MICROBIOLOGICAL QUALITY, SAFETY, MYCOTOXINS AND HEAVY METALS LEVELS IN UNDERPRIZED KALAHARI TRUFFLE, *TERMITOMYCES SCHIMPERI* AND *GANODERMA* MUSHROOM SPECIES

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Abstract

In many African countries, edible mushrooms are used as a source of food and income. In Namibia, *Ganoderma* mushrooms are barely utilised. Kalahari truffles and *Termitomyces schimperi* are the common harvested for domestic consumption and/or selling in the informal markets. Though truffles in western markets fetch very high prices, the cost of truffles in Namibia is barely reported. The microbial quality, safety and heavy metal level in the edible Namibian mushrooms is not known. This study investigated the cost of truffles at a select informal market in north-central Namibia and assessed the effect of washing and drying of truffles on the microbial quality, safety, mycotoxin and heavy metal levels. The microbial quality, safety, mycotoxin and heavy metal levels were also determined in the *Ganoderma* and *Termitomyces schimperi* mushrooms collected from the wild. Microbial analyses were carried out using culture techniques. Mycotoxin analysis was carried out using ELISA kits. Heavy metal analysis was done using the ICP-OES. The price of Kalahari truffle ranged from N$ 29 to N$ 71 per kg. Truffles in Namibia are underpriced than in developed nations and has no objective price consistent with a unit mass. Generally, all mushrooms samples had total aerobic counts that are mostly within the acceptable total aerobic count limit of <5.7 log cfu/g. No yeast and *Salmonella* detected in *Ganoderma* samples. The levels of fumonisin B_1 (17.4 to 142.1 µg/kg) in truffles irrespective of washing were within the permissible limits (200-300 µg/kg). The levels of all the analysed mycotoxins in *Termitomyces schimperi* were above the permissible limits. Nickel and mercury were not detected in truffles or *Termitomyces schimperi*. There is a need for appropriate processing to take place before consumption of majority of the studied mushrooms.

List of Publication(s)/Conference(s) proceedings

T.A. Hainghumbi, K. K.M. Nantanga, P.N. Kadhila & L. Iipumbu (2018) *Kalahari truffle: A prized but highly perishable delicacy*. A paper presented at International Conference on Agriculture and Natural Resources (ICANR), University of Namibia, Neudamm campus.

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List of Abbreviations

AACC: American Association for Clinical Chemistry

AOAC: Association of Official Analytical Chemists

ASS: Atomic Absorption Spectrometry

A_w: Water Activity

CAC: Codex Alimentarius Commission

Cfu/g: Colony forming unit per gram

Da: Dalton

DNA: Deoxyribonucleic Acid

EC: European Commission

ELISA: Enzyme-Linked Immunosorbent Assay

FAO: Food and Agriculture Organisation

FDA: Food and Drugs Association

HPLC: High Performance Liquid Chromatography

ICP-MS: Inductively Coupled Plasma-Mass Spectrometry

ICP-OES: Inductively Coupled Plasma-Optical Emission Spectrometry

MPN: Most Probable Number

OD: Optical Density

SD: Standard Deviation

TLC: Thin Layer Chromatography

WHO: World Health Organisation

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Dedication

This thesis is dedicated to my father, Immanuel Shinjaame Hainghumbi. Thank you for your love and support throughout my life.

Declarations

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Tukuna. A. Hainghumbi

Chapter 1: Introduction

1.1. Orientation of the proposed study

In developed nations, edible mushrooms are regarded as a delicacy with high nutritional and functional value (Valverde, Hernández-Pérez, & Paredes-López, 2015). They are also valued for their exceptional sensory attributes including unique aroma and flavour (Villares, Mateo-Vivaracho, & Guillamón, 2012).

A mushroom is a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eye and can be picked by hand (Miles & Chang, 2004).

Mushroom are found in many parts of the world and countries such as China, Japan, Europe, the Middle East such as Saudi Arabia, Israel, Turkey, South America such as in Mexico, Africa such as Malawi, Tanzania (Boa, 2004) and Namibia. Some species have been cultivated and others grow and are collected from the wild (Gry & Andersson, 2014).

Traditionally, some mushrooms have been used for their physiological effects such as anti-cancer, anti-diabetes and mostly as food supplements (Boa, 2004; Loyd et al., 2018) and not consumed for their flavour or taste or nutritional attributes (Boa, 2004). These include *Ganoderma* mushrooms. Other than used as food, mushrooms could be alternative sources of new antimicrobial compounds (Valverde et al., 2015). Wild mushrooms are more often used as bio-resources for identifying antimicrobials than cultivated mushrooms (Shen, Shao, Chen, & Zhou, 2017).

Mushrooms are rich in proteins relative to most vegetables and their amino acid composition can be compared to that of animal proteins (Danell & Eaker, 1992). Mushrooms contain leucine and lysine amino acids which commonly lack in many staple cereal foods (Reid, Munyanyi, & Mduluza, 2017). This study focused on three mushrooms indigenous to Namibia, namely the Kalahari truffle (*Kalaharituber pfeilii*), *Termitomyces schimperi* (*Owowa*) and *Ganoderma* species.

Truffles are fungi that form fruit bodies below the ground (hypogeous) (Danesh, 2015). They are not only harvested for their culinary properties but for their nutritional value as well (Kagan-Zur & Roth-Bejerano, 2008; Shavit & Volk, 2007).

Kalahari Desert truffles are found in the Southern part of Africa in countries like Namibia, Botswana and South Africa (Trappe, Claridge, Arora, & Smit, 2008). Other desert truffles are found in other parts of Africa such as Morocco, Algeria, Tunisia, Libya, Egypt and in the Middle East in countries around the Mediterranean region such as Israel and countries of the Arabian Peninsula such as Jordan, Syria, Saudi Arabia, Iraq, Bahrain, and Kuwait (El Enshasy, Elsayed, Aziz, & Wadaan, 2013).

The prices of truffles can be quite high and vary from season to season in response to supply and demand (Lefevre & Hall, 2000). However, in Africa and in Namibia the cost of underutilized Kalahari truffle is almost nothing compared to commercially marketed truffles in Europe (Trappe et al., 2008). For example, the market price for *Tuber melanosorum* is $1760 per kg (Bonito et al., 2013) as compared to $23-$330 per kg market price for Desert truffles (Morte, Andrino, Honrubia, & Navarro-Ródenas, 2012). The *Ganoderma* industry has been reported to be quite profitable (Loyd, Richter, Jusino, Truong, Smith, Blanchette, & Smith, 2018).

Termitomyces is a genus that comprises edible mushrooms commonly consumed in Africa and Asia among the mushrooms collected from the wild (Hsieh & Ju, 2018). *Termitomyces* mushrooms form symbiotic relationships with termites and are found around the bases of termite mounds (Van Der Westhuizen & Eicker, 1991).

Some mushrooms such as *Ganoderma lucidum* have medicinal properties and their fruiting bodies can be processed into dietary supplements (Miles & Chang, 2004). Among the wild growing mushrooms in Namibia, truffles and *Termitomyces* are the common food mushrooms collected for both home consumption and for sale in the informal local markets or along roadsides for a limited time when they are in their fresh state. Edible fungi are usually cooked and eaten when fresh and less commonly dried in Southern Africa (Boa, 2004). Truffles are often cooked by boiling the cleaned sliced truffles or can be stir-fried (Patel, 2012). *Ganoderma* fruiting bodies can be soaked in boiled water to make *Ganoderma* tea (Ekandjo & Chimwamurombe, 2012). *Termitomyces* mushrooms are usually made into a relish or can be dried for later consumption (Sangvichien & Taylor-Hawksworth, 2001).

1.2. Statement of the problem

Fresh and unprocessed mushrooms contain a moisture content of 83-93% (g/100 g) and a water activity (a_w) of about 0.98 and a neutral pH (Venturini, Reyes, Rivera, Oria, & Blanco, 2011). These characteristics of fresh mushrooms make them an ideal medium for microbial growth. For example, total microbial counts ranging from 4.4 to 9.4 log cfu/g and total coliform bacteria was detected in 23.4% of the mushroom samples of different species collected from formal markets in Spain (Venturini et al. (2011).

Bacterial and unwanted fungal populations in fresh mushrooms can cause quality deterioration and reduce the shelf-life of fresh mushrooms. Some microorganisms indicate the safety of foods and thus it is important to reduce and/or eliminate the presence of certain microbes in foods including in mushrooms.

Another safety aspect of mushrooms is the presence of mycotoxins. These are natural chemical substances produced by toxigenic moulds growing as contaminants on some foods. Mycotoxins tend to affect the immune system, liver, kidneys, and blood. Some mycotoxins were found to be carcinogens (Scott, 1984). Mycotoxins of importance in food include aflatoxins, ochratoxin, and *Fusarium* toxins (Misihairabgwi, Ezekiel, Sulyok, Shephard, & Krska, 2019). Toxigenic moulds have been detected in dried mushrooms (Ezekiel et al., 2013). As far as this project has scanned the literature, there is no data on the microbial quality and safety of mushrooms species indigenous to Namibia.

Moreover, mushrooms contain minerals. These include manganese, iron, copper, zinc, arsenic, cadmium, and lead (Lenntech, 2017). Although micro-minerals such as iron, zinc have tolerable daily intake of 0.8 mg/kg day^{-1} body weight (EFSA, FAO/WHO, 2010) and 0.43 mg/kg day^{-1} body weight (SCF, 2003) respectively (Asomugha et al., 2016), consumption of mushrooms containing high metal concentrations can have life-threatening complications in humans (Lenntech, 2017). Hence the permissible limits of 43 mg/kg for iron (FAO/WHO, 2011) and 60 mg/kg for zinc in food (WHO, 1982).

In a study by Sarikurkcu, Copur, Yildiz, and Akata (2011), heavy metals such as lead, cadmium, iron, manganese, copper, chromium, nickel and cobalt were detected in wild edible fresh and unprocessed mushrooms. The mineral composition of domesticated

Namibian *Ganoderma* mushrooms has been studied by Mhanda, Kadhila-Muandingi, and Ueitele (2015). However, no literature was found that quantified the heavy metals in wild growing *Ganoderma* mushrooms, Kalahari truffle and *Termitomyces schimperi*. Thus, this project investigated the microbial quality and safety, mycotoxins and heavy metals levels in selected wild growing edible mushrooms indigenous to Namibia.

1.3. Objectives

The objectives of this study were to:

a) To determine the prices of Kalahari truffle in select open markets in Namibia.

b) To assess the microbiological quality of Kalahari truffle before and after washing, *Ganoderma* and *Termitomyces schimperi* mushrooms.

c) To quantify the levels of mycotoxins and heavy metals in Kalahari truffle, and *Termitomyces schimperi.*

d) To quantify the levels of mycotoxin in *Ganoderma* mushroom species.

1.4. Hypotheses

The hypotheses of this study were:

a) There is no significant difference in the price of Kalahari truffle sold by different vendors at the select informal markets in Namibia

b) There is no significant difference in the microbiological quality of Kalahari truffle, *Ganoderma* and *Termitomyces schimperi* mushrooms.

c) There is no significant difference in levels of mycotoxins and heavy metals in Kalahari truffle and *Termitomyces schimperi* mushrooms.

d) There is no significant difference in levels of mycotoxins in *Ganoderma* mushroom species.

1.5. Significance of the study

The findings of the study provide baseline information of microbial, fungal, mycotoxins and heavy metals levels in mushrooms which in turn would lay a foundation for creation of public and institutional awareness of microbiological, heavy metals and mycotoxin contamination of wild edible mushrooms in Namibia. The results of this study also provide baseline information for creation of safety standards. The study also contributes knowledge that can lead to improved shelf-life and thus reduce postharvest losses.

1.6. Limitation of the study

Reduced funding for this project due to national budget cuts limited the scope of study to focus on collecting samples from few regions than was originally envisaged.

Chapter 2: Literature review

2.1. Categories of mushrooms

Mushrooms are divided into four categories namely: (1) edible mushrooms; (2) medicinal mushrooms; (3) poisonous mushroom; and (4) miscellaneous mushrooms.

Edible mushrooms are either wild mushrooms or cultivated mushrooms, and which are suitable for use as food after appropriate processing (Gry & Andersson, 2014) such as truffles and *Termitomyces schimperi*. Not all edible mushrooms are used as food though. For example, *Ganoderma* mushrooms are used for their medicinal properties in a form of supplements (Loyd et al., 2018).

Poisonous mushrooms on the other hand are proven to be, or suspected of being poisonous, for example *Amanita phalloides* (Miles & Chang, 2004). The miscellaneous category includes a large number of mushrooms whose properties remain less defined and may be tentatively grouped together as “other mushrooms” (Miles & Chang, 2004). Ecologically, mushrooms can be grouped into three groups namely; mycorrhizal, parasitic and saprophytic (Rasalanavho, Moodley, & Jonnalagadda, 2019). Mycorrhizal symbioses are mutualistic interactions between plant roots and the mycelium of fungi (Wang & Qiu, 2006). For example, truffles form symbiotic root associations with host plant roots taking up and releasing soil nutrients to the host and receiving sugars in return to fuel their growth (Danesh, 2015; Trappe et al., 2009). A saprophytic fungus obtains nutrients from dead organic matter (plant and animal remains). For example, *Ganoderma* mushrooms. Parasitic fungi derive their nutrients from living plants and animals, consequently causing harm to the hosts. For example, *Pleurotus cornucopiae* is known to attack and consume living nematodes (Miles & Chang, 2004).

2.2. Production share of edible mushrooms and truffles

Asia (80.3%) has had the highest production share of mushrooms and truffles by continent, Europe (13.3%), Americas (5.5%), Africa (0.3%) and Australia/Oceania (0.5%) over period of 23 years (1994-2017) as per FAOSTAT (2019). The leading producers of mushrooms are China, USA, Netherlands, Poland, Spain, Canada, France, UK, India and Germany (Table 1).

Table 1: Production of mushrooms: top 10 producers (FAOSTAT, 2019)

Country	**Production average (tonnes) 2016-2017**
China	7 674 518
USA	424 566 5
Netherlands	300 000
Poland	296 656
Spain	153 527.5
Canada	131 706.5
France	100 522.5
UK	99 732.5
India	87 123
Germany	72 797.5

2.3. Processing of edible mushrooms

Mushrooms are highly perishable goods with a shelf life of about 24 hours in their fresh state. This is because mushrooms have high moisture content and rich in nutrients that can lead to fast spoilage (Jaworska & Bernaś, 2009; Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004).

The choice of processing methods for edible mushrooms is generally influenced by preference rather than the impact on nutritional or health benefits (Asamoa, Essel, Agbenorhevi, & Oduro, 2018). Mushrooms can be processed into canned products (Campo, Marco, Oria, Blanco, & Venturini, 2017; Gry & Andersson, 2014), can be dried and used as a flavouring in soups (Shavit & Volk, 2007). Edible mushrooms are commonly cooked by boiling or stir-frying especially when fresh than in a dried state (Boa, 2004).

2.4. Preservation of edible mushrooms

Several physiological and morphological changes occur after harvest cause spoilage of mushrooms hence there is a need for preservation. Sun drying is one of the drying methods used to preserve mushrooms. However it yields unhygienic and poor quality dried products (Bala, Morshed, & Rahman, 2009). On the contrary, drying mushrooms does not cause a significant loss of the nutritional value (Reid et al., 2017). Methods such as freezing, drying, and sterilization are used for long-term preservation of mushrooms (Bernaś, Jaworska, & Kmiecik, 2006).

2.5. Truffles

Truffles are epigeous fungi (form fruiting bodies below ground) (Danesh, 2015). They are mycorrhizal, known to form mutually beneficial associations with the roots of plants (Morte, Zamora, Gutiérrez, & Honrubia, 2009). Truffles are members of the Ascomycota (Giovanni et al., 2015). Desert truffle refers to members of the genera *Terfezia* and *Tirmania* of the *Terfeziaceae*, order *Pezizales* which thrives in arid and semi-arid areas (Shavit & Volk, 2007). It also includes *Kalaharituber pfeilii*, which was until recently regarded as a *Terfezia pfeilii* (Trappe et al., 2008).

Desert truffles (about 20% dry matter) reportedly have a protein content in the range of 20-27% of which 85% is digestible by humans; 3-7.5% fat content (saturated and unsaturated); 60% carbohydrate, 2-5% ascorbic acid; and 7-13% crude fiber (Murcia et al., 2003).

2.5.1. Economic importance of truffles

Wild-harvested and cultivated truffles are reportedly sought after and some species command remarkable prices (Berch, 2013). For example, Hall, Brown, and Zambonelli (2007) quoted a retail price for Italian white truffle (*Tuber magnatum)* up to €6,000 per kilogram and €3,000 for Perigord black truffle (*Tuber melanosporum).* Bonito et al. (2013) indicated that the market price in USA in 2009 ranged from $110 USD for *Tuber indicum* to $ 5060 for *Tuber magnatum.* The market price per kg for Desert truffles has been reported to be $26 to $33 (Table 2). Table 2 shows economically important truffle species determined by market prices (USD).

Table 2: Economically important truffle species determined by market prices (USD) adapted from (Berch, 2013)

Species	**Market price per kg**	**Source**
Tuber indicum	$ 110	Asia
Tuber canaliculatum	$220	North America
Tuber gibbosum	$220	North America
Tuber lyonii	$220	North America
Tuber oregonense	$220	North America
Tuber borchii	$440	Europe
Tuber brumale	$660	Europe
Tuber macrosporum	$660	Europe
Tuber melanosporum	$1760	Europe
Uber magnatum	$5060	Europe

Desert truffles	$26-$330	Europe, Africa

2.5.2. Health benefits of truffles

Traditionally, truffles have been used as medicine for various ailments. Dried powder of truffles has been used for stomach ailments and open cuts whereas the juice has been used for eye infection treatment (Shavit & Volk, 2007). Truffles are an unlimited source of therapeutic compounds with anti-inflammatory, immunosuppressor, antimutagenic and anticarcinogenic properties, antioxidant properties, antimicrobial and steroidal glucoside with polyhydroxy ergosterol nucleus (Bradai, Neffar, Amrani, Bissati, & Chenchouni, 2015). Moreover, antibacterial substances have been isolated from the juice of a variety of desert truffles. For example, a potential peptide antibiotic has been isolated from the juice of *Terfezia claveryi*, which effectively inhibited the growth (in vitro) of *Staphylococcus aureus* by 66.4% (Shavit & Volk, 2007).

2.5.3. Ecosystem functions of truffle Fungi

Truffle fungi are key players in numerous forest ecosystem processes. They perform critical soil processes that sustain ecosystem productivity. Above all, they fill in as key biological links that connect multifaceted food webs between subterranean and over-the-ground living organisms (Trappe et al., 2009).

Fungal hyphae release polysaccharides (sugars) that stabilize the soil. In this procedure, they form the fabric of soil by creating and increasing soil aggregation and soil micropores, consequently enabling soil aeration and water movement which is vital for myriad oxygen requiring soil organisms and plants (Claridge, Trappe, & Hansen, 2009). Truffles form symbiotic root associations (ectomycorrhizae) with host plant roots. They

supply host plants with nutrients and receive sugars in return (Danesh, 2015; Trappe et al., 2009). Fungi are also actively involved in forest food webs and biodiversity pathways. For example, insects and mites feed on fungal mycelium (Trappe et al., 2009).

2.5.4. Processing of truffles

Truffles are used as food and are eaten raw after washing and peeling (Rivera, Blanco, Oria & Venturini, 2010)**.** They are also commonly cooked by boiling or roasted over the fire or buried in hot ashes (Leistner, 1967; Story, 1958). Peeling of truffles has been reported to significantly reduce the protein, fat, ash, ascorbic acid and mineral elements especially calcium and iron (Pegler, 2002).

2.5.5. Preserving truffles

Due to the seasonality of truffles, several storage methods are used to ensure their availability throughout the year (Culleré, Ferreira, Venturini, Marco, & Blanco, 2013).

The preservation technique to be used should be able to retain the nutritional composition of the truffles and should be able to reduce the microbial population (Ntshakaza, 2013). Canning (Murcia, López-Ayerra, & García-Carmona, 1999) ,drying and freezing (Al-Ruqaie, 2006) are some of the techniques used to preserve truffles.

2.5.5.1. Drying

This method involves removal of water from food products, consequently inhibiting the growth of microorganisms. Since bacteria, yeasts and moulds require water in foodstuffs to grow, effective drying prevents microorganisms from surviving in the food (Murcia et al., 2014). Sun drying of mushrooms is one of the cheapest preservation methods.

However, sun dried products are generally unhygienic and of poor quality (Bala et al., 2009).

2.5.5.2. Canning

Canning is one of the common postharvest preservation technique used to maintain the quality of foodstuff (Murcia et al., 2014). During canning, truffles are put into jars filled with hot (typically 85°C) filling medium (e.g. 20 g NaCl per litre of water). The jars are then closed and finally heated at 121 °C for 30 minutes before cooling them in water (Murcia et al., 1999). The disadvantage of canning is that it may cause softening of the texture, aroma changes (Campo et al., 2017) and antioxidant losses (Murcia et al., 1999).

2.5.5.3. Low temperature storage

Freezing involves the transformation of the liquid water in food into ice at -18°C or lower (Murcia et al., 2014). Low temperatures inhibit the growth of microorganisms and retard the kinetics of chemical and enzymatic reactions (Murcia et al., 2014). Low temperature storage can be divided into refrigeration (4°C) and freezing (-20°C) (Saltarelli, Ceccaroli, Cesari, Barbieri, & Stocchi, 2008). Storage at 4°C may not be suitable for all truffles. For example the microbial population of fresh *Tuber borchii* (white truffles) increased within 4-8 days and reaching a quiescent of about 10^{10} cfu/g after 15 days of storage at 4°C (Saltarelli et al., 2008).

2.6. Kalahari Desert truffles

Kalahari desert truffle (*Kalaharituber pfeilli*) formerly known as (*Terfezia pfeilli)* is an edible mycorrhizal fungus (Figure 1) found in the Southern part of Africa in countries like Namibia, Botswana and South Africa (Taylor, Thamage, Baker, Roth-Bejerano, &

Kagan-Zur, 1995). Kalahari truffles inhabit sandy soil, require less water as well as host plants to complete their life cycles (Taylor et al., 1995). In northern Namibia, Kalahari truffles are found towards the end of June. In the central and northern Kalahari desert, the fruiting of desert truffles is from April to July (Story, 1958).

2.6.1. Structure/ Morphology

The fruiting bodies of desert truffles found have round or irregular shape (Figure 1) and resemble small sandy potatoes (Shavit & Volk, 2007; Trappe et al., 2008). However, truffles are usually covered with sand and this masks their true colour. All desert truffles are found to possess plenty of large, inflated, thin-walled cells in the peridium and gleba (Trappe et al., 2008) (Figure 2). Kalahari truffle exhibits a pale brown outer peridium and interior whitish pockets (Trappe et al., 2008).

Figure 1: Fruiting bodies of Kalahari tuber

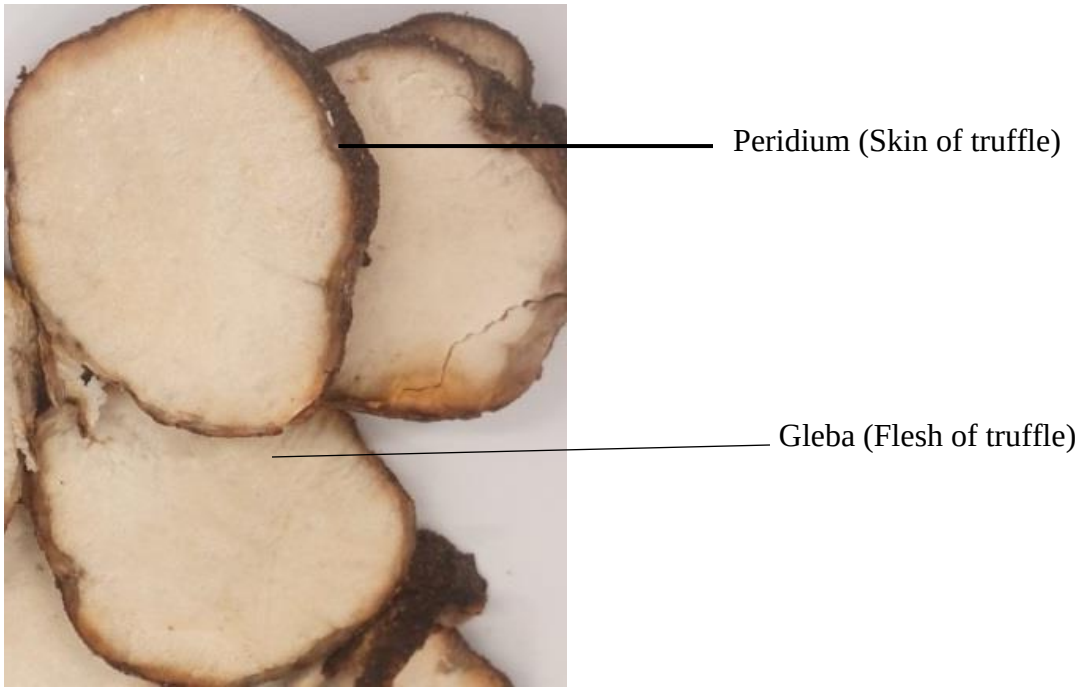

Figure 2: Peridium and gleba of Kalahari truffle tuber

2.6.2. Ecology and life cycle of Kalahari Desert truffles

The life cycle of truffles has three phases. The first phase is growth as a filamentous mycelium; second phase is symbiotic association of the fungal hyphae with the host root (ectomycorrhiza) and the final phase is the organization of a hypogeous fruit body or ascocarp (Saltarelli et al., 2008).

Similarly to Australian truffles, plants associated with Kalahari desert truffles are diverse and comprise both herbaceous and woody species (Trappe et al., 2008). Desert truffles could be considered as transitional between true ectomycorrhizal and true endo-mycorrhizal (Kagan-Zur & Roth-Bejerano, 2008).

In the northern part of Namibia, Kalahari truffle is found in cultivated fields of pearl millet, watermelons, and sorghum (Trappe et al., 2008). Association with shrubs of

Acacia hebeclada has also been reported (Trappe, Claridge, & Kagan-Zur, 2014). On the other hand, several investigations highlighted the positive, negative and neutral effects of soil microorganisms on mycorrhizal formation hence there is a proposed tripartite association between the plant, mycorrhizal fungus and those bacteria that exhibit positive effects on the association (Adeleke & Dames, 2014).

Desert animals such as foxes and meerkats have been observed to eat Kalahari Desert truffles. It is then assumed that these animals disperse the spores (Dighton & White, 2005). On the other hand, spore dispersal of truffles by wind has been reported by (Trappe et al., 2008).

2.6.3. Factors that affect Kalahari truffle growth

2.6.3.1. Soil/substrate

Kalahari truffles are solely associated with calcareous sands but can also be found in arenosol within the top 5-20 cm of the soil (Trappe et al., 2008). In southern Kalahari, truffles require fairly compact to compact, pink or infrequently white, slightly calcareous sands (Trappe et al., 2008). The pH of the soil in which Kalahari truffles grow ranges from 5.5-6.5 but can go up to 7.2 (Trappe et al., 2008).

2.6.3.2. Rainfall

Fruiting of truffles requires adequate and well-distributed rainfall. In Kuwait, *Terfezia* and *Tirmania* species fruit in years with a well-distributed rainfall (from October through March) and minimum rainfall of about 180 millimeters. As for the southern African and other hemisphere species, there is no similar data published but tradition and experience confirm the need for water to initiate fruiting (Trappe et al. 2008).

Sudden rainfall after a dry period is perceived to be one of the most important factors in the appearance of truffles (Trappe et al., 2014).

2.6.3.3. Fire

Fire in the Kalahari Desert is caused by both human activities as well as by lightning strikes. The effects of fire on truffle production in the Kalahari Desert depend on how fire affects the truffle host plants, especially the sensitive ones (Trappe, et al., 2014). Nevertheless, the entire topic of fire impacts on truffle production needs to be explored more carefully (Trappe, et al., 2014).

2.6.3.4. Overgrazing

Overgrazing is detrimental to soils, water, and plants. The increase of cattle population impacts on native wildlife, and concentrated grazing opens the landscape to evasion of undesirable exotic plants and damages truffle grounds (Trappe et al., 2014).

2.6.3.5 Climate change

Climatic factors are among the most important drivers of desert truffle occurrence (Kagan-Zur & Roth-Bejerano, 2008). Climate change may affect Kalahari truffle-producing areas. High temperatures and high evaporation may increase aridity. Increased erodibility and wind energy could bury significant areas of truffle production (Trappe et al., 2014).

2.6.4. Locating Kalahari Desert truffle

To collect desert truffles, one does not have to rely on trained dogs or pigs as is the case with European forest truffles. Fruit bodies develop close to the soil surface. As they

swell, they manifest by lifting up the soil to form cracked little mounds recognisable to the trained eye (Kagan-Zur & Roth-Bejerano, 2008).

2.6.5. Cultivation of desert truffles

Cultivation of desert truffles is not trivial, and for decades was not a priority because desert truffles are regarded as inferior to the much-praised forest truffles (Kagan-Zur & Roth-Bejerano, 2008). However, desert truffles are becoming more popular and there is research on their cultivation (Kagan-Zur & Roth-Bejerano, 2008). Other truffle species have been cultivated. For example, *Terferzia claveryi* has been cultivated in symbiosis with *Helianthemum almeriense* (Kagan-Zur & Roth-Bejerano, 2008).

2.7. *Ganoderma* mushrooms

This is a large and diverse genus of wood decaying fungi (Figure 3) (Baby, Johnson, & Govindan, 2015; Loyd et al., 2018) with hard fruiting bodies (Baby et al., 2015) belonging to the division of Basidiomycota, a Homobasidiomycetes class, order: Aphyllophorales, family: Polyporaceae (*Ganodermataceae*) and genus of *Ganoderma* (Richter, Wittstein, Kirk, & Stadler, 2015). Over 300 species within genus *Ganoderma* have been reported in several taxonomic studies, and they are widely distributed in tropical and temperate regions (Richter et al., 2015) such as North and South America, Europe, Africa and Asia, growing either as a parasite or saprotroph on a wide range of trees (Moncalvo, Wang, & Hseu, 1995).

Ganoderma is consumed as a health tonic and as a dietary supplement (Chang & Mshigeni, 2001). The medicinal properties of *Ganoderma* include antitumor, immunomodulatory, prevention and treatment of cardiovascular diseases, antihepatotoxic, and antinociceptive effects among others (Miles & Chang, 2004).

Ganoderma mushrooms are used as dried whole, powder or can be used in a form of capsules or tablets (Oei, 2003). Among medicinal mushrooms, *Ganoderma lucidum* has been regarded to be of superior grade (Miles & Chang, 2004).

The Namibian wild *Ganoderma* has been reported to grow on a wide range of tree species such as *Acacia sieberana, Acacia spp., Baikiaea plurijuga, Colophospermum mopane, Combretum collinum, Combretum frarans, Combretum zeyheri Croton gratissimus, Grewia retinervis, Mundulea sericea, Sclerocarya birrea, Terminalia prunioides, Terminalia sericea,*(Kadhila-Muandingi, 2010).

In Namibia, *Ganoderma* species are not widely used and are not sold. If anything, they are collected from the wild. Local people in the north-eastern parts of Namibia use *Ganoderma* mushrooms for three medicinal purposes namely; to halt nose bleeding, boost immunity and strengthen infant bones (Ekandjo & Chimwamurombe, 2012). *Ganoderma* can be prepared in different ways before use as medicine. *Ganoderma* is usually burned on a clean surface and the smoke will be inhaled by a nose bleeding individual to prevent nose bleeding. *Ganoderma* cap can also be soaked in boiled water until the water has turned reddish to extract a remedy known as *Ganoderma* tea which can be taken by pregnant women as an immune booster for both mother and child. Lastly, *Ganoderma* can be ground to powder which is then rubbed on infants' forehead to strengthen the skull bones (Ekandjo & Chimwamurombe, 2012).

Figure 3: Fruiting body of *Ganoderma lucidum* (Kadhila-Muandingi, 2010)

2.7.1. Nutritional composition of *Ganoderma* mushrooms

Based on the qualitative and quantitative analysis of *Ganoderma lucidum* harvested from Vom in Nigeria, *G. lucidum* mushrooms contained high amounts of crude fibre (34.7%), protein (13.3%), fat (2.6%) and some bioactive compounds like saponins and resins (Ogbe, 2008). On the other hand, Taofiq et al. (2017) found dried *G. lucidum* fruiting bodies to contain lipids (2.50 g/100g), proteins (6.27 g/100g), ash (2.4 g/100g), available carbohydrate (38.0 g/100g) and (88.0 g/100g) total carbohydrates.

Mhanda et al. (2015) reported 1.9 (g/100g) fats and oils, 18.2 (g/100g) crude protein, 2.6 (g/100g) ash, 2.6 (g/100g) carbohydrates and 45 (g/100 g) crude fibre in the Namibian

cultivated *Ganoderma* species The chemical compounds and nutrients of *Ganoderma* mushrooms play a role in nutrition, maintaining good health and physiological functions of the body (Ogbe et al., 2009).

2.7.2. Applications of *Ganoderma lucidum* antimicrobials

Gram positive bacteria are reportedly more susceptible to antimicrobial activities of *Ganoderma lucidum* as compared to Gram negative bacteria (Kamble, Venkata, & Gupte, 2011; Shen et al., 2017). Fifteen types of Gram-positive and Gram-negative bacteria have been reported to have been inhibited by the aqueous extract from the carpophores of *G. lucidum* (Yoon, Eo, Kim, Lee, & Han, 1994).

2.8. *Termitomyces* mushrooms

The genus *Termitomyces* comprises edible mushrooms commonly consumed in Africa and Asia among the mushrooms collected from the wild (Hsieh & Ju, 2018). In some countries, *Termitomyces* mushrooms are made into relish that is eaten with maize meal porridge (Sangvichien & Taylor-Hawksworth, 2001). They can also be preserved by drying (Sangvichien & Taylor-Hawksworth, 2001).

2.8.1. *Termitomyces* and termites interaction

Termitomyces mushrooms live in a mutualistic symbiosis with termites (Tibuhwa, 2012) in the termite nests, where they produce different enzymes to help termites digest lignocellulosic substrates (Hsieh & Ju, 2018). *Termitomyces* are fully dependent on the termites and are never found free-living (Darlington, 1994). Several experiments have shown that termites are unable to survive without the *Termitomyces* fungus (Aanen et al., 2002; Rouland-Lefèvre, 2000; Sands, 1956).

2.8.2. The socio-economic importance of *Termitomyces* mushrooms

Termitomyces mushrooms are regarded as a delicacy collected for both home consumption and for sale in local markets or along roadsides as a source of income (Sangvichien & Taylor-Hawksworth, 2001). In Ghana, a 1 kg bundle of *Termitomyces schimperi* can cost about US$1.2-US$2 (Apetorgbor, Apetorgbor, & Nutakor, 2005). In Namibia, larger specimens of *Termitomyces* can go for US$2 (N$30) (Rothman, 2018)

Apart from their distinctive flavour and quite firm texture (Sangvichien & Taylor-Hawksworth, 2001), *Termitomyces* mushrooms serve as important source of nutrients including vitamins and minerals in areas where staple foods with low nutrition values are consumed (Hsieh & Ju, 2018). They contain about 26-36% protein and 10-18 mg/g ascorbic acid (Hsieh & Ju, 2018).

2.8.3. Nutritional composition of *Termitomyces* mushrooms

Table 3 shows the nutritional composition of some *Termitomyces* species studied by Kansci, Mossebo, Selatsa, and Fotso (2003). The lipids content is found to be variable among the species and it averaged 3.51 g/100g dry weight. The protein content of *Termitomyces* species was high and averaged 16.73 g/100g (dry weight) in all the species studied. However, this protein content is relatively low compared to other mushrooms such as *Pleurotus* (20 g/100 g dry weight) and cultivated *Agaricus* (30 g/100 g dry weight). Carbohydrate contents varied from 43.7 g/100 g dry weight in *Termitomyces le.testui* to 57.4 g/100 in *Termitomyces schimperi* (Kansci et al., 2003).

Table 3: Nutrient content (g/100 g of dry weight) of some mushroom species of the genus *Termitomyces* (Kansci et al., 2003)

Species	**Lipids**	**Ash**	**Protein**	**Crude fibre**	**Carbohydrates**
T. subclypeatus forme bisporus sp. nov.	2.50	6.30	17.84	18.98	54.38
T. le Testui	5.14	8.45	19.13	23.13	43.65
T. aurantiacus	2.70	9.56	16.62	24.68	46.44
T. mboudaena sp. nov	2.63	10.68	17.26	24.10	45.33
T. schimperi	2.64	5.17	14.48	20.29	57.42
T. mammiformis	5.42	14.39	15.07	17.56	47.56
Average	3.51	9.03	16.73	21.46	49.13

2.8.4. *Termitomyces* of Namibia

Only one species known as *Termitomyces schimperi* (*omajowa*) is commonly consumed in Namibia (Rothman, 2018). This mushroom species grows in groups around the bases of tall termite mounds (Van Der Westhuizen & Eicker, 1991). Other three edible *Termitomyces* species (*Termitomyces umkowaani, Termitomyces microcarpus, and Termitomyces tyleranus)* have been reported in Namibia (Rothman, 2018). Figure 4 shows *Termitomyces* mushrooms growing around the base of a termite mound (Rothman, 2018).

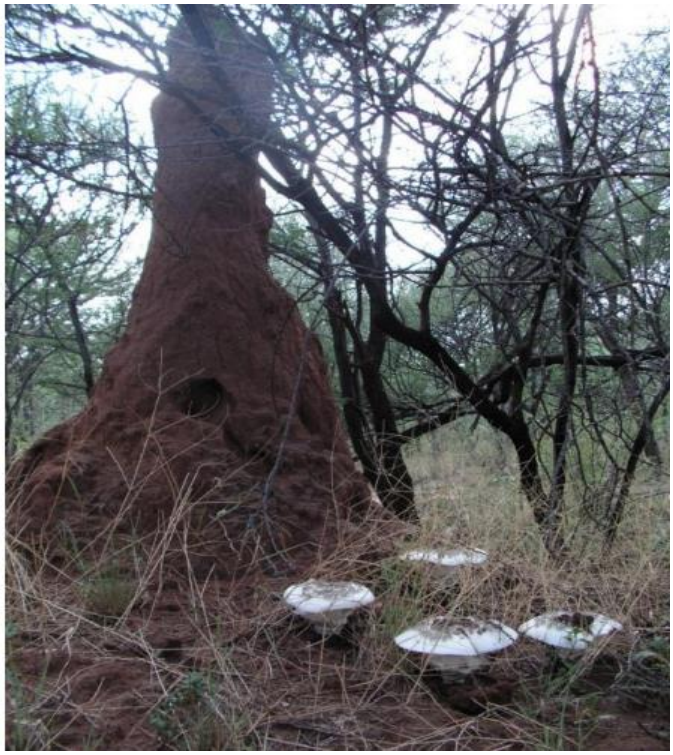

Figure 4: *Termitomyces* mushrooms (Rothman, 2018)

2.8.4.1. Morphology and occurrence

On emergence, the fruiting bodies of *Termitomyces* are the "size of a man's fist" and expand to 15-28 cm and the diameter may reach 40 cm (Van Der Westhuizen & Eicker, 1991). The fruiting bodies (caps) are originally white but the soil of the mound usually stain the thick, soft scales yellow to reddish brown (Van Der Westhuizen & Eicker, 1991). The sporocarps (fruit bodies) appear in groups of up to 50 around one mound. They tend to appear after good soaking rains of 12 mm or more in spring however, development begins in January throughout March (Van Der Westhuizen & Eicker, 1991).

2.9. Contamination and safety of Mushrooms

Fresh and unprocessed mushrooms are likely to convey food-borne bacteria resulting from various sources. Contamination of cultivated mushrooms with pathogenic bacteria may take place during all growth, harvest and sorting stages as well as the substrate where they grow (Venturini et al., 2011) or from irrigation water (Gry & Andersson, 2014). Wild mushrooms have a high possibility to become contaminated with foodborne bacterial pathogens compared to cultivated varieties. This could be attributed to the fact that wild mushrooms are hand-harvested from the forest and woodlands ecosystems which are silvipastoral systems on which livestock and wild animals usually graze (Venturini et al., 2011). Soil is generally a potential contaminator for mushrooms.

Moreover, mushrooms could also be attacked by infecting microfungi which may consequently produce some toxic compounds (Gry & Andersson, 2014).

Kim et al. (2016) detected aerobic counts of 4.2 log cfu/g, coliforms (0.5 log cfu/g) and yeast and mouls (2.2 log cfu/g) in whole dried Shiitake mushrooms. Furthermore, contamination of mushrooms with *Salmonella* has been reported (Samadpour et al., 2006).

2.9.1. Microbial contamination of truffles

Truffles grow in the soil and this increases the likelihood of truffle contamination with soil microbial communities (Rivera et al., 2010) including human pathogens (Rivera, Blanco, Oria, & Venturini, 2007). In a study by Rivera Blanco, Oria & Venturini, (2010), an average total microbial count of 8.3 log cfu/g and 8.4 log cfu/g in whole ascocarps of *Terfezia. aestivum* truffles and *T. melanosporum,* respectively. Both species investigated were forced air dried.

Rivera, Venturini, Oria, and Blanco (2011) reported an average of 6.1 log cfu/g of *Enterobacteriaceae* (includes coliforms) in fresh summer truffles. *Enterobacteriaceae* family in dried *T. aestivum* and *T. melanosporum* had average counts of 6.3 and 5.1 log cfu/g in a study by Rivera et al., (2010). Average yeast and moulds counts in fresh summer truffles did not exceed 4.5 log cfu/g (Rivera et al., 2011). In dried truffles (*T. aestivum* and *T. melanosporum),* reported mould counts in the range of 2.0 to 5.4 log cfu/g. Yeast population obtained an average of 3.4 log cfu/g for both truffle species.

2.10. Decontamination of truffles

Since truffles are found to have high initial microbial load including the presence of pathogenic microorganisms, this resulted in the need to employ early decontamination procedures that reduce this initial microbial load. Truffle surface decontamination methods can be physical and/or chemical (Rivera et al., 2007).

Physical decontamination methods include irradiation with ultraviolet light and application of sonication whereas chemical decontamination includes washing using ethanol, hydrogen peroxide and sodium hypochlorite (Rivera et al., 2007).

2.11. Coliforms

Coliforms are a group of bacteria that are part of *Enterobacteriaceae* (Jay, 2000). They are rod-shaped gram-negative non-spore forming bacteria found in soil, vegetation and aquatic environment as well as in the faeces of warm-blooded animals (Sivaraman, 2018). The presence or detection of coliforms is used as an indicator of sanitary quality of foods and water (Feng et al., 2002; Sivaraman, 2018). Moreover, total coliforms can also be used as an indicator for the possible presence of pathogens in foods (Noble, Moore, Leecaster, McGee, & Weisberg, 2003).

2.12. Yeast

Yeasts are unicellular eukaryotic fungal microorganisms. Yeast species found in foods include the fermentative *Saccharomyces cerevisiae*. Yeast that grow and unfavourably impact foods where they are not usually associated are referred as contaminant yeasts (Howell, 2016). Yeast contamination of 5-6 log cfu/g counts is perceived to cause food spoilage. Turbidity, swelling, slime formation, discolouration, and off-flavours are the main effects of yeast growth (Hernández et al., 2018).

Yeasts pathogenic to humans such as *Candida albicans* and *Cryptococcus neoformans* are often not associated with food and beverage ecosystems. Nevertheless, evidence that the presence of yeasts in foods can lead to human allergic and hypersensitive reactions such as asthma, migraines, respiratory problems, and gut and bowel syndromes exists (Howell, 2016).

2.13. Moulds

In nature, moulds are ubiquitous (Pitt, 2000) and grow almost anywhere, indoors and outdoors (Matumba et al., 2016). Various fungal species play a significant role in food spoilage and can produce mycotoxins during distinct development phases (Matumba et al., 2016; Pitt, 2000). There are three genera dominating the natural fungal flora existing in conjunction with food production namely; *Aspergillus, Fusarium* and *Pencillium* (Pitt, 2000). It is generally suspected that in most instances, the existence of moulds on foodstuff shows a high likelihood of at least one toxin being present (Matumba et al., 2016).

2.14. *Salmonella*

Salmonella is a gram-negative bacterium mainly found in the intestinal tract of animals and can thrive in various foods due to its simple nutritional requirements and ability to grow under both aerobic and anaerobic conditions (Jones, 1992). It can grow in foods with a pH of between 4 and 9 at temperatures ranging between 8 °C and 45 °C and water activity above 0.94 (Silva & Gibbs, 2012). Most *Salmonella* strains are heat sensitive and drying or freezing does not eliminate all of them (Jones, 1992).

2.15. Microbiological legislation of mushrooms

Based on European Union Commission Recommendation (directive 2004/24/EC), acceptable limits for total aerobic count, total coliforms, yeast and moulds are $<5 \times 10^5$ cfu/g (< 5.7 log cfu/g), < 1 cfu/g, <3.7 log cfu/g, ($< 5 \times 10^3$ cfu/g) and <3.7 log cfu/g, ($< 5 \times 10^3$ cfu/g), respectively (Ajis, Chong, Tan, & Chai, 2017). Food Safety Authority of Ireland (2006) regulations states that *Salmonella* should not be present in 25 g portions of raw mushrooms.

There does not seem to be established limits of microbiological quality and safety criteria specific for truffles yet. However, the current legislation based on Economic Comission for Europe (2006) makes some recommendations namely; 1) the preparation and packing of truffles pieces must comply with the rules of hygiene for food products, 2) truffles should be marketed immediately after harvest and, 3) truffles must be cleaned to remove excess soil. It is important to note that these recommendations do not necessarily cater for the adequate removal of pathogenic organisms from the truffles (Rivera, Blanco, Oria & Venturini, 2010)

2.16. Mycotoxins of foods

Mycotoxins are natural chemical substances produced by fungi growing as contaminants on some foods (Ezekiel et al., 2013; Scott, 1984). The contamination of food by mycotoxins can occur before production, during storage, processing, transportation or marketing of the food products (Darwish, Ikenaka, Nakayama, & Ishizuka, 2014). Mycotoxins of concern to human and animal health are produced by the *Aspergillus*, *Penicillium* and *Fusarium* genera (Reddy, Reddy, & Muralidharan, 2009). However, the presence of moulds in commodities does not necessarily show the occurrence of mycotoxins since moulds could be present in a commodity without producing toxins (Ashiq, 2015). The most important mycotoxins found in foods are aflatoxins, ochratoxin A, patulin,deoxynivalenol, zearalenone, and fumonisins (Ezekiel et al., 2013).

2.16.1. Prevalence of mycotoxins in Africa

Aflatoxins, fumonisins, ochratoxins, trichothecenes and zearalenones are the major mycotoxins of significance in terms of health and economy from an African continent perspective. The occurrence of these mycotoxins in major food and feed may be aggravated by favorable climatic conditions in the continent (Gbashi et al., 2018).

Climate change, poverty, limited/lack of awareness, pro-regulation and legislation, poor agricultural practices, amongst others are the major factors contributing to the prevalence of mycotoxins in African food and feed commodities (Gbashi et al., 2018).

2.16.2. Mycotoxins structures

Structures of mycotoxins range from single heterocyclic rings with molecular weights of about 50 Da to groups of irregularly arranged 6 or 8 membered rings with molecular weight over 500 Da. These relatively small molecules induce no response in the human

immune system, consequently a major potential danger of mycotoxins in the human diet resides in the inability of human bodies to detect them biologically (Pitt, 2000).

2.16.2.1. Deoxynivalenol

This mycotoxin belongs to a very large group of chemically related mycotoxins produced by *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium sporotrichioides* (Reddy, Salleh, Saad, Abbas & Shier, 2010). Deoxynivalenol (Figure 5) is known to have adverse effects such as vomiting both in animals and humans (European Food Safety Authority, 2013).

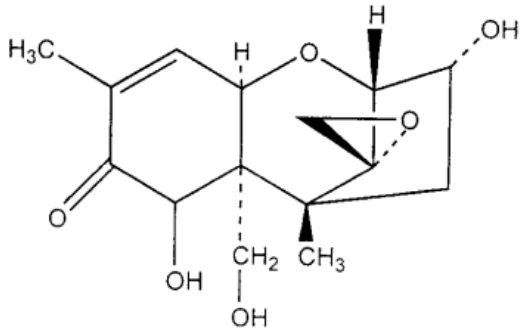

Figure 5: Deoxynivalenol structure (Bennett & Klich, 2003)

2.16.2. Fumonisins

These are heat-stable, water-soluble mycotoxins synthesised by *Fusarium verticillioides* and *F. proliferatum.* The structures of fumonisins consist of a 20 carbon aliphatic chain with two ester linked hydrophilic side chains (Figure 6). These chains resemble an essential phospholipid in cell membranes called sphingosine. It appears that the toxic action of fumonisins results from competition with sphingosine in sphingolipid metabolism (Pitt, 2000). Fumonisins are often found to co-occur with other mycotoxins

(mostly aflatoxin and trichothecenes, especially deoxynivalenol). The toxicity of this group is associated with fumonisin B_1 and B_2 (Mostrom, 2016).

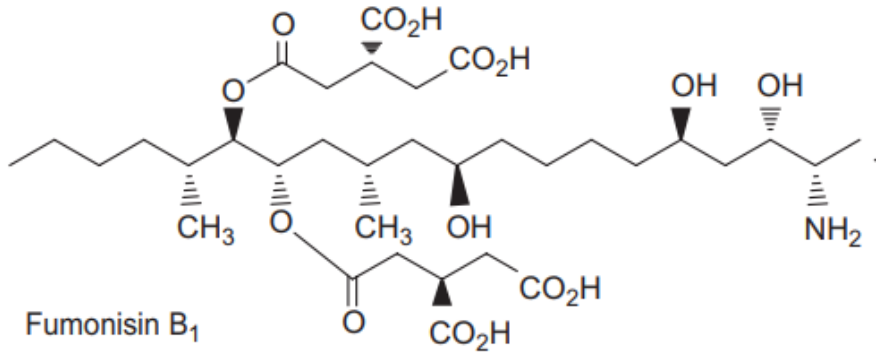

Figure 6: Structure of fumonisin B_1 (Reddy et al., 2010)

2.16.3. Ochratoxin A

Ochratoxin A is fat soluble (Pitt, 2000) secondary metabolites produced by *Aspergillus* sp. and *Penicillium* sp. (Bennett & Klich, 2003; Selvaraj et al., 2015). The ochratoxin family comprises ochratoxin A, B and C. However, ochratoxin A is of great health concern to humans and animals owing to immunosuppressive, nephrotoxic, carcinogenic and teratogenic effects (Bennett & Klich, 2003; Selvaraj et al., 2015). Ochratoxin A is light sensitive and is also efficiently oxidised by atmospheric oxygen (Shugalei, Ilyushin, Sudarikov, & Kapitonenko, 2014) Figure 7 shows the structure of Ochratoxin A.

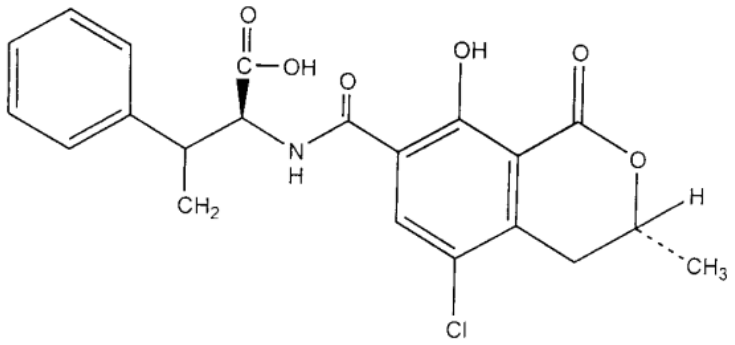

Figure 7: Structure of Ochratoxin A (Bennett & Klich, 2003)

2.16.4. Aflatoxins

These are difuranoccoumarin derivatives produced by a polyketide pathway by numerous strains of *Aspergillus flavus* and *A. parasiticus* (Klich, Mullaney, Daly, & Cary, 2000). Although aflatoxins comprise about 20 related fungal metabolites, only aflatoxins B_1, B_2, G_1 and G_2 that are common in food and feed (Coffey & Cummins, 2008). Aflatoxins have carcinogenic, mutagenic, teratogenic and immunosuppressive effects on humans and animals thus they are of great concern (Zinedine & Mañes, 2009).

Some of the factors that influence aflatoxin production comprise of drought stress and rainfall, crop genotype adaptation to climate conditon, insect damage, and agricultural practices (Montagna, De Giglio, & Napoli, 2012) Aflatoxins can be produced during storage, transportation, and food processing (Montagna et al., 2012).

Teratogenic and immunosuppressive effects of aflatoxins are exerted through the reaction with the nucleophilic sites in DNA, RNA, and proteins (Shugalei et al., 2014). In southern Africa, afllatoxin contamination has been strongly linked with child undernutrition, increased mortality and morbidity (Katerere, Shephard, & Faber, 2008). This is due to the negative effects of aflatoxins on micronutrient absorption and immune

function (Gbashi et al., 2018). The structures of aflatoxins G_1 and G_2 are shown in Figure 8.

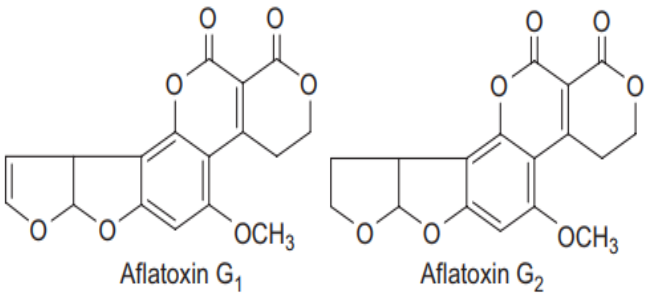

Figure 8: Chemical structures of Aflatoxins G_1 and G_2 (Reddy et al., 2010)

2.16.5. Zearalenone

This is a nonsteroidal, phenolic resorcyclic acid lactone estrogenic toxin (Pico´, 2016) produced by *Fusarium graminearum* and related species. It was formerly known as F-2 (Mostrom, 2016). Zearalenone exhibit interactions with estradiol-binding receptors in target cells (Shugalei et al., 2014). Zearalenone is water and aqueous alkali soluble. The solubility of zearalenone in hexane is slight whereas it is progressively more in acetone, acetonitrile, benzene, chloride, ethanol, methanol and methylene (Picó, 2016). Zearalenone usually co-occurs with one or more of the trichothenes due to the ability of its producing fungi to synthesise more than one mycotoxin (Gbashi et al., 2018). Figure 9 shows the structure of Zearalenone.

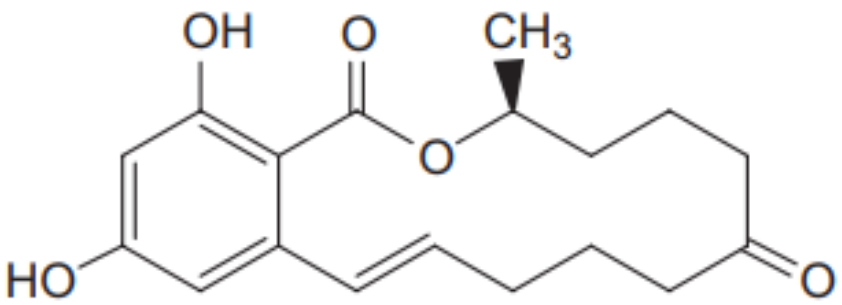

Figure 9: Structure of zearalenone (Reddy et al., 2009)

2.17. Factors affecting mycotoxin production

Temperature and humidity are the most significant environmental factors that affect fungal growth and mycotoxin production (Patriarca, & Pinto, 2017). Consequently, mycotoxin build up both before and after harvest generally reflects climatic conditions. The factors affecting mycotoxin production can be divided into biological, physical and chemical factors. Crops in tropical and subtropical areas where humidity and temperature are high are prone to mycotoxin contamination (Patriarca & Pinto, 2017).

2.17.1. Biological factors

There is little known about the biological factors affecting mycotoxin production in mushrooms but it may be similar to what is known for cereals. In terms of cereals, there is a likelihood of seeds colonisation by different fungal species in the field or at harvest. Subsequently, fungal interaction may influence mycotoxin production. A direct correlation between colonisation with A. *flavus* and aflatoxin B_1 contamination of maize kernels has been reported (D'Mello & Macdonald, 1997).

2.17.2. Physical factors

Interacting factors such as temperature, humidity (D'Mello & Macdonald, 1997; Patriarca, & Pinto, 2017), the extent of insect infestation and time could have an impact on mycotoxin production in the field and during storage (D'Mello & Macdonald, 1997). This could apply to mushrooms before harvest and during storage.

2.17.3. Chemical factors

The use of fungicides to control fungal diseases in crops may have an effect on mycotoxin production. Sub-lethal concentrations of fungicides may enhance mycotoxin production. There is also a possibility of synergism between physical and chemical factors in relation to mycotoxin production (D'Mello & Macdonald, 1997). Fungicides may contaminate area in which mushrooms grow and this might lead to mycotoxin production. Different mycotoxins grow best at different temperatures and water activity are shown in Table 4.

Table 4: Optimal environmental conditions for mycotoxin production (Patriarca & Pinto, 2017).

Mycotoxin	**Temperature (ºC)**	**Water activity (A_w)**
Ochratoxin A	25-37	0.94-0.98
Aflatoxions	30-33	0.99
Fumonisins	15-30	0.9-0.995
Zearalenone	25	0.96
Deoxynivalenol	26-30	0.995

2.18. Effects of mycotoxins on human health

Mycotoxins have a negative effect on the immune system, liver, kidneys and blood while some have carcinogenic effects (Scott, 2004). Diseases caused by the growth of fungi on animal hosts are collectively known as mycoses whereas mycotoxicoses is a collective name for diseases caused by dietary, respiratory, dermal and other various exposure to poisonous fungal metabolites (Bennett & Klich, 2003).

The toxicity of mycotoxins can be divided into four basic kinds, namely; acute, chronic, mutagenic and teratogenic (Pitt, 2000). Acute toxicity refers to adverse effects manifested within a relative short period ranging from almost immediately to several days following exposure or dosing whereas chronic toxicity involves irreversible adverse effects following a low-dose exposure of toxicant over a long period of time (James, Roberts, & Williams, 2000). Mutagenic effects are characterised by changes in the genetic makeup of the cell (DNA) after exposure to toxicant(s) leading to increased frequency of mutations. Teratogenicity involves development malformations of a fetus (Pitt, 2000).

Table 5: Mycotoxins, fungal sources and main effects on humans (D'Mello & Macdonald, 1997; Mostrom, 2016)

Mycotoxin	**Fungal source**	**Effects of ingestion**
Aflatoxin B_1	*Aspergillus parasiticus* *Aspergillus flavus*	Carcinogenic, immunosuppressive, hepatotoxic, nephrotoxic
Ochratoxin A	*Aspergillus ochraceus* *Penicillium viridicatum Penicillium cyclopium*	Carcinogenic immunosuppressive, nephrotoxic
Fumonisin B_1	*Fusarium verticillioides*	Carcinogenic, dermatotoxic, neurotoxic
Deoxynivalenol	*Fusarium culmorum* *Fusarium graminearum Fusarium*	Carcinogenic, dermatotoxic, neurotoxic

	sporotrichioides	
Zearalenone	*Fusarium culmorum* *Fusarium graminearum Fusarium sporotrichioides*	Oestrogenic, dermatotoxic

2.19. Impact of mycotoxins on food security in Africa

Mycotoxins pose a significant threat to achieving food security and safety in Africa (Gbashi et al., 2018). The supplies of food are limited and often of poor quality, with mycotoxins proliferation frequently implicated as the cause. Moreover, it has been reported that approximately 35% of global food and feed produce is contaminated by mycotoxins which in turn results in food losses/wastages of up to 1 billion metric tons annually with majority of these losses come from Africa (Gbashi et al., 2018).

2.20. Economics of mycotoxins

Mycotoxin have attracted global interest not only due to their perceived effect on human health but additionally because of the economic losses accruing from contaminated foods (Ostry, Malir, Toman, & Grosse, 2017). The economic impacts of mycotoxins to human society can be thought of in two ways. Firstly, in terms of direct market costs associated with lost trade or reduced revenues owing to contaminated food as well as to the costs linked to prevention and decontamination strategies (Bullerman & Bianchini, 2007; Pitt et al., 2012). The second impact is human health losses from adverse effects associated with mycotoxin consumption (Pitt et al., 2012).

2.21. Effect of food processing on mycotoxins

Mycotoxins are stable and moderately heat-resistant compounds (Bullerman & Bianchini, 2007). Consequently, pasteurisation or ordinary cooking conditions, for instance boiling and frying result in little or no reduction in total toxin levels (Kabak,

2009) thus mycotoxins remain nearly intact after processing (Bullerman & Bianchini, 2007).

The degree and extent of toxin degradation is affected by factors such as; 1) pH and ionic strength of food, 2) the initial level of contamination, 3) the heating temperature and time employed, 4) the type and concentration of the mycotoxin, and 5) the degree of heat penetration along with the moisture content (Kabak, 2009).

2.22. Modified mycotoxins

The word "modified mycotoxins" refers to any modification of the basic chemical structure of mycotoxins through chemical or biological modifications (Rychlik et al., 2014).

Food processing can chemically alter mycotoxins, however most of the resulting compounds are found to be less toxic (Berthiller et al., 2013). For example, during extrusion of grits, fumonisin B_1 was transformed into small amounts of hydrolysed fumonisin B_1 and N-carboxymethyl (Bullerman & Bianchini, 2007).

Similarly, the use of microorganisms to degrade mycotoxins in food products may transform mycotoxins into chemically different compounds (Berthiller et al., 2013). For example, *Pseudomonas putida* isolated from sugarcane metabolised and bio-transformed aflatoxin B_1 to new compounds (aflatoxin D_1, aflatoxin D_2 and aflatoxin $D_{3)}$ which are less toxic compounds (Samuel, Sivaramakrishna, & Mehta, 2014).

2.23. Reducing exposure to mycotoxins

Good agricultural and manufacturing practices are some of the approaches used to minimize the levels of mycotoxins in food (Galvano, Piva, Ritieni, & Galvano, 2001).

However, even under good manufacturing practices conditions, aflatoxins and ochratoxin A may still be considered as inevitable contaminants of food and feed (Wen, Kong, Hu, Wang, & Yang, 2014). Another approach used to reduce exposure to mycotoxins is by avoiding contaminated foodstuffs which can be achieved by modifying the diet (Galvano et al., 2001).

2.24. Dietary strategies to counteract effects of mycotoxins

Several chemical, physical, and biological approaches have been experimented in an attempt to lessen the toxicity of mycotoxin. However, some chemical detoxification methods may produce toxic residues and/or alter the nutritional composition of the final products (Dvegowda, Raju, & Swamy, 1998).

Some dietary approaches that could be used to counteract the effects of mycotoxins have been investigated (Galvano et al., 2001). Some of these approaches include but not limited to supplementation of nutrients, food components, or additives with protective properties against mycotoxin toxicity and addition of nonnutritive sorbents or bacteria, yeast and modified yeasts cells capable of reducing the bioavailability of mycotoxins (Galvano et al., 2001).

2.25. Mycotoxins regulated in food in Africa

Food legislation helps to safeguarding the health of food consumers and the economic interests of food producers and traders (Ashiq, 2015; van Egmond & Jonker, 2004). Availability of toxicological data and availability of data on the incidence of mycotoxins in several commodities and analytical methods are some of the factors that determine how the mycotoxin limits are chosen and for which commodities (Egmond &Dekker, 1994).

Figure 10 shows the occurrence of the regulatory limits for several mycotoxins in food in 2003. Only fifteen countries were known to have mycotoxin regulations whereas the majority of the African countries, such regulations are hard to find and probably do not simply exist. Most of the existing mycotoxin regulations in Africa concern the aflatoxins (van Egmond & Jonker, 2004). As for Namibia, there is no information available.

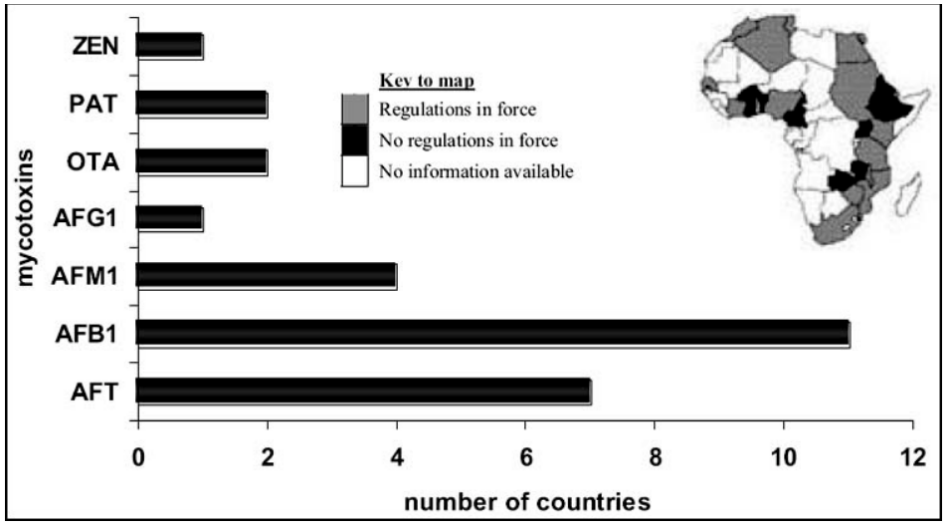

Figure 10: Mycotoxins regulated in Africa in 2003 (van Egmond & Jonker, 2004)

2.26. Mycotoxin regulations for Food and Drug Administration (FDA), European Commission (EC) and Codex Alimentarius Commissison (CAC)

Regulatory limits or advisory levels or tolerance levels for different mycotoxins in foodstuff and animal feed for sale or import have been set or established by different governments or regulatory bodies. There are currently no national mycotoxin regulations documented for Namibia. The United States Food and Drugs Administration (FDA) has set the regulatory levels for total aflatoxin at 20 µg/kg (FAO, 2004), deoxynivalenol (500 µg/kg), fumonisins 2-3 µg/g (2000-3000 µg/kg) in foodstuffs and no specifications

for zearalenone and ochratoxin A. The European Union has regulation limits of 5µg/kg for ochratoxin A, advisory levels of 5 µg/kg for deoxynivalenol(Food and Agriculture Organisation, 1997). The European Union limits for zearalenone and fumonisin exist for cereals and cereal based products but not for mushrooms. Codex Alimentarius Commission has established a limit of 15 µg/kg for aflatoxins (Codex Alimentarius Commissison, 2001).

2.27. Mycotoxin regulations in Asia

Regulatory limits for mycotoxins exist in some of the Asian countries. All the Asian countries that have mycotoxin regulations in place at least have regulatory limits for aflatoxins since aflatoxins are regulated most strictly and worldwide (Anukul, Vangnai, & Mahakarnchanakul, 2013). Table 6 shows the maximum allowable limits for aflatoxin for some Asian countries. The levels differ from country to country. For instance, total aflatoxin limits in foods in both Malaysia and Indonesia are set at 35 µg/kg, whereas Singapore has more strict limit of 5 µg/kg (Anukul et al., 2013). For other mycotoxins, the limits in place are specific to products such as cereals and spices. The maximum limits for zearalenone in Thailand for all foodstuff range from 30-1000 µg/kg (Anukul et al., 2013).

Table 6: Maximum limits for total Aflatoxins in some Asian countries (Anukul et al., 2013)

Country	**Maximum limits (µg/kg)**	**Foodstuff**
Indonesia	35	All
Malaysia	35	All
Sri Lanka	30	All
Philippines	20	All

Thailand	20	All
Japan	10	All
Taiwan	10	All
Vietnam	10	All
Singapore	5	All

2.28. Techniques for mycotoxins analyses

Since mycotoxin toxicity can occur at very low concentrations, sensitive and reliable methods for their detection are required (Rahmani, Jinap, & Soleimany, 2009; Turner, Subrahmanyam, & Piletsky, 2009). The methods used for mycotoxin analysis should be able to address issues such as detection limits, repeatability, reproducibility and the percentage of recovery (Mathoto, 2007). Moreover, the detection method should be robust and be highly flexible over a wide range of compounds (Turner et al., 2009). Since mycotoxins have different structures, one standard technique cannot be used to detect all mycotoxins (Turner et al., 2009).

2.28.1. Enzyme-linked immuno-sorbent assay

ELISA method is used to quantify specific antigens (mycotoxins). The antigen (mycotoxin) binds to selective antibodies forming a specific antigen-antibody complex (Wolf & Schweigert, 2018). The optical density of the samples is then interpolated into a standard curve to determine the concentration of the antigen (mycotoxins).

Immuno assays are rapid, simpler and cheaper than the HPLC methods (Anfossi, Giovannoli, & Baggiani, 2016).They do not require sample extract purification and allows handling of many samples in a single experiment. However, cross reactivity of related mycotoxins has been found to occur with most ELISA (Berthiller et al., 2013).

Other drawbacks of immuno assays is the rare possibility of false positives and they are semi-quantitative (Scott & Trucksess, 1997). False positives are observed if the test is sensitive to other similar compounds such as metabolites (Watson, 2001) thus experimental specificity may minimize false positives (Wong & Lewis Sr, 2017).

2.28.2. High performance liquid chromatography (HPLC)

This is a separation technique based on the mass transfer between the stationary phase and mobile phase. The mobile phase (liquid) is moved by a pump through the column under high pressure. Separation of sample components is depended on the chemical interactions of the sample components with the mobile and stationary phases (Kanpur, 2012).

2.28.3. Principle of TLC

This technique is grounded on a heterogeneous equilibrium established during the flow of the mobile phase (solvent), through an immobile stationary phase to separate components from the mixture carried by the solvent (Touchstone & Dobbins, 1983).

2.28.4. *Comparison of enzyme-linked immunosorbent assay, thin layer chromatography and high-performance liquid chromatography*

Alternatives to the methods used in this study (Elabscience ELISA kits) for mycotoxin analysis could be TLC and HPLC. TLC technique is frequently used for screening of samples contaminated with mycotoxins and it yields qualitative or semi-quantitative results (Krska et al., 2008). TLC analysis of mycotoxins has advantages such as high throughput of samples, low operating cost and ease of identification (Turner et al., 2009). HPLC is found to be more quantitative and faster. The drawbacks of HPLC

method are that it requires sophisticated and expensive equipment and running the analysis is costly.

Table 7: Advantages and disadvantages of analytical techniques for mycotoxin analysis (Mathoto, 2007)

Technique	**Advantages**	**Disadvantages**
TLC	High throughput; good screening technique	Semi-quantitative
HPLC	Precise and accurate; automated; simultaneous detection of different mycotoxins	Requires well trained personnel expensive technology
Immunoassays	Minimum sample preparation; quick results	Semi-quantitative; false positives

2.29. Heavy metals in foodstuffs

Living organisms require trace amounts of some heavy metals (essential) such as iron , cobalt, copper , manganese, chromium and zinc (Liu et al., 2015). These metals have an important role in the biological systems (El-Kady & Abdel-Wahhab, 2018). Some other metal elements such as arsenic, cadmium and lead are considered to be harmful (Liu et al., 2015).

Heavy metals can enter food via human activities such as industrial and agricultural processes (Toxicology Factsheet, 2009). Heavy metals are non-degradable and resistant to natural biodegradation thus they tend to accumulate in all media including soil and aquatic environments (El-Kady & Abdel-Wahhab, 2018).

2.29.1. Heavy metal contamination in mushrooms

Edible mushrooms require a variety of nutrients from the surrounding environment for growth and reproduction (Zou et al., 2019). The fruiting bodies of mushrooms are known to bioaccumulate metal ions (Chiocchetti et al., 2019; Liu et al., 2015).

Accumulation of heavy metals in mushrooms is influenced by factors such as metal concentration in the soil, organic matter, and contamination by atmospheric deposition (Garcia, Alonso, Fernández, & Melgar, 1998). In addition, fungal factors such as fungal structure, biochemical composition, decomposition activity, development of mycelium and fruit bodies are also found to influence the accumulation of heavy metals in microfungi (Garcia et al., 1998). Owing to controllable sources of raw materials, cultivated mushrooms are generally less susceptible to chemical contamination (Zou et al., 2019).

Sarikurkcu et al. (2011) detected contents of trace element in wild edible mushrooms in the ranges of 0.7-4.2 mg/kg lead, 0.31-54.2 mg/kg cadmium, 29-146 mg/kg zinc, 138-1714 mg/kg iron, 10-77 mg/kg manganese, 6-187 mg/kg copper, 0.0-21.6 chromium, 0.7-4.2 mg/kg nickel and 0.0-.2 mg/kg cobalt. In studies by (Rudawska & Leski, 2005a, 2005b), iron levels of 400-500 mg/kg were detected in mushrooms. Fang et al. (2014) detected lead content of 0.038–2.539 mg/kg in dried mushrooms and Falandysz et al. (2001) detected zinc of 0.59 mg/kg in dried mushrooms.

2.29.2. Effects of heavy metals on human health

Excessive intake of heavy metals such as cadmium, chromium, lead, mercury, nickel, manganese and essential elements such as zinc and iron can have health complications in human beings (Wu et al., 2018). Cadmium can cause renal tubular dysfunction or anaemia skeletal damage (Liang et al., 2019). Moreover, chronic exposure of cadmium has been reported to cause adverse effects such as lung cancer, kidney dysfunction bone fractures (WHO, 2007). Lead is non-essential to human body and excessive intake of lead can cause nervous, skeletal, circulatory, endocrine and immune system damage

(Pareja-Carrera, Mateo, & Rodríguez-Estival, 2014). Mercury has been reported to cause brain damage and disruption of the nervous system whereas nickel is found to be carcinogenic (Mahurpawar, 2015).

Chronic exposure to essential metals such as zinc and iron can impair health (Mendil, Tuzen, Yazıcı, & Soylak, 2005). Excessive intake of zinc has been linked to electrolyte imbalance, nausea, anaemia and lethargy (Fairweather-Tait, 1988). Excessive iron intake can lead to iron poisoning (Osweiler, Carson, Buck, & Van Gelder, 1985). In addition, excessive iron intake can lead to conditions such as coagulation of blood in the vessels, hypertension and drowsiness (Davies, Allison, & Uyi, 2006).

2.29.3. Heavy metal limits in mushrooms

The permissible limits for cadmium, iron, manganese, lead in food are 0.2, 43, 5.5 and 1 mg/kg, respectively as per (FAO/WHO, 2011) regulations. The permissible limit for zinc in food is 60 mg/kg as per (WHO, 1982) regulations. For chromium, Czech Republic has statutory limits of 4.0 mg/kg in place (Kalač, 2010).

2.29.4. Heavy metal analysis techniques

Methods that are used include Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Flame Atomic Absorption spectrometry (Flame AAS), Graphite Atomic Absorption Spectrometry (Graphite furnace AAS).

2.29.4.1. Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)

The principle of ICP-OES is based on the energy from a plasma converting molecules to atoms and ions. The plasma energy then raises atom from ground to excited state.

Emission of energy at specific wavelength is measured as excited atoms return to a lower energy state. The amount of energy emitted is proportional to the concentration of specific element (Harris & Marshall, 2017). ICP-OES method requires the samples to be ashed before analysis. Ashing methods include wet and dry ashing. Dry ashing involves the use of muffle furnace that can maintain temperatures of 500-600 °C. Dry ashing principle is based on water and volatiles being vaporised, and organic substances being burned in the presence of oxygen to form CO_2 and oxides of N_2. This step removes organic materials from the sample (Harris & Marshall, 2017). Wet ashing is a preparation procedure for the analysis of minerals that might be volatalised and lost during dry ashing. Organic substances are removed from samples using strong acids such as hydrochloric acid, sulfuric acid, nitric acid and perchloric acid (Harris & Marshall, 2017).

2.29.4.2. Principle of Flame Atomic Absorption Spectrometry

This method uses a nebulizer-burner to convert a sample solution into atomic vapour. The nebulized sample solution is then mixed with an oxidant and burned in a flame produced by oxidation of the fuel. As the sample solution goes through desolvation, vaporisation, atomisation and ionisation, atoms and ions are formed within the hottest portion of the flame. The analyte element is quantified by determining the attenuation of a beam of radiation passing through the flame, as a result of atomic absorption of incident radiation by the analyte element (Yeung, Miller, & Rutzke, 2017).

2.29.4.3. Principles of Graphite Furnace Atomic Absorption Spectroscopy

This method is based on heating the sample electrically in stages in a graphite furnace to achieve atomisation. The tube is aligned to the path of the radiation beam to be absorbed by the atomised sample and absorbance is determined (Yeung et al., 2017).

2.29.4.4. Comparison of ICP-OES, ICP-MS, Flame AAS and GAAS

ICP-OES method has a better detection limit as compared to Flame AAS and Graphite furnace AAS. Moreover, ICP-OES is easy to use and can be used to analyse multiple elements in large number of samples with a single aspiration (Yeung et al., 2017).

ICP-MS technique, on the other hand has overall best detection limits as compared to other techniques (Yeung et al., 2017). Moreover, it can also be used to analyse elements at ultra-trace levels in all types of matrices (Otles & Senturk, 2015). The major limitation of ICP-MS is cost, which can be two to four times higher than ICP-OES counterparts (Yeung et al., 2017).

Flame AAS technique is relatively easy to use and has good detection limits. However, it has low sensitivity, uses explosive fuel gas and has limitations on multi element analysis (Yeung et al., 2017). Graphite furnace AAS has better detection limits than Flame AAS (Yeung et al., 2017).

Chapter 3: Research

Microbiological quality and safety, mycotoxins, heavy metals in underprised Kalahari truffle, *Termitomyces schimperi* and *Ganoderma* mushrooms

3.1. Abstract

Mushrooms can be edible and are used as food and as nutraceuticals. The cost of mushrooms can be quite high in developed countries but less so in developing nations. Mushrooms are mostly collected from the wild with a relatively high water activity, thus prone to microbial contaminations and their safety can be precarious. Therefore, this study determined the cost of Kalahari truffle, microbiological quality and safety, mycotoxins and heavy metals contents of Kalahari truffle, *Termitomyces schimperi a*nd *Ganoderma* species collected in Namibia. Truffles and *Termitomyces schimperi* were purchased from informal markets where they are almost exclusively found when in season. *Ganoderma* samples were collected from the wild. The prices of Kalahari truffles ranged from N$ 28.77- N$ 70.09 per kg. The prices of truffles increased towards the end of the growing season. Total aerobic count in unwashed truffles ranged between 4.4 and 7.3 log cfu/g. Total aerobic counts in *Ganoderma* samples ranged between 2.5 and 5.7 log cfu/g. For *T. schimperi*, total aerobic count was 4.7 log cfu/g. Total coliform counts detected in truffles, *T. schimperi* and *Ganoderma* were 6.0, 5.4 and 6.6 log cfu/g respectively. Total coliform counts were above the acceptable limits of <1 cfu/g set by the European Union Commission Recommendation. *Salmonella* was not detected in any of the mushrooms. Doxynivalenol was the most prevalent mycotoxin in truffles, *Termitomyces schimperi* and *Ganoderma* with the levels as high as 25933.3 µg/kg, 48897.1 µg/kg and 48973.3µg/kg, respectively Fumonisin B_1 levels ranged between 17.4 and 142.1 µg/kg in unwashed truffles. In washed truffles, fumonisin B_1 levels ranged between 18.1 to 65.3 µg/kg. Ochratoxin A levels in unwashed truffles ranged between 0.1 to 48.5 µg/kg. Total aflatoxin levels for truffles were between 26.3 and 27.5 µg/kg. Zearalenone levels in truffles ranged between 45.0 and 9680 µg/kg. For *Ganoderma* mushrooms, the levels of deoxynivalenol, fumonisin B_1, ochratoxin A, total aflatoxin and zearalenone ranged between 46675.2 and 48973.3 µg/kg, 48.0 and 17536.4

µg/kg, 4.7 and 19320 µg/kg, 91.7 and 100.6 µg/kg, 105.0 and 5478 µg/kg, respectively. For *T. schimperi*, the levels of deoxynivalenol, fumonisin B1, ochratoxin A, total aflatoxins and zearalenone were 48897.1 µg/kg, 17462.2 µg/kg, 19391.4 µg/kg, 101.0 µg/kg and 5529.5 µg/kg, respectively. The iron content of unwashed and washed truffles was up to 746.72 mg/kg. Cadmium, lead and zinc were detected in the studied samples whereas mercury and nickel were not detected in any sample. Not all mushroom studied samples are of suitable quality to consume without further appropriate processing.

Keywords: Mycotoxins, truffles, *Ganoderma*, *Termitomyces schimperi*

3.2. Introduction

Mushrooms can be edible, medicinal, poisonous (Rasalanavho et al., 2019). Kalahari truffles including *Kalaharituber pfeilli,* the common truffle found in Namibia are underpriced as compared to other truffles in other parts of the world. In Namibia, truffles are harvested for household consumption and/or for selling in the informal market. In Europe, the market price for truffles ranges from US$ 220.5 to US$ 3305.30 per kg (Álvarez-Lafuente, Benito-Matías, Peñuelas-Rubira, & Suz, 2018). Similarly, *Termitomyces schimperi* in Namibia is used at household level and is primarily sold along the roadsides. Larger specimens of *Termitomyces schimperi* can go for N$ 30.00 (US$ 2) (Rothman, 2018). *Ganoderma* mushrooms in Namibia are mostly used at household levels for their medicinal properties whereas in some countries, *Ganoderma* mushrooms are utilised commercially by processing them into supplements in a form of capsules or tablets (Oei, 2003). The annual sale of products from *Ganoderma lucidum* in Asian countries is estimated to be more than US$ 2.5 billion (Li et al., 2013). Namibia is one of the countries that imports *Ganoderma* products from Asia, although there are indigenous *Ganoderma* mushrooms in Namibia.

Truffles are telluric and seasonal (Trappe et al., 2008). *Ganoderma* mushrooms grow in the wild on dead and decaying trees (Baby et al., 2015; Loyd et al., 2018). *Termitomyces schimperi* is also a seasonal mushroom species that grows around the termite mounds in the wild (Van Der Westhuizen & Eicker, 1991).

For one to preserve mushrooms, there is a need to understand their microbial profile since fresh mushrooms are found to have a high moisture content and water activity which make them an ideal medium for microbial growth (Venturini et al., 2011). For

instance, Venturini et al. (2011) detected total microbial counts in the range of 4.4 to 9.4 log cfu/g and total coliform bacteria in 23.4% of different mushroom species sampled. Ezekiel et al. (2013) detected toxigenic moulds in dried mushrooms. Consequently, the presence of bacterial and unwanted fungal populations in fresh mushrooms can cause quality deterioration and reduce the shelf-life of fresh mushrooms (Venturini et al., 2011).

Moreover, proliferation of toxigenic moulds in foodstuff could lead to the production of mycotoxins. Mycotoxins have a negative impact on the immune system, liver, kidneys and blood whereas some mycotoxins are found to be carcinogens (Scott, 1984). Another safety aspect of mushrooms is the presence of heavy metals. High levels of heavy metals can cause health complications in humans (Lenntech, 2017). Heavy metals such as lead, cadmium, manganese, copper, nickel and cobalt have been detected in wild fresh and unprocessed mushroom species (Sarikurkcu et al., 2011). This study therefore investigated the cost of Kalahari truffle, the microbial quality and safety, mycotoxins and heavy metals in wild growing edible mushrooms Kalahari truffle (*Kalaharituber pfeilli*), *Ganoderma* species and *Termitomyces schimperi* in Namibia.

3.3. Materials and methods

3.3.1. Sample collection and treatment

Truffles are sold fresh in subjectively vendor-determined batches (groups of individual truffles, Figure 11). A batch in this study was considered as a sub-population of the purchased truffles. A total of eight batches of fresh Kalahari Desert truffles (*Kalaharituber pfeilli*) were purchased from different vendors at the informal market in Omuthiya, Ondangwa and Casablanca. One batch was obtained from each of a total of

eight vendors. Six batches (T_1, T_2, T_3, T_4, T_7 and T_8) of truffles were purchased during the peak of the growing season (mid May 2018) while batches T_5 and T_6 were purchased towards the end of growing season (around June). Truffle batches (T_1-T_6) were bought from different vendors at Omuthiya gwIipundi in Oshikoto region whereas batch T_7 was bought from Ondangwa in Oshana region. Batch T_8 was purchased from Casablanca, Oshikoto region. Table 8 shows the sample codes and places were Kalahari Desert truffles were purchased.

Table 8: Sample code, place where Kalahari Desert truffles were purchased

Sample code	**Place of collection**
T_1	Omuthiya
T_2	Omuthiya
T_3	Omuthiya
T_4	Omuthiya
T_5	Omuthiya
T_6	Omuthiya
T_7	Ondangwa
T_8	Casablanca

To determine the prices of Kalahari truffle, the vendors at the selected open market in Namibia were asked how much they were selling the truffle batches for. The price per weight for sample T_8 was however not determined, due to removal of some truffles for different uses before the weight of the sample was recorded.

The number of truffles in each batch was counted except for batch 8. Truffles in each batch were then weighed individually. Each batch was divided into two portions. One

portion from each batch was washed under running water whereas the other remained unwashed. Each batch therefore resulted into 2 samples (washed and unwashed). Both unwashed and washed truffles were then sliced and spread on separate trays. Drying was done at ambient conditions in the Food Processing Laboratory for five consecutive days. Dried truffles were then ground and kept frozen. A portion of sample T_6 (fresh) was frozen without drying.

Ganoderma fruiting bodies (8) (in a dry form) were collected between April and May 2018 with the explicit permission and guidance from the owners of the village plots in Oshana and Ohangwena regions. Collection of *Ganoderma* mushrooms was randomly done from any host tree on which the fruiting body was seen. The fruiting bodies were transported to Windhoek in Khaki bags, a day after collection. The fruiting bodies were then cleaned the following day using a dry paper towel to remove soil, dust and grass. The fruiting bodies of *Ganoderma* were then sun dried for at least eight (8) hours and packaged in clean khaki bags. The bags were stored at room temperature until analyses. Fifteen (15) *Ganoderma* mushrooms out of the 23 collected were successfully identified using DNA extraction. Six (6) identified species and two (2) unidentified species were subjected to Mycotoxin analysis and microbial analysis. For sample G_8 (Table 9), microbial analysis was not carried out due to unforeseen inadequate quantity of the sample. *Ganoderma* mushroom samples were not subjected to heavy metal analysis. Dried (under laminar flow) *Termitomyces schimperi* samples were obtained from Zero Emissions Research Initiatives. Sample codes, place of collection and identified names of *Ganoderma* mushrooms are given in table 9.

Table 8: Sample code, location and name of *Ganoderma* species

Sample code	**Place of collection (village and region)**	**Species**
G_1	Oshoongela, Oshana	*Ganoderma enigmaticum*
G_2	Ondeihaluka, Ohangwena	*Ganoderma enigmaticum*
G_3	Ondeihaluka, Ohangwena	*Ganoderma enigmaticum*
G_4	Ondeihaluka, Ohangwena	*Ganoderma enigmaticum*
G_5	Oshoongela, Oshana	*Ganoderma lucidum*
G_6	Oshoongela, Oshana	*Ganoderma enigmaticum*
G_7	Ondeihaluka, Ohangwena	Not identified
G_8	Ondeihaluka, Ohangwena	Not identified

Due to limited reagents and/or amounts of samples, not all mushrooms samples were subjected to all the analyses in this study. For each analysis, the samples subjected to it are stated in that respective section.

3.3.2. Moisture content analysis

Moisture content of truffles was determined using the AACC International Method 44-15.02 (AACC International. 1999). Approximately 2 g of sample was weighed and heated in an oven at 130°C for 60 minutes and cooled for 45 minutes in a desiccator before weighing and moisture determination.

3.3.3. Microbial analyses

T_1-T_8 Kalahari truffle, G_1-G_7 *Ganoderma species* and *Termitomyces schimperi* samples were subjected to microbial analyses.

3.3.3.1. Aerobic plate count

Total aerobic plate count was carried out following a method described by Larry and Peeler (2001). Five (5) grams of mushroom sample was placed in a sterile stomacher bag and homogenized using a stomacher (Seward) in 45 mL buffered peptone water for 30 seconds. Tenfold serial dilutions were prepared, and appropriate dilutions were pour-plated onto plate count agar (Acumedia lab, UK) at 35°C for 48 hrs.

3.3.3.2. Coliforms

Coliforms enumeration was carried out using a method described by Feng et al. (2002). Dilution 10^{-1} was prepared by homogenising five grams of mushroom sample into 45 mL of buffered peptone water (Acumedid lab, UK) for 30 seconds. Tenfold serial dilutions were prepared, and appropriate dilutions were pour-plated in onto Violet Red Bile agar. (Acumedia lab, UK) A second overlay was performed with the Violet Red Bile agar and plates were incubated at 35°C for 48 hours.

3.3.3.3. Yeast and moulds

Yeasts and moulds enumeration were done using the AOAC Official Method 997.02 (AOAC International, 2016). Five (5) grams of mushroom sample was homogenised into 45 mL of buffered peptone water (Acumedia lab, UK) for 30 seconds. Tenfold serial dilutions were prepared and spread-plated in duplicates onto Rose Bengal chloramphenicol agar (Acumedia lab, UK) plates. The plates were incubated at 25˚C for five days.

3.3.3.4. Qualitative detection of Salmonella

Detection of *Salmonella* was carried out by modifying AOAC Official methods of Analysis 995.20:2016 (AOAC International, 2016). Sterile lactose broth (45 mL) was added to five (5) grams of mushroom samples. The mixture was incubated overnight at 35°C. After incubation, 0.1 ml of the incubated mixture was transferred to Rappaport-Vassiliadis medium (Scharlau lab, Spain) and incubated for 24 hours at 42°C. Three (3) mm loop was then streaked on selective enrichment media plates of Xylose lysine desoxycholate (XLD) agar. (Scharlau lab, Spain) The plates were examined for *Salmonella* colonies after 24 hours of incubation at 35°C.

3.3.4. Mycotoxins analyses

T_1, T_5, T_6, T_7 and T_8 of truffles, G_1 to G_8 of *Ganoderma* and *Termitomyces schimperi* samples were analysed for mycotoxins due to limited access to ELISA kits. All kits were obtained from Elabscience®, USA. Each kit was used according to the manufacturer's instruction.

3.3.4.1. Sample preparation for deoxynivalenol analysis

One (1) gram of Kalahari Truffle, *Ganoderma* and *T. schimperi* homogenates were separately weighed in duplicates into 50 mL EP tube. Five (5) mL of deionised water was added to tubes containing Kalahari truffle homogenates whereas 10 mL of deionised water was added to tubes containing *Ganoderma* and *Termitomyces schimperi* homogenates. The tubes were oscillated for 5 minutes and centrifuged at 4000 r/min at room temperature. The supernatant (0.1 mL) was taken and 0.9 mL of Reconstitution Buffer was added and mixed fully. Fifty (50) µL was taken for detection and analysis.

3.3.4.2. Sample preparation for fumonisin B_1 analysis

One (1) gram of the three mushroom species were separately weighed in duplicates into the 50 mL EP tubes then 2.5 mL of 70% methanol was added to the tubes containing truffle samples. Ten (10) mL of 70% ethanol was added to tubes of *Ganoderma* and *Termitomyces schimperi*. The tubes were oscillated for 5 minutes and then centrifuged at 4000 r/min for ten minutes at room temperature. Fifty (50) µL was then taken for detection and analysis.

3.3.4.3. Sample preparation for ochratoxin A analysis

One (1) gram of Kalahari Truffle, *Ganoderma* and *Termitomyces schimperi* homogenates were separately weighed in duplicates into 50 mL EP tubes. Five (5) mL of 70% methanol was added to tubes containing Kalahari truffle samples whereas 10 mL of 70% was added to tubes containing *Ganoderma* and *Termitomyces schimperi* samples. The tubes were swirled and centrifuged at 4000r/min for 1 minute at room temperature. The supernatant (0.5 mL) was obtained and 1 mL of 0.1 M $NaHCO_3$ solution was added. Fifty (50) µL was taken for detection and analysis.

3.3.4.4. Sample preparation for total aflatoxin

One (1) gram of the samples was separately weighed in duplicates into 50 mL EP tubes. Five (5) mL of 70% methanol was added and the tubes were oscillated for 5 minutes. The tubes were centrifuged for 10 minutes at room temperature. The supernatant (0.5) mL was taken and 0.5 mL of deionized water was added and mixed fully. Fifty (50) µL was taken for detection and analysis.

3.3.4.5. Sample preparation for zearalenone analysis

One gram of the three different mushrooms homogenates was separately weighed in to a 50 mL tube. Eight (8) mL and 10 mL of 90% methyl alcohol were added to tubes containing truffle and *Ganoderma* homogenates, respectively. Ten (10) mL of 90% methyl alcohol was also added to *T. schimperi* homogenate. The tubes were oscillated for 5 minutes and then centrifuged at 4000 r/min for 10 minutes at room temperature. Supernatant (0.5 mL) was obtained and 2 mL of deonised water was added and mixed. Finally, fifty (50) µL was taken for detection and analysis.

The assays were performed in 96-microwell plates pre-coated with mycotoxin of interest. The optical density (OD) value of each well was determined by reading with a micro-plate reader (SpectraMax 190) set at 450 nm.

Absorbance (%) was determined using the formula:

Absorbance (%) = A/A_0 x 100

Where, A= average absorbance of standard or sample, A_0 = average absorbance of 0 ppb standard. A standard curve was created by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot according to the manufacturer's manual. The average absorbance values of samples were added to standard curve to obtain corresponding concentration. The concentration calculated from the standard curve was multiplied by the dilution factor for each sample.

3.3.5. Heavy metal analysis

Heavy metal analysis was carried out according to the method described by (Giron, 1973). Three (3) grams of the samples was in a porcelain crucible was placed in a muffle furnace. Ashing was done overnight at 550ºC. The samples were then cooled and dissolved in 5 ml of 6 M hydrochloric acid (Promark, USA). Evaporation to dryness was then carried out on a water bath and 5 ml of Nitric acid (HNO_3) (Merk, Germany) was added. The crucible was heated on a water bath and removed as soon as the solution has started to boil. The solution was filtered through a filter paper into 100 ml volumetric flask. The filter paper was then washed with warm water. The solution was the cooled and diluted with deionized water and mixed well. The quantitative determination of elements was carried out using ICAP Series (ICP Spectrometer) (Thermo Scientific, USA). Kalahari truffle samples (T_1-T_8) and *Termitomyces schimperi* samples were subjected to heavy metal analysis.

3.3.6. Statistical analyses

All samples were analysed in duplicates. Results represent the average of duplicates and are presented as means ±standard deviation. Data obtained was subjected to an analysis of variance (one-way ANOVA) and Duncan's least significant differences (LSD) test using SPSS Statistics Software, Version 25 (IBM, USA).

3.3.7. Research Ethics

Kalahari truffle samples were purchased from the open market. *Ganoderma* samples were collected from Oshana and Ohangwena with the explicit permission and guidance from the owners of the village plots. Microorganisms that were analysed belong in the risk group 1 (non-pathogenic) and the laboratory safety levels were safety level 1 (open

bench) and safety level 2 (biosafety cabinet/laminar flow hood). These are all available at the University of Namibia. There were no human participants involved. The study did not use animals or animal tissues or fluids, human tissues or biological fluids as well as no radioactive hazards were used. All sources were acknowledged and referenced appropriately.

3.4. Results and discussion

3.4.1. Prices, weights of truffles

In Namibia, truffles are sold at open markets (figure 11) in vendor-determined batches. Table 10 shows the number of Kalahari truffles, weight and cost of Kalahari truffles sold in open market in Namibia. Different batches of truffles had different number of truffles. The batches with small sized truffles tended to have more counts whereas batches with large sized truffles had fewer counts. On the other hand, the batches with large sized truffles had less total weight as compared to batches with small sized truffles, but they had the same price. Batches T_5 and T_6 were bought towards the end of the growing season and the prices increased to N$ 40.00 and N$ 60.00, respectively. The prices of truffles followed the general trend of supply and demand. Truffles in Europe are generally highly prized and expensive as compared to desert truffles. The market price of desert truffles has been reported to be $26 to $330 per kg (Morte et al., 2012) as compared to the market price of truffles in Namibia ($ 1.96 to $ 4.76 per kg). This then implies that the prices for truffles in Europe can cost as much as over 60 times more compared to truffles in Namibia. The market price of some truffle species in Europe is also higher than the market price of truffles in Namibia. For examples, the market price of *Tuber melanosporum* is reportedly $1760 (Bonito et al., 2013) which is $369.75

higher than the market price of Kalahari truffles. This difference could be attributed to type of species, different characteristics and functionality of truffle species.

Figure 11: Batches (11) of truffles sold at the open market during the 2018 growing season

Table 9: Prices, weights of Kalahari truffles sold in open markets (Ondangwa and Omuthiya) in Namibia in 2018

Batches	**Count**	**Total weight (g)**	**Average weight (g)**	**Median weight (g)**	**Minimum weight (g)**	**Maximum Weight (g)**	**Cost/batch (N$)**	**Cost/kg (N$/kg)**
T_1	7	336.6	48.1	38.7	21.8	117.8	20	59.42
T_2	24	506.3	21.1	20.8	8.0	42.0	20	39.50
T_3	39	621.9	16.0	14.0	2.7	48.4	20	32.16
T_4	11	695.1	63.2	52.0	21.6	184.4	20	28.77
T_5	12	647.4	54.0	27.5	8.3	165.5	40	61.78
T_6	22	874.0	39.7	23.0	12.0	270.0	60	68.65
T_7	15	856.0	57.1	52.0	30.0	122.0	60	70.09

[1]Count=Total in a batch

[2]Average weight =mean

3.4.2. Moisture content

Moisture content of unwashed and washed truffles is given in table 11. Moisture content in dried unwashed truffles ranged from 10.0-17.6 % whereas in washed truffles, the moisture content in dried washed truffles was in the range of 7.8 and 17.5 %. In fresh samples, the moisture content was 75.5% and 70.0% in unwashed and washed truffles, respectively. There was no significant difference ($p > 0.05$) in moisture content between unwashed and washed samples except in sample T_1.

Table 10: Moisture (%) content in Kalahari truffles

[1]Sample	[2]Unwashed	Washed
T_1 (dried)	12.5^{bc} ±3.5	17.5^{a} ±3.5
T_2 (dried)	12.5^{bc}±3.5	13.95^{bc}±0.5
T_3 (dried)	10.0^{bc}± 0.0	12.05^{bc}±4.2
T_4 (dried)	17.6^{b}±4.6	15.4^{bc}±0.6
T_5 (dried)	10.0^{bc} ±0.0	10.0^{bc}±0.0
T_6 (dried)	15.0^{bc}±7.1	7.25^{c}± 3.9
T_6 (fresh)	72.5^{a}±3.5	70.0^{a}±7.1
T_7 (dried)	12.5^{bc}±3.5	15.0^{bc}±2.1
T_8 (dried)	14.0^{bc}±0.5	7.8^{c}±3.1

[1]T_1-T_8 = Truffles from different vendors

[2]Values are means of two replicates ± standard deviation

Values with different superscript letter (^{abc}) in a row differ significantly ($p< 0.05$)

3.4.3 Aerobic, total coliforms, yeast, mould, *salmonella* in truffles

Aerobic, total coliforms, yeast, mould, counts in truffles are given in table 12. Even though washing of truffles reduced the aerobic counts in truffles, the counts did not differ significantly ($p \geq 0.05$) between washed and unwashed truffles except for sample T_8 where washing had significantly reduced the total aerobic counts ($p < 0.05$).

The aerobic counts detected in both unwashed and washed truffle samples were less than the 8.4 log cfu/g that Rivera et al., (2010) detected in other truffle species. The differences in the microbial counts could be linked to the heterogeneity of the sample, the harvesting season, mechanical damage or internal parasitisation (Rivera, D. Blanco, R. Oria, & M. E. Venturini, 2010). Another reason that could explain the differences is that some truffles may have been parasitised and spoiled and yet included in the microbial analysis. Parasites inside ascocarps increase internal contamination and allow entry of microorganisms (Rivera et al., 2010).

The European Union Commission Recommendation (directive 2004/24/EC) (Ajis et al., 2017) states that the acceptable total aerobic count limit is <5.7 log cfu/g. Based on the European Union Commission regulation, six (T_2, T_3, T_4, T_5, T_6 and T_6 (fresh) unwashed truffle samples had counts below the acceptable limits and only three unwashed (T_1, T_7 and T_8) truffle samples had counts above the acceptable limits. For washed truffles, only sample T_6 (fresh) out of seven had counts above the acceptable limits. Overall, the results indicate that majority of truffle samples were of good sanitary quality and may have a relatively longer shelf life compared to the freshly harvested ones.

Table 11: Aerobic counts, total coliforms, yeasts and mould in truffles

	[2]Aerobic count		Total coliforms		Yeast		Mould	
[1]Sample	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
T_1	6.4^{ab} ±0.0	5.5^{bc} ±0.0	6.2^{a} ±0.0	3.9^{bcd} ±0.1	7.2^{a} ±0.1	5.7^{bcd} ±0.5	5.8^{ab} ±0.7	4.7^{bcde} ±0.5
T_2	5.2^{bcd} ±0.5	4.6^{cd} ±1.2	4.5^{abcd} ±0.6	2.7^{cd} ±0.0	4.7^{def} ±0.8	4.5^{def} ±0.0	4.7^{bcde} ±0.2	3.7^{def} ±0.5
T_3	4.8^{cd} ±0.2	4.5^{cd} ±0.1	5.0^{ab} ±1.6	4.4^{abcd}±0.5	6.6^{ab} ±0.03	5.0^{cde} ±0.7	5.4^{bc} ±0.1	4.6^{bcde} ±0.4
T_4	5.5^{bc} ±0.0	4.9^{cd} ±0.7	3.4^{bcd} ±0.6	4.5^{abc} ±0.6	4.9^{cdef} ±1.5	5.3^{bcd} ±0.0	4.4^{cde} ±1.7	5.2^{bc} ±0.3
T_5	4.8^{cd} ±0.0	4.6^{cd} ±0.8	4.3^{abcd} ±0.7	ND	ND	3.5^{f} ±0.9	5.0^{bcd} ±0.6	4.7^{bcde} ±0.7
T_6	4.9^{cd} ±0.4	4.5^{cd} ±0.2	4.1^{abcd} ±1.1	2.4^{d}±0.0	3.7^{ef} ±0.3	4.9^{cdef} ±1.0	4.3^{cde} ±0.0	3.6^{ef} ±0.2
T_6 ($_{fresh}$)	4.6^{cd} ±1.2	6.3^{ab} ±0.1	[3]ND	ND	ND	ND	2.9^{f} ±0.1	3.6^{ef} ±0.2
T_7	6.2^{ab} ±0.2	5.2^{bcd} ±0.5	5.1^{ab} ±0.0	2.5^{d}±2.0	ND	6.3^{abc} ±0.6	6.9^{a} ±0.5	5.5^{bc} ±0.7
T_8	7.3^{a} ±0.1	4.0^{d} ±0.0	3.6^{bcd} ±0.2	4.3^{abcd} ±1.0	ND	6.4^{ab} ±0.8	4.8^{bcde} ±0.1	4.8^{bcde} ±0.0

[1]T_1-T_8 = Truffles from different vendors

[2]Values are mean of two replicates ±Standard deviation (n=2)

Values with different letter superscript in columns (^{abc}) are significantly ($p < 0.05$) different from each other

[3]ND: Not detected, counts below the detection limit

Total coliform counts did not differ significantly ($p \geq 0.05$) between unwashed and washed truffles in samples T_2, T_3, T_4, T_6 and T_8 whereas in samples T_1 and T_7, total coliform counts differed significantly ($p < 0.05$) between washed and unwashed truffles.

Total coliform counts for unwashed truffles ranged from not detected to 6.2 log cfu/g whereas in washed truffles the counts ranged from not detected to 4.4 log cfu/g. Unwashed truffle samples (T_1, T_2, T_3 and T_8) had high total coliform counts compared to the total coliforms counts (4.0 log cfu/g) that Reale et al. (2009) detected in fresh black truffles. In washed samples, only sample T_4 had higher total coliform counts than the counts detected by Reale et al. (2009). These differences could be linked to the type of method used to remove the soil surrounding the truffles and the preservation method used (Rivera et al., 2010). Other reason could be the type of species and the level of soil contamination from which the truffles were harvested.

The presence of coliforms in wild growing truffles could be due to domestic and wild animals that can freely enter fields where truffles grow and consequently increase the risk of faecal contamination. Another reason could be that the conditions under which truffles grow are favourable for the development of *Enterobacteriaceae* organisms since truffles have high amount of glucose that could be fermented by *Enterobacteriaceae* consequently altering the taste and aroma (Rivera et al., 2010).

Acceptable limit for total coliforms based on European Union Commission Recommendation (directive 2004/24/EC) is <1 cfu/g (Ajis et al., 2017). Based on these recommendations, only sample T_6 (fresh) and sample T_5 (washed) had total coliform counts below the acceptable limits. The presence of coliforms in foodstuff may indicate

poor hygienic quality of truffles. This then suggests that truffles should not be eaten raw and should be subjected to further processing such as cooking.

Yeast counts were below the detection limit in samples T_5, T_6 (fresh), and T_7 and T_8 for unwashed truffles. The counts for these samples (T_5, T_6 (fresh), T_7 and T_8) were significantly ($p < 0.05$) higher in the washed counterparts. For unwashed truffles, yeast counts detected were in the range of 3.7 to 7.2 log cfu/g whereas in washed truffles yeast counts ranged between 3.5 and 6.4 log cfu/g. The results were higher than the 3.4 log cfu/g yeast counts that Rivera et al., (2010) detected in dried *T. aestivum* and *T. melanosporum* truffles. The presence of yeast in food products is not a hazard to health (Guidelines for Environmental Health Officers on the interpretation of microbiological analysis data of food, 2000). However, consumption of yeast contaminated food could lead to allergic reactions and food spoilage (Howell, 2016). Acceptable limit of yeast and mould count based on European Union Commission Recommendation (directive 2004/24/EC) is $< 5 \times 10^3$ cfu/g (< log 3.7 cfu/g) (Ajis et al., 2017). Irrespective of washing of truffles, majority of the samples had yeast counts above the acceptable limits of the European Union Commission. This could indicate a shorter shelf life of truffles and further preservation may be necessary.

Washing of truffles generally reduced mould counts. However, the mould counts between unwashed and washed truffles did not differ significantly ($p \geq 0.05$) except for sample T_7. For most of the samples irrespective of washing, mould counts were higher than the 3.7 log cfu/g that Rivera et al. (2011) detected in fresh *Tuber aestivum* truffles.

Regardless of washing of truffles, only three samples had mould counts below the acceptable limit (<log 3.7 cfu/g) based on the European Union Commission

Recommendation (directive 2004/24/EC) (Ajis et al., 2017) thus might not be fit for consumption since the presence of moulds in foodstuff may lead to the production of mycotoxins which can cause health complications.

No *Salmonella* was detected in 10 grams of truffle samples. This is in agreement with the study by Reale et al. (2009). Based on Food Safety Authority of Ireland (2006) regulations, *Salmonella* should not be present in 25 g portions of raw mushrooms. This then implies that the truffle samples were in compliance with the regulations with the (Food Safety Authority of Ireland, 2006) regulations with regard to *Salmonella*.

3.4.4. Aerobic counts, total coliforms, yeast, mould, and *Salmonella* in *Ganoderma* mushrooms

Aerobic, total coliforms, yeast and mould counts in *Ganoderma* mushrooms samples are given in table 13. Sample G_3 had significantly different ($p < 0.05$) aerobic counts as compared to samples G_1, G_2, G_4 and G_6. The Aerobic counts in *Ganoderma* samples ranged between 2.5 and 5.7 log cfu/g. Samples G_1, G_2, G_3, G_4 ,G_5 and G_7 had aerobic counts below the acceptable limits (< 5.7 log cfu/g) as per European Union Commission Recommendation (directive 2004/24/EC) whereas sample G_6 had aerobic counts above the acceptable limits. This then indicate a good sanitary quality and further processing to reduce the microbial load may not be required for all samples except one.

Table 12: Aerobic, total coliforms, yeast, mould counts (log cfu/g) in *Ganoderma* mushrooms

[1]Sample	[2]Aerobic count	Total coliforms	Mould
G_1	4.0^{bcd} ±0.3	[3]ND	7.3^{a} ±0.1
G_2	4.1^{bc} ±0.3	6.6^{a} ±0.02	3.8^{d} ±0.1
G_3	2.5^{e} ±0.1	ND	3.2^{e} ±0.1
G_4	4.8^{ab} ±0.9	5.7^{a} ±1.1	5.3^{b} ±0.1
G_5	3.1^{de} ±0.0	ND	4.7^{c} ±0.01
G_6	5.7^{a} ±0.6	ND	5.2^{b} ±0.04
G_7	3.4^{cde} ±0.0	4.9^{a} ±0.01	ND

[1]G_1= *Ganoderma enigmaticum*, G_2= *Ganoderma enigmaticum*, G_3= *Ganoderma enigmaticum*, G_4= *Ganoderma enigmaticum*, G_5= *Ganoderma lucidum*, G_6= *Ganoderma enigmaticum*, G_7 = unidentified

[2]Values are mean of two replicates ±Standard deviation (n= 2)

Values with different letter superscript in columns (per parameter) are significantly ($p < 0.05$) different from each other

[3]ND= Counts below the countable range

Coliform counts were only detected in samples G_2, G_4 and G_7 and did not differ significantly ($p \geq 0.05$). The coliform counts detected were above the acceptable limits (<1 cfu/g) as per European Union Commission Recommendation (directive 2004/24/EC) (Ajis et al., 2017). On the contrary, samples G_1, G_3, G_5 and G_6 had total coliform counts below the acceptable limits. Further processing may be required for samples G_2, G_4 and G_7. No references on the microbial profile of *Ganoderma* mushrooms have been found in literature.

Yeast counts were not detected in *Ganoderma* mushrooms. This indicates that the samples were in compliance with the European Union Commission Recommendation

(directive 2004/24/EC) Ajis et al. (2017) which states that the acceptable limit for yeasts is < log 3.7 cfu/g. This may also indicate minimal yeast spoilage of *Ganoderma* mushrooms and a better shelf life.

Maximum mould count detected in *Ganoderma* mushrooms was 7.3 log cfu/g. The counts differed significantly ($p < 0.05$) in *Ganoderma* samples except between samples G_4 and G_6. No mould counts were detected in sample G_7. Based on the European Union Commission Recommendation (directive 2004/24/EC), samples G_1, G_2, G_4, G_5 and G_6 had mould counts above the acceptable limits whereas samples G_3 and G_7 had mould counts below the acceptable limit (< 3.7 log cfu/g) as per European Union Commission Recommendation (directive 2004/24/EC) (Ajis et al., 2017). Further preservation may be required to reduce yeast spoilage and extend the shelf life of *Ganoderma* mushrooms.

Salmonella was not present in 10 grams of *Ganoderma* mushrooms thus the samples were of acceptable quality with regards to *Salmonella* contamination. The absence of *Salmonella* in *Ganoderma* samples could be due to antimicrobial properties of *Ganoderma* mushrooms (Kamble et al., 2011; Shen et al., 2017). The samples were in compliance with the regulations of the Food Safety Authority of Ireland (2006) which states that *Salmonella* should not be present in 25 g portions of mushrooms.

3.4.5. Aerobic, total coliforms, yeast, and mould counts, *Salmonella* in *Termitomyces schimperi*

Aerobic counts detected in *Termitomyces schimperi* was 4.7 log cfu/g. This is slightly higher than the 4.2 log cfu/g that Kim et al. (2016) detected in dried shiitake mushrooms. The difference could be linked to the contamination during preparation or storage conditions.

Aerobic counts detected in *T. schimperi* mushroom in this study were below the acceptable limits (<3.7 log cfu/g) based on the European Union Commission Recommendation (directive 2004/24/EC) (Ajis et al., 2017). This then indicates *T. schimperi* mushrooms were of good sanitary quality.

Total coliform counts in *T. schimperi* were 5.4 log cfu/g. This is higher than the counts reported by (Ajis et al., 2017; Kim et al., 2016) in dried mushrooms. The counts were also above the acceptable limits (<1 log cfu/g) of the European Union Commission Recommendation (directive 2004/24/EC)(Ajis et al., 2017) . The presence of coliforms may indicate potential contamination of pathogens. It is therefore necessary to subject the mushrooms to further processing processes to minimise the risk of food-borne illness.

Yeasts were not detected in *Termitomyces schimperi*. The samples were in compliance with the regulations of the European Union commission with regards to maximum acceptable limits of yeasts (< log 3.7 cfu/g). This indicates that the samples could be less susceptible to yeast spoilage and thus have a potential longer shelf life.

Mould counts of 4.9 log cfu/g were detected in *T. schimperi* samples. This is higher than the counts reported by in (Ajis et al., 2017; Kim et al., 2016) in dried mushroom. The samples had mould counts above the acceptable limits (< 3.7 log cfu/g) of the European Union Commission (Ajis et al., 2017). Therefore, there is a need to investigate optimal preservation methods for *T. schimperi.*

Salmonella was not detected in 10 grams of *T. schimperi* samples. This could indicate good handling practices. Another reason could be due to the antimicrobial properties of

Termitomyces species which has been reported by Gebreyohannes, Nyerere, Bii, and Sbhatu (2019). Based on the regulations of Food Safety Authority of Ireland (2006), *Termitomyces* mushroom was of acceptable quality with regards to *Salmonella* contamination.

3.4.5. Mycotoxins in truffles

The results of mycotoxins in unwashed and washed truffles are presented in table 14. The levels of deoxynivalenol differed significantly ($p < 0.05$) between unwashed and washed truffles. Washing of truffles had significantly reduced the levels of deoxynivalenol. Food and Drug Administration, (2001) has set the regulatory levels for deoxynivalenol at 500 µg/kg in foodstuffs. Based on these regulations, all the truffle samples had deoxynivalenol levels above the allowable limits. Since mycotoxins are stable chemicals, decontamination of truffles should be carried out to reduce the levels of mycotoxins to safe levels.

The levels of fumonisin B_1 in unwashed and washed truffles did not differ significantly ($p > 0.05$). Noteworthy is the increase of the levels of fumonisin B_1 after washing truffles in sample T_1. The occurrence of fumonisin B_1 in truffles has not been reported in literature. Nevertheless, fumonisin B_1 has been reported in some tubers. Amri and Lenoi (2016) reported the occurrence of fumonisin B_1 (12.34 to 267.86 µg/kg) in dried sweet potato chips. The United States Food and Drugs Administration (FDA) have the regulatory levels for fumonisins (2000- 3000 µg/kg) in foodstuffs (Food and Drug Administration, 2001). Based on FDA regulations, the levels of Fumonisin B_1 detected in truffles irrespective of washing were below the maximum allowable limits. This

indicates that truffles were of good quality and safe for consumption with regards to fumonisin $B_{1.}$

Ochratoxin A levels differed significantly ($p < 0.05$) between unwashed and washed samples in samples T_1 and T_5. The levels of ochratoxin A in samples T_5, T_6, T_7 and T_8 increased after washing truffles but the levels did not differ significantly ($p \geq 0.05$). This could be that the toxigenic moulds that produce mycotoxins could have penetrated deep into the food and not only on the surface where the mycotoxin content or load could have been reduced by washing. Another reason could be that the toxigenic moulds could have produced mycotoxins after washing.

Although ochratoxin A occurrence in truffles has not been reported, Wen et al. (2014) reported ochratoxin A levels in the ranges of 0.32 to 5.7 µg/kg and 0.31 to 0.4 µg/kg in mouldy fresh ginger and ginger powder, respectively. Even though the processing of ginger differs from that of truffles, it is logical to compare the levels of ochratoxin A in the two commodities since they all grow underground. The advisory limits of ochratoxin A in foodstuffs for European Union is set at 5 µg/kg (Food and Agriculture Organisation, 1997). Based on the European Union regulations, only samples T_1 (unwashed) and T_5 (washed) had levels above the advisory limits, while 80% of the samples had ochratoxin A levels below the acceptable limits.

Total aflatoxin levels between unwashed and washed truffles did not differ significantly ($p \geq 0.05$). This indicates that washing of truffles had no significant effect on total aflatoxin levels in truffles. Even though occurrence of aflatoxin in truffles has not been reported in literature, Jonathan and Esho (2010) detected aflatoxin B1 (1.93µg/kg to

4.21µg/kg) in dried and stored Nigerian Oyster mushrooms. In foods stored for an extended period, there is a higher chance of aflatoxins production. FDA established regulatory limits of 20 µg/kg for total aflatoxin in foodstuffs (FAO, 2004). Based on these regulations, all unwashed and washed truffles investigated in this study had total aflatoxin levels above the allowable limits therefore the truffles were not fit for human consumption.

Although washing of truffles reduced the levels of zearalenone in samples T_5, T_6, T_7 and T_8, the levels of zearalenone did not differ significantly ($p \geq 0.05$) between unwashed and washed samples except in sample T_1 where the levels increased significantly ($P < 0.05$) from 59.0 to 9680.0 µg/kg after washing of truffles. The occurrence of zearalenone in truffles has not been reported in literature. However, zearalenone occurrence in medicinal dried rhizomes and root tubers has been reported by (Koul & Sumbali, 2008) and were in the range of 520 to 14510 µg/kg roots, tubers and truffles all grow underground. The levels of zearalenone in unwashed truffles was in the ranged of 59.0 to 271.0 µg/kg whereas in washed truffles, zearalenone levels ranged between 45.0 and 9680 µg/kg. The levels of zearalenone in truffles were less than the levels detected by Koul and Sumbali (2008) in dried rhizomes and root tubers.

The European Commission has maximum limits in place for zearalenone mostly for cereals and cereal based products. For instance, the maximum limits for cereals other than maize is 100 µg/kg (European Commission, 2006). Thailand established the maximum limits for ZEN (30-1000 µg/kg) in foodstuffs (Anukul et al., 2013). Based on zearalenone regulations for Thailand, only sample T_1 (washed) had zearalenone levels

were above the acceptable limits. This indicates that 90% of the truffle samples were fit for consumption with regards to zearalenone levels.

Table 13: Mycotoxins (µg/kg) (as is) in truffles

	Deoxynivalenol		**Fumonisin B_1**		**Ochratoxin A**		**Total aflatoxin**		**Zearalenone**	
Sample	Unwashed	washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
T_1	25462.2^{c} ±109	26113.7^{a} ±30	17.4^{b} ±6.5	65.3^{ab} ±55.7	48.5^{a} ±9.2	0.1^{c} ±0.1	26.5^{a} ±0.4	27.5^{a} ±1.3	59.0^{b} ±4.0	9680.0^{a} ±9438.0
T_5	25887.7^{b} ±167	25130.3^{d} ±98	142.1^{a} ±115.6	23.9^{ab}±2.4	1.7^{c} ±2.2	19.1^{b} ±0.7	27.3^{a} ±1.1	26.5^{a} ±0.3	117.0^{b} ±10.0	63.0^{b} ±15.0
T_6	25933.3^{a} ±55	25556.2^{c} ±58	86.1^{ab} ±66	35.8^{ab}±27.7	0.8^{c} ±0.1	4.0^{c} ±2.2	26.6^{a} ±0.2	26.7^{a} ±0.1	89.0^{b} ±3.0	59.0^{b} ±9.0
T_7	24965.7^{d} ±101	25596.2^{c} ±95	73.0^{ab} ±21.7	18.1^{b}±4.0	0.6^{c} ±0.7	3.6^{c} ±2.0	27.7^{a} ±0.3	26.5^{a} ±0.0	271.0^{b} ±109.0	50.0^{b} ±5.0
T_8	25882.2^{b} ±53	2552.3^{c} ±73	140.4^{ab} ±13.1	49.2^{ab}±48.9	0.2^{c} ±0.2	3.2^{c} ±3.1	26.8^{a} ±0.1	26.3^{a} ±0.3	99.0^{b} ±18.0	45.0^{b} ±3.0

[1]T_1, T_5, T_6, T_7, T_8= Truffles from different vendors

[2]Values are means of two replicates ±Standard deviation (n=2)

Values with different letter superscript (^{abc}) in columns per mycotoxin are significantly ($P < 0.05$) different

3.4.6. Mycotoxins (µg/kg) in *Ganoderma* mushrooms

The results of mycotoxins in *Ganoderma* mushrooms are given in table 15. The levels of deoxynivalenol ranged from 46675.2 to 48973.3 µg/kg. The levels of deoxynivalenol in *Ganoderma* samples did not differ significantly ($p \geq 0.05$). In addition, the levels of deoxynivalenol detected in *Ganoderma* mushrooms were above the maximum limits set by (Food and Drug Administration, 2001).

There were significant ($p < 0.05$) variations in the levels of fumonisin B_1 among some *Ganoderma* mushrooms. The maximum acceptable limits for fumonisins is 2000-3000 µg/kg (Food and Drug Administration, 2001). Based on these regulations, samples G_3, G_4 and G_5 had fumonisin B_1 levels below the maximum acceptable limits. On the other hand, samples G_1, G_2, G_6 G_7 and G_8 had fumonisin levels above the maximum acceptable limits set by (Food and Drug Administration, 2001).

Ochratoxin A levels in *Ganoderma* samples ranged between 4.8 and 19320.5 µg/kg. The levels of ochratoxin did not differ significantly ($p \geq 0.05$). Of note is that only sample G_4 had levels below the advisory limits based on the European Union regulations for ochratoxin A as stated by (Food and Agriculture Organisation, 1997).

The levels of total aflatoxin in *Ganoderma* mushrooms ranged between 91.7 and 100.6 µg/kg. The levels did not differ significantly except in sample G_5. The levels of total aflatoxin in *Ganoderma* mushrooms were above the maximum allowable limits (20 µg/kg) as per regulations of (Food and Drug Administration, 2001).

Zearalenone levels in *Ganoderma* mushrooms ranged from 105.0 to 5478.4 µg/kg. The levels of zearalenone in *Ganoderma* mushrooms differed significantly ($p < 0.05$) in

some samples. The European Union has zearalenone limits in place for specific foodstuffs such as cereals and not for all foodstuffs. Thailand on the other hand has set limits for zearalenone at 30-1000 µg/kg (Anukul et al., 2013). Based on the regulations for Thailand with regards to Zearalenone, all the *Ganoderma* mushrooms analysed in this study had levels exceeding the maximum allowable levels. This shows that the mushrooms were not of acceptable quality with regards to zearalenone.

Table 14: Mycotoxins (µg/kg) in *Ganoderma* mushrooms

[1]Sample	[2]*Deoxynivalenol*	Fumonisin B1	*Ochratoxin A*	*Total aflatoxin*	Zearalenone
G_1	$47806.4^{a} \pm 1935.8$	$8989.7^{ab} \pm 12313.4$	$9840.6^{a} \pm 13916.6$	$95.9^{ab} \pm 7.1$	$2781.2^{ab} \pm 3794.4$
G_2	$48973.3^{a} \pm 4.4$	$17454.2^{a} \pm 81.7$	$19122.8^{a} \pm 646.8$	$99.3^{ab} \pm 0.01$	$5467.9^{a} \pm 10.4$
G_3	$46675.2^{a} \pm 309.8$	$48.0^{b} \pm 16.8$	$13.6^{a} \pm 15.4$	$94.7^{ab} \pm 0.4$	$114.3^{b} \pm 3.7$
G_4	$47132.2^{a} \pm 200.7$	$53.4^{b} \pm 7.5$	$4.7^{a} \pm 3.9$	$93.1^{ab} \pm 0.1$	$109.6^{b} \pm 6.4$
G_5	$46830.0^{a} \pm 104.0$	$698.2^{b} \pm 603.9$	$7349.3^{a} \pm 10380.5$	$91.7^{b} \pm 1.0$	$105.0^{b} \pm 1.3$
G_6	$47392.5^{a} \pm 2146.4$	$8876.0^{ab} \pm 12311.5$	$9724.5^{a} \pm 13752.6$	$97.1^{ab} \pm 5.5$	$2915.9^{ab} \pm 3656.5$
G_7	$48859.6^{a} \pm 7.7$	$17345.4^{a} \pm 324.7$	$19320.5^{a} \pm 245.1$	$100.6^{a} \pm 0.0$	$5256.9^{a} \pm 10.0$
G_8	$48972.5.6^{a} \pm 1.1$	$17536.4^{a} \pm 123.1$	$19016.4^{a} \pm 80.4$	$100.2^{a} \pm 0.01$	$5478.4^{a} \pm 5.2$

[1]G_1= *Ganoderma enigmaticum*, G_2= *Ganoderma enigmaticum*, G_3= *Ganoderma enigmaticum*, G_4= *Ganoderma enigmaticum*, G_5= *Ganoderma lucidum*, G_6= *Ganoderma enigmaticum*, G_7 = unidentified, G_8 = unidentified

[2]Values are mean of two replicates ± standard deviation (n= 2)

Values with different letter superscript in columns per mycotoxin are significantly ($P < 0.05$) different from each other

3.4.7. Mycotoxins in *Termitomyces schimperi*

The levels of deoxynivalenol, fumonisin B_1, ochratoxin A, total aflatoxin and zearalenone in *Termitomyces schimperi* were 48897.1, 17462.2, 19391.4, 101.0, and 5529.5 µg/kg, respectively. Based on the regulations of United States Food and Drugs Administration (Food and Drug Administration, 2001), the levels of deoxynivalenol, fumonisin B_1, ochratoxin and total aflatoxin in *T. schimperi* were above the acceptable limits. FDA does not have regulations for zearalenone for all foodstuffs in place. Thailand however has set the limits for zearalenone in all foodstuffs at 30-1000 µg/kg (Anukul et al., 2013). Based on the regulations for Thailand, zeralenone levels in *T. schimperi* were above the acceptable limits. It is therefore necessary to decontaminate mushrooms to reduce mycotoxin content to safe levels.

3.4.8. Heavy metals (mg/kg) in Kalahari truffles

Heavy metal contents in unwashed and washed truffles are given in table16. Cadmium levels in truffles differed significantly ($p < 0.05$) between unwashed and washed truffles in samples T_2, T_4, T_5, T_6, T_6 (fresh), T_7, and T_8. However, cadmium levels did not differ significantly ($p \geq 0.05$) between washed and unwashed truffles in samples T_1 and T_3. Washing of truffles had reduced cadmium levels in samples T_5, T_6, T_7 and T_8. Cadmium levels in unwashed truffle samples ranged from 0.14 to 0.81 mg/kg whereas in washed counterparts, the levels ranged from not detected to 1.35 mg/kg. The levels of cadmium in truffles were lower than the 0.31 to 54.2 mg/kg cadmium levels that Sarikurkcu et al. (2011) detected in wild edible mushrooms. These differences can be attributed to levels of cadmium contamination of the fields where truffles were harvested.

The permissible limits for cadmium in food samples is 0.2 mg/kg (FAO/WHO, 2011). Based on FAO/WHO (2011) regulations, 69% of truffle samples had cadmium levels above the maximum permissible limit irrespective of washing.

Chromium levels in truffles did not differ significantly ($p \geq 0.05$) between unwashed and washed samples except for sample T_6 (fresh). The levels of chromium in unwashed truffle samples ranged between 1.13 and 14.50 mg/kg whereas in washed truffles, chromium levels were between 0.28-16.67 mg/kg. Sarikurkcu et al. (2011) reported chromium levels in the range of not detected-21.6 mg/kg in mushrooms. Czech Republic has set the limits for chromium in wild growing mushrooms at 4.0 mg/kg dry matter (Kalač, 2010). Based on these regulations all the unwashed truffle samples had levels above the acceptable limits. For washed truffles, samples T_1, T_2, T_3, T_6 and T_8 had chromium levels above the acceptable limits whereas samples T_4, T_5, T_6 (fresh) and T_8 had chromium levels below the acceptable limits. Washing of truffles had reduced chromium levels to safe levels in samples T_4, T_5, T_6 (fresh) and T_8.

The levels of iron in unwashed and washed truffles differed significantly ($p < 0.05$) except in sample T_6 (fresh). The highest levels of Iron detected were 521.04 and 746.72 mg/kg in unwashed and washed truffles, respectively. This was higher than the iron levels (400-500 mg/kg) detected in *Amanita rubescens* mushrooms by (Rudawska & Leski, 2005a, 2005b). Majority of truffle samples irrespective of washing had iron levels above the permissible limit of 43 mg/kg set by (FAO/WHO, 2011). Excess amount of Iron may cause rapid increase in pulse rate and coagulation of blood in the vessels, hypertension and drowsiness (Davies et al., 2006).

Mercury and nickel were not detected in all truffle samples. However, Fang et al. (2014) detected 0.02 mg/kg in dry mushrooms. The permissible levels for mercury in food is 0.6 mg/kg as per (FAO/WHO, 2011) standard. The absence of mercury and nickel could be an indication that the fields from which the truffles were harvested are not contaminated with mercury and nickel.

The levels of manganese in truffles differed significantly ($p < 0.05$) between washed and unwashed truffles in samples T_1, T_2, T_3, T_4, T_6, T_6 (fresh) and T_8. On the other hand, the levels of manganese in truffles did not differ significantly ($p > 0.05$) between unwashed and washed truffles in samples T_5 and T_7. The levels of manganese ranged from 0.57 to 5.61 mg/kg in unwashed truffles whereas for washed truffles, the levels ranged between 0.66 and 9.56 mg/kg. Manganese levels detected in the present study were lower than the 10-77 mg/kg that (Rudawska & Leski, 2005a, 2005b) reported in wild growing mushroom species. The maximum permissible limit for manganese in foodstuff set by FAO/WHO (2011) is 5.5 mg/kg. Seventy eight percent (78%) of the samples had manganese levels below the maximum permissible levels set by (FAO/WHO, 2011). This indicates that majority of truffle samples were safe for human consumption with regards to manganese contamination.

For unwashed truffles, lead was not detected in samples T_2, T_4, T_5, T_6, and T_6 (fresh) whereas for washed truffles, lead was not detected in samples T_3, T_4, T_5, T_6, and T_6 (fresh). The highest lead level detected in unwashed truffles was 20.76 mg/kg whereas for washed truffles, the highest level was 24.44 mg/kg. Fang et al. (2014) detected lower lead content in the range of 0.038–2.539 mg/kg in dried mushrooms as compared to the results of this study. These differences could be due to the fact that lead contents in

saprophyte mushrooms tend to be higher than those of mycorrhizal species (Garcia et al., 1998).

The maximum permissible limit for lead in foodstuffs is 1.0 mg/kg. Although lead was not detected in (56%) of the samples, 44% of the samples had lead levels above the permissible limit of (FAO/WHO, 2011). Consumption of foodstuff contaminated with lead may pose health risks.

The levels of zinc differed significantly ($p < 0.05$) between unwashed and washed truffles in samples T_1, T_3, T_4, T_5, T_7 and T_8. Oppositely, the levels of zinc did not differ significantly between unwashed and washed truffles in sample T_2, T_6 and T_6 (fresh). Zinc levels ranged between 11.70 to 153.24 mg/kg in unwashed truffles whereas in washed counterparts, zinc levels were in the range of 8.25 to 136.63 mg/kg. Falandysz et al. (2001) detected zinc as high as 0.59 mg/kg in dried mushrooms. Zinc is found to be an antagonist of other metals such as cadmium, lead and nickel thus its presence in some mushrooms reduces the risks associated with other toxic metals at high concentrations (F. WHO, 1996) thus the high levels of zinc could possibly reduce the toxicity of other metals. In the present study, nine samples had zinc levels above the permissible limit and the other remaining samples had zinc levels below the maximum acceptable limit of 60 mg/kg set by (WHO, 1982).

Table 15: Heavy metals (mg/kg) in truffles

	Cadmium		Chromium		Iron		Lead		Manganese		Zinc	
Sample	**Unwashed**	**Washed**	**Unwashed**	**Washed**	**Unwashed**	**Washed**	**Unwashed**	**Washed**	**Unwashed**	**Washed**	**Unwashed**	**washed**
T_1	0.14^{f}± 0.01	0.26^{def}± 0.06	10.1^{de} ±0.5	16.67^{a} ±1.3	521.04^{b}± 36.4	746.72^{a}± 40.0	20.76^{ab} ± 1.1	5.98^{c}± 0.6	5.61^{c} ±0.5	6.10^{b}±0.1	153.24^{a}±9 .2	103.61^{c} ±3.0
T_2	0.81^{b} ± 0.2	0.34^{de}± 0.1	9.31^{def} ±1.1	5.75^{g}±0. 3	300.03^{e} ±27.4	166.64^{g}± 2.5	ND	1.12^{d} ± 0.03	3.15^{f}±0.3	2.67^{g} ±0.01	67.93^{e} ±3.3	65.02^{e}±1. 9
T_3	0.28^{def}± 0.09	0.21^{ef}± 0.02	10.74^{cd}±0. 3	14.07^{b} ± 0.4	434.34^{c}± 15.5	531.44^{b}± 15.2	18.43^{b} ±2.2	ND	4.75^{d}± 0.2	5.57^{c}±0.0 3	135.17^{b}±7 .1	51.98^{gh} ± 0.3
T_4	0.39^{cd}±0.0	0.16^{f}± 0.08	14.50^{b}±0.5	2.23^{h}± 0.2	404.30^{cd}± 17.8	120.03^{h}± 0.01	ND	ND	3.84^{e}± 0.1	1.50^{ij}±0. 01	49.56^{gh} ±2.8	61.79^{ef}± 1.7
T_5	0.27^{def}± 0.0	ND	12.14^{c}±1.3	2.57^{h}± 0.05	259.21^{f}± 4.1	147.62^{gh} ±15.1	ND	ND	2.79^{fg}±0.0 3	2.76^{fg}±0. 04	56.13^{fg}± 0.6	46.26^{h}±0. 2
T_6	0.25^{def} ± 0.02	ND	5.66^{g} ± 0.5	8.16^{f}±1.6	108.96^{h}± 22.5	377.33^{d}± 30.2	ND	ND	1.77^{hi} ±0.3	2.84^{fg}±0. 3	45.33^{h} ± 2.9	46.43^{h}±3. 5
T_6 (fresh)	0.16^{ef} ± 0.06	ND	1.13^{hi}±0.0 5	0.28^{i}± 0.03	42.66^{i}± 3.7	55.19^{i}± 1.8	ND	ND	0.57^{k}±0.03	0.66^{k} ±0.0	11.70^{i}±1. 0	8.25^{i}± 0.2
T_7	0.53^{c}±0.07	ND	4.59^{g} ±0.3	2.09^{h}± 0.3	241.34^{f} ± 11.1	117.18^{h}± 2.2	9.5^{c} ±2.2	9.04^{c}±0. 5	1.98^{h}±0.1	1.22^{j} ±0.1	85.57^{d} ±4.8	65.09^{e}± 1.3
T_8	0.27^{def}± 0.09	1.35^{a}± 0.06	8.62^{ef}± 0.6	10.65^{cd}± 0.8	366.67^{d}±1 4.5	444.27^{c}± 12.9	2.07^{d}±0.5	24.44^{a}± 3.1	5.20^{c}±0.2	9.56^{a}±0.0 1	52.61^{gh}± 1.3	136.63^{b}± 0.4

[1]T_1-T_8= Truffles from different vendors

[2]Values are means of two replicates ± standard deviation

Values with different superscript letters in rows per heavy metal differ significantly ($p < 0.05$)

3.3.9. Heavy metals in *Termitomyces schimperi*

The levels of cadmium, chromium, iron, manganese and zinc in *T. schimperi* mushroom were 0.66, 0.15, 57.47, 21.56 and 116.84 mg/kg, respectively. The levels of cadmium and iron in *T. schimperi* were above the 0.2 mg/kg and 43 mg/kg limits set by FAO/WHO (2011) respectively. Chromium levels detected in *Termitomyces schimperi* mushrooms were below the acceptable limits of 4.0 mg/kg of Czech Republic (Kalač, 2010). The levels of manganese and zinc detected in *T. schimperi* in the current study was above the permissible limits of 5.5 mg/kg (FAO/WHO, 2011) and 60 mg/kg (WHO, 1982) Moreover, nickel, lead and mercury were not detected in *T. schimperi* mushrooms. Overall, the samples were not safe for human consumption in terms of heavy metals.

3.5. Conclusions

Kalahari truffles in Namibia are underprized underpriced as compared to truffles in other African countries and beyond. Similarly, there is underutilisation of *Ganoderma* mushrooms as compared to Asian countries. *Termitomyces schimperi* mushrooms are commonly harvested for household consumption and for selling along the roadsides. It appeared that the pricing of Kalahari Desert truffle depends on the vendor's intuition as the prices were not consistent regarding weight. Irrespective of washing of truffles, 87.5% of the samples had aerobic counts below the acceptable limits. On the other hand, 81%, 69% and 88% of the truffle samples had total coliforms, yeast, and mould counts above the acceptable limits regardless of washing of truffles. *Salmonella* was not detected in all the three (3) sampled mushroom species. For *Ganoderma* samples, aerobic counts were below the acceptable limits and no coliforms counts were detected

in 57% of the samples. For *Termitomyces schimperi*, aerobic total coliform and mould counts were below the acceptable limits. Deoxynivalenol levels detected in truffles, *Ganoderma* and *Termitomyces schimperi* were above the acceptable limits. Majority of truffle samples had fumonisin B_1 levels below the acceptable limits. On the other hand, fumonisin B_1 levels for *Ganoderma* and *Termitomyces schimperi* were above the acceptable limits. Ochratoxin A levels detected in majority of truffle samples were below the acceptable limits. The levels of total aflatoxin, and zearalenone were above the acceptable limits for truffles, *Ganoderma* and *Termitomyces schimperi*. Majority of truffle samples had cadmium, chromium, iron and manganese levels above the permissible levels. Lead was not detected in most truffle samples. For *Termitomyces schimperi,* the levels of cadmium, iron and zinc were above the permissible limits whereas the levels of chromium were below the permissible limit. Lead was not detected in *Termitomyces schimperi* mushrooms. Nickel and mercury were neither detected in truffles or *Termitomyces schimperi* mushrooms. The synergic effects of microorganisms, mycotoxins and heavy metals in underprised indigeneous mushrooms may impair human health thus they should be subject to appropriate processing before consumption.

Chapter 4: General discussion, Conclusions and Recommendations

4.1. Prices and socio-economic importance of Kalahari truffles, *Termitomyces schimperi* and *Ganoderma* mushrooms in Namibia

Kalahari truffles and *Termitomyces* in rural communities in Namibia are harvested for use at household level and also for selling in the informal markets. *Ganoderma* is generally used for in rural communities as a remedy for several ailments. Truffle harvesters could earn more if they had better access to even low-level technology such as simple packaging equipment or refrigeration technology. Preservation and processing of Kalahari truffles, *Ganoderma* and *Termitomyces schimperi* mushrooms into high value export products and marketing has a potential to penetrate the world market and contribute to the economic growth of countries where it currently is underutilised and not well researched.

4.2. Sustainability of Kalahari truffles, and *Ganoderma and Termitomyces* mushrooms

The production of Kalahari truffles is reported to be declining mostly in places where livestock is concentrated (Trappe et al., 2008). Some land practices such as ploughing may also pose a threat to truffle production. Some practices such as clearing land for cultivation may result in the destruction of *Ganoderma* mushrooms host species which may pose a threat to the sustainability of *Ganoderma* mushrooms. From a personal observation, in some communities such as northern Namibia, the termite mounts soil is used to make floors of traditional huts. The destruction of termite mounts may lead to unsustainable production of *Termitomyces* mushrooms.

4.3. Microbial load of truffles, *Ganoderma* and *Termitomyces*

Since washing of truffles reduced bacterial and fungal counts in truffles, it could be used to reduce bacterial populations, and to improve the microbiological safety of truffles. Synergistic combinations of different treatments could also be employed to extend the shelf life of truffles. For example, a combination of blanching in 4% Nacl solution for 4 minutes and immediate flash freezing at –18°C was reported to be the best preservation method with regard to overall quality of truffles as compared to freezing, soaking in vinegar and blanching for 2 or 4 minutes and blanching for 2 minutes in 2 or 4 % boiling salt solution (Al-Ruqaie, 2002). *Ganoderma* and *T. schimperi* mushrooms could also be subjected to decontamination procedures to reduce their microbial load.

4.4. Mycotoxin in truffles, *Termitomyces schimperi* and *Ganoderma* mushrooms

Fleshy, underground tubers and rhizomes appear to be more susceptible to mycotoxin contamination since they are rich in mineral salts and carbohydrates which are reported to be stimulants for mycotoxin production (Jarvis 1971). Truffles are also found to have high carbohydrate content Murcia et al. (2003) which may have stimulated mycotoxin production. Although the levels of some mycotoxins were below the maximum acceptable limits, it is a concern because truffles are consumed by all age groups including young children and individuals with compromised immune systems which put them at a high risk. Chilaka, De Boevre, Atanda, and De Saeger (2018) highlighted that the co-occurrence of mycotoxins in Cassava and Yam may lead to synergistic health effects. This could also apply to individuals consuming truffles and *T. schimperi* mushrooms contaminated with mycotoxins.

Wild growing *Ganoderma* mushrooms are generally exposed to conditions that may be optimal for mycotoxin production thus the high levels of mycotoxins detected in *Ganoderma* samples in this study. Since *Ganoderma* mushrooms can be processed into capsules and *Ganoderma* tea, there is a need to carry out mycotoxin screening prior to processing and on the final products. *Ganoderma* mushrooms can also be processed into a powder form and rubbed on infants' foreheads to strengthen the skull bones. This could also put the infants at a risk of dermal exposure of mycotoxins.

4.5. Mycotoxin analytical challenges

Since mycotoxins have different structures, one standard technique cannot be used to detect all mycotoxins (Turner et al., 2009). Another challenge is the transformation of the original mycotoxin (masked or conjugated) into a different structure, example Deoxynivalenol-3-glucoside (DON-3-G) is viable and could result unreported by traditional analytical methods due to its detectability (Pascari, Gil-Samarra, Marín, Ramos, & Sanchis, 2019).

4.6. Heavy metals in truffles and *Termitomyces schimperi*

Since washing of truffles reduced the levels of some heavy metals in some samples, washing could be used to reduce heavy metal levels in truffles. High levels of heavy metals detected in some unwashed truffle samples could be a result of contaminated soil surrounding truffles. Although lead and nickel were not detected in *Termitomyces schimperi* mushooms, the levels of cadmium, chromium, iron, manganese and zinc were above the acceptable limits. This could mean that the mushrooms were collected from contaminated fields.

4.7. How choice of processing could have influenced the microbiological quality

Washing of truffles may have served as a decontamination procedure. With regards to (above ground mushrooms), washing mushrooms with water can remove soil fragments but produces a higher deterioration of mushrooms owing to the harm to the hyphae caused by water absorption, encouraging microbial growth and the incidence of bacterial blotch (Sapers, Miller, Miller, Cooke, & Choi (1994). This could then imply that washing of truffles may have influenced the microbial quality of the truffles.

Despite the fact that drying inactivates some microorganisms, some microorganisms recover in dried foods, particularly in cases where drying is not well managed (Pittia & Antonello, 2016) Drying of Kalahari truffles and *Termitomyces schimperi* could have had an effect on the microbial results of this study.

Freezing is found to kill microorganisms by physical and chemical effects and perhaps through induced genetic changes (Archer, 2004).Therefore, freezing of Kalahari truffles and *Termitomyces schimperi* could have caused unintended microbial injury and death which could in turn influence the microbiological quality of the truffles. This could then imply that the microbial results obtained may not indicate the initial microbial load of truffles and *Termitomyces schimperi*. Generally, chemical analysis may not have been affected by freezing and drying thus the results provides the initial contents.

On the contrary, Mushrooms (including truffles and *Termitomyces schimperi)* have a short lifespan. Truffles in the rural communities are harvested at the time when people are also busy threshing Mahangu thus they may opt to prepare truffles by washing, cutting and cooking. From a personal observation, truffles in the rural communities are

also washed, cut into small pieces and preserved by drying for later use. Moreover, if truffles or *Termitomyces schimperi* are to be composited then one would wash, dry and freeze.

In a study by Saltarelli, Ceccaroli, Cesari, Barbieri, & Stocchi, (2008) fresh truffles were washed with distilled water, cut into small pieces. The pieces were then frozen and subjected to microbial analysis. The current study partially followed the sample preparation used by Roberta et al., 2008).

4.8. Conclusions

In Namibia, indigenous Kalahari truffle, *Ganoderma* and *Termitomyces schimperi* mushrooms are not utilised to their full potential as compared to other nations. There was no consistency in pricing of Kalahari truffles. Aerobic counts in most truffles, *Ganoderma* and *Termitomyces scimperi* mushrooms were below acceptable limits. Total coliforms were not detected in *Ganoderma* mushrooms but were above the acceptable limits in truffles. *Termitomyces schimperi* mushrooms had total coliform counts below the acceptable limits. Mould and yeasts were detected in the mushrooms. *Salmonella* was not detected in either of the mushrooms. Mycotoxins were detected in the mushrooms and deoxynivalenol was the overall prevalent mycotoxin in the mushrooms. Traces of heavy metals were also detected, and some such as cadmium was above the acceptable limits. The study presented data on the microbial, mycotoxins and heavy metals of Kalahari truffle, *Ganoderma* and *Termitomyces schimperi* mushrooms indigenous to Namibia that adds to scientific knowledge and has potential to influence policy, standards and opportunities for quality management and upscale of the three mushroom species.

4.9. Recommendations

Based on the results, the study recommends that the strategies that could be used to reduce bacterial populations and to improve the microbiological safety of truffles should be extensively investigated. Washing of truffles under running water reduced the bacterial and fungal populations in some samples and could therefore be used to reduce bacterial populations. The study also recommends that truffles should not be eaten raw due to safety issues.

Furthermore, since wild growing fungi are an important source of additional income, especially in less favoured rural areas, the study recommends that more research needs to be done in order to get a deeper insight into the market chain and to get more accurate information on the wider social, economic and environmental issues that concern wild growing mushrooms. The study also recommends that as trade increases, wild growing fungi should be taken into account in forest management programs in order to guarantee their sustainable development.

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