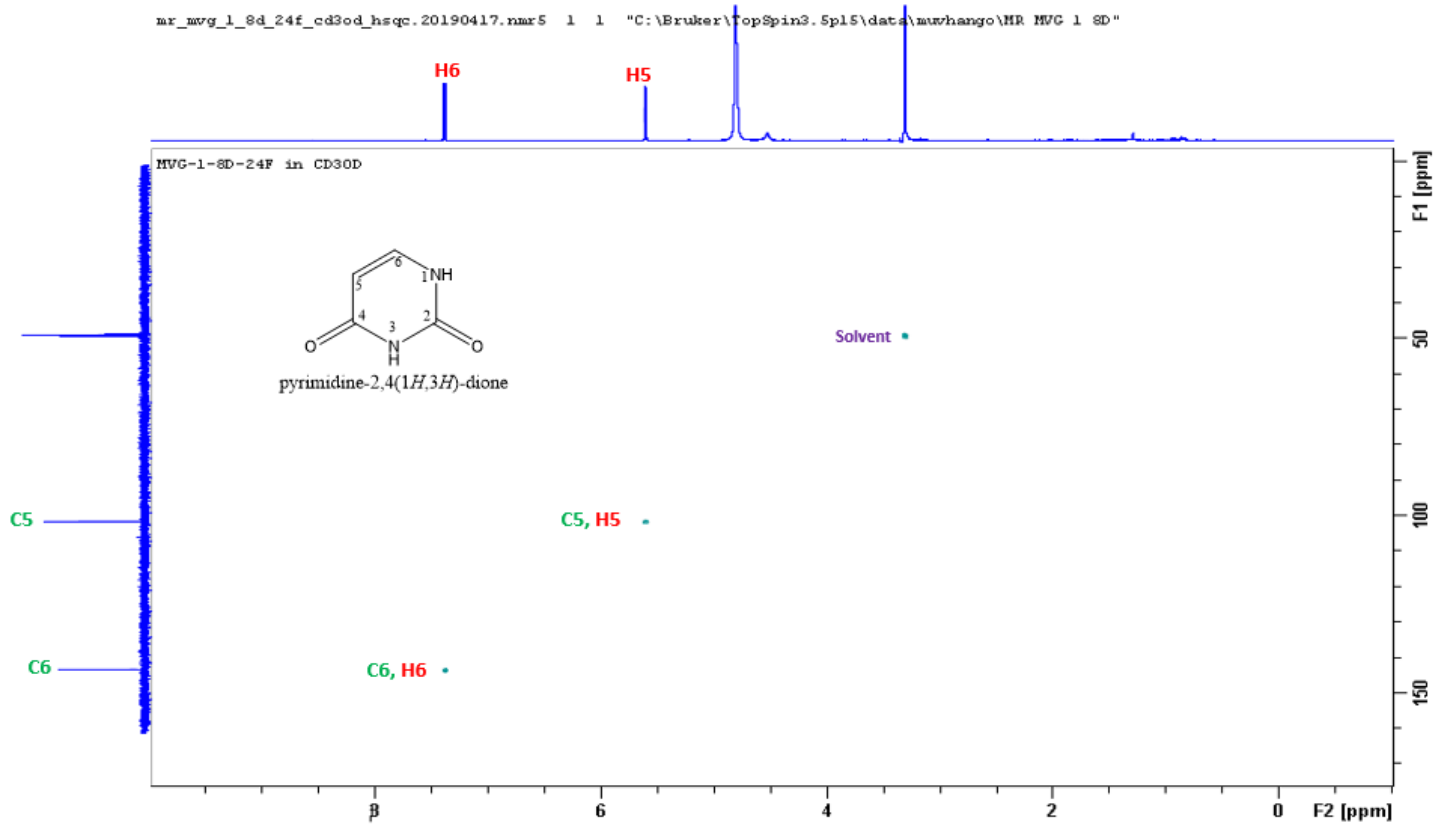


Figure A5. 32 The heteronuclear correlation (HSQC) spectrum of uracil in CD_3OD.