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# Assessment of Microbiological Properties, Mycotoxins, and Heavy Metals in Underprized Raw Kalahari Truffles Sold in Namibia

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# HIGHLIGHTS

Article type

- Total aerobic count of unwashed truffles ranged from 4.4 to 7.3 log Colony Forming Unit (CFU)/g.
- Ochratoxin A levels in unwashed truffles ranged from 0.1 to  $48.5 \,\mu g/kg$ .
- Total aflatoxin levels were 26.3 to 27.5 μg/kg, while zearalenone levels ranged from 45.0 to 9,680 μg/kg.

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Acronyms and abbreviations CFU=Colony Forming Unit

# ABSTRACT

**Background:** Kalahari truffle (*Kalaharituber pfeilii*) is found in the Kalahari desert and nearby regions (Africa). This study assessed the microbiological quality and safety, mycotoxins, and heavy metals contents of raw Kalahari truffle sold in Namibia.

**Methods:** Batches of Kalahari truffles were purchased from informal markets and different vendors in Namibia. Total aerobic, coliform, yeast, and moulds counts, and *Salmonella* were assessed. Also, some mycotoxins and heavy metals were determined. Data were analyzed using SPSS Statistics Software, Version 25.

**Results:** Total aerobic count of unwashed truffles ranged from 4.4 to 7.3 log Colony Forming Unit (CFU)/g. Total coliform counts detected in truffles were 6.0 log CFU/g. *Salmonella* was not detected. Doxynivalenol was the most prevalent mycotoxin. Fumonisin B<sub>1</sub> levels ranged from 17.4 to 142.1  $\mu$ g/kg. Ochratoxin A levels in unwashed truffles ranged from 0.1 to 48.5  $\mu$ g/kg. Total aflatoxin levels were 26.3 to 27.5  $\mu$ g/kg, while zearalenone levels ranged from 45.0 to 9,680  $\mu$ g/kg. The iron content was up to 746.72 mg/kg. Cadmium and zinc were detected in the studied samples, but mercury and nickel were no detectable in any samples.

**Conclusion:** The studied truffle samples were safe in terms of *Salmonella*, mercury, and nickel. However, some of the detected microorganisms, mycotoxins, and heavy metals in underprized Kalahari truffles may impair the safety, shelf life, and human health. Thus, they should be subjected to appropriate processing before consumption.

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## Introduction

Truffles are edible ectomycorrhizal, hypogenous mushrooms found in many countries such as Italy, Germany, France, USA, China, Saudi Arabia, Namibia, Botswana, and Zambia. In some developing countries such as those in southern Africa, truffles are seasonal and are harvested for primarily household consumption and for sale at in

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formal markets and along the main roads. Kalahari truffle (*Kalaharituber pfeilii*) is found in the Kalahari desert and nearby regions (Africa). They are underpriced as compared to other truffles in other parts of the world (Álvarez-Lafuente et al., 2018; Trappe et al., 2008).

Fresh mushrooms including truffles have a high moisture content and water activity which make them an ideal medium for microbial growth. For instance, Venturini et al. (2011) detected total microbial counts in the range from 4.4 to 9.4 log Colony Forming Unit (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 (Ünüsan, 2019). Another safety aspect of mushrooms is the presence of heavy metals. High levels of heavy metals can cause health complications in humans. These include neurotoxicity, binding to proteins and enzymes and the promotion of oxidative stress that damageimportant molecules such as the DNA, proteins, and lipids (Paithankar et al., 2021). Heavy metals such as lead (Pb), cadmium (Cd), manganese (Mn), copper (Cu), nickel (Ni), and cobalt (Co) have been detected in wild fresh and unprocessed mushroom species (Sarikurkcu et al., 2011). To preserve and understand the safety of these seasonal and telluric delicacies, this study investigated the microbial quality, mycotoxins, and heavy metals in wild growing edible raw Kalahari truffle, one of the least studied truffles.

#### Materials and methods

## Sample collection and treatment

From May to June 2018, eight batches of fresh raw Kalahari Desert truffles (*Kalaharituber pfeilii*) were purchased from different vendors at the main informal markets where Kalahari truffles are seasonally sold in Namibia (Figure 1), including Omuthiya, Ondangwa, and Casablanca open markets. 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 near 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.

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 two 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 one sample (T<sub>6</sub>; fresh) was frozen without drying.

#### Moisture content

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 min and cooled for 45 min in a desiccator before weighing and moisture determination.

## Microbial analyses

#### -Aerobic plate count

Total aerobic plate count was carried out following a method described by Maturin and Peeler (2001). Five g of mushroom sample was placed in a sterile stomacher bag and homogenized using a stomacher (Seward) in 45 ml buffered peptone water (Acumedia lab, UK) for 30 s. Tenfold serial dilutions were prepared, then appropriate dilutions  $(10^{-6})$  were pour plated onto plate count agar (Acumedia lab, UK) and incubated at 35 °C for 48 h.

#### -Coliforms

Coliforms enumeration was carried out using a method described by Feng et al. (2002). Dilution  $10^{-1}$  was prepared by homogenising 5 g of mushroom sample into 45 ml of buffered peptone water (Acumedia lab, UK) for 30 s. Tenfold serial dilutions were prepared, and appropriate dilutions ( $10^{-6}$ ) 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 h.

## -Yeast and moulds

Five g of mushroom sample was homogenised into 45 ml of buffered peptone water for 30 s. Tenfold serial dilutions  $(10^{-1}-10^{-6})$  were prepared and spread-plated in duplicates on Rose Bengal chloramphenicol agar (Acumedia lab, UK) plates. The plates were incubated at 25 °C for 5 days.

## -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 5 g 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 h at 42 °C. After culturing on selective enrichment media plates of Xylose Lysine Desoxycholate (XLD) agar (Scharlau lab, Spain), the plates were assessed for *Salmonella* after 24 h of incubation at 35 °C.

## Mycotoxins analyses

Truffles (T<sub>1</sub>, T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>, and T<sub>8</sub>) were analysed for deoxynivalenol, fumonisin B<sub>1</sub>, ochratoxin A, total aflatoxins, and zearalenone using Enzyme-Linked ImmunoSorbent Assay (ELISA) kits (Elabscience<sup>®</sup>, USA). Each kit was used for extraction and analysis, according to the manufacturer's instruction. 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 microplate reader (SpectraMax 190) set at the respective wavelength.

#### Heavy metal analysis and daily intake of metals

Heavy metals (Cu, Fe, Zn, Cd, Mn, Ni, Pb, Hg, Cr) analyses was carried out according to the method described by Giron (1973). The quantitative determination of elements was carried out using ICAP Series (ICP Spectrometer; Thermo Scientific, USA). Daily Intake of metal was calculated for the maximum detected level per analyzed metal following the equation and assumptions reported by Sarikurkcu et al. (2020). Briefly, the amount of mushroom consumed by an average adult person (assumed to be 30 g dried) was multiplied with the metal concentration in the dried mushroom. This mathematical product was then divided by the average body weight of the consumer, assumed to be 70 kg.

#### Statistical analyses

All tests were done in duplicates. Data was subjected to an analysis of variance (one-way ANOVA) and Duncan's least significant differences (p<0.05) test using SPSS Statistics Software, Version 25 (IBM, USA).

## Results

## Moisture content

The moisture contents of unwashed and washed truffles are given in Table 1. The moisture content of dried unwashed truffles ranged from 10.0 to 17.6%, whereas that of dried washed truffles was in the range of 7.8 and 17.5%. Fresh samples, unwashed, and washed truffles had moisture contents ranged from 70.0% to 72.5%.

#### Aerobic count, total coliforms, yeast, mould, Salmonella

The aerobic count, total coliforms, yeast, and mould counts in truffles are given in Table 2. Washing of truffles reduced the aerobic counts, but the counts did not differ significantly ( $p \ge 0.05$ ) from those of the unwashed truffles except for sample T8 where washing had significantly reduced the total aerobic counts (p < 0.05). The total coliform counts were not significantly ( $p \ge 0.05$ ) different between unwashed and washed truffles for samples T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>6</sub>, and T<sub>8</sub>, whereas for samples T<sub>1</sub> and T<sub>7</sub>, the total coliform counts differed significantly (p < 0.05) between washed and unwashed truffles. There was no detected yeast in the unwashed samples  $T_5$ ,  $T_6$ (fresh), T7, and T8. The yeast counts for these samples (T<sub>5</sub>, T<sub>7</sub>, and T<sub>8</sub>) were significantly (p < 0.05) higher in the washed counterparts. There was no Salmonella in each 10 g of truffle sample.

#### **Mycotoxins**

The results of mycotoxins in unwashed and washed truffles are presented in Table 3. Washing of truffles significantly (p<0.05) reduced the levels of deoxynivalenol, but the levels of fumonisin B<sub>1</sub> in unwashed and washed truffles had no significant (p>0.05) difference. The ochratoxin A levels differed significantly (p<0.05) between unwashed and washed samples in samples T<sub>1</sub> and T<sub>5</sub>. Although the levels of ochratoxin A in samples T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>, and T<sub>8</sub> increased after the washing of truffles, this increase was not statistically significant (p≥0.05). Regarding the total aflatoxin and zearalenone, there were no significant (p≥0.05) differences between the levels analyzed in the unwashed and the washed truffles.

#### Heavy metals

The levels of studied heavy metals in unwashed and washed truffles are given in Table 4. Noteworthy is that Hg and Ni were detected in none of the truffle samples. The levels of Cd differed significantly (p<0.05) between the unwashed and the washed truffles in all samples except for samples T<sub>1</sub> and T<sub>3</sub>. Essentially, washing of truffles reduced the levels of Cd in samples T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>, and T<sub>8</sub>. The Cr levels in truffles did not differ significantly (p≥0.05) between unwashed and washed samples except for sample T<sub>6</sub> (fresh). The levels of Fe in unwashed and washed truffles differed significantly (p<0.05) in all the samples except for sample T<sub>6</sub> (fresh). The levels of Mn differed significantly (p<0.05) between washed and

unwashed truffles in all samples except for samples T<sub>5</sub> and  $T_{7}$ . The levels of Zn differed significantly (p < 0.05) between unwashed and washed truffles in all truffle samples except for truffle sample  $T_2$ ,  $T_6$ , and  $T_6$  (fresh). Based on the highest levels determined for each heavy metal in this study, the maximum daily metal intakes (µg/kg body weight day or one serving per day) were calculated. The maximum daily intakes for Cd, Cr, Fe, Mn, and Zn were 0.17, 7.14, 320.02, 4.10, and 65.67, respectively.



Figure 1: Batches of truffles sold at the open market in Namibia

Table 1: Moisture (%) content of Kalahari truffles sold in Namibia

Sample	Unwashed	Washed		
T <sub>1</sub> (dried)	12.5 <sup>bc</sup> ±3.5	17.5 <sup>a</sup> ±3.5		
T <sub>2</sub> (dried)	12.5 <sup>bc</sup> ±3.5	13.95 <sup>bc</sup> ±0.5		
T <sub>3</sub> (dried)	$10.0 ^{bc} \pm 0.0$	12.05 <sup>bc</sup> ±4.2		
T <sub>4</sub> (dried)	17.6 <sup>b</sup> ±4.6	15.4 <sup>bc</sup> ±0.6		
T <sub>5</sub> (dried)	10.0 <sup>bc</sup> ±0.0	10.0 <sup>bc</sup> ±0.0		
T <sub>6</sub> (dried)	15.0 <sup>bc</sup> ±7.1	7.25 °±3.9		
$T_6$ (fresh)	$72.5^{a} \pm 3.5$	$70.0^{a} \pm 7.1$		
T <sub>7</sub> (dried)	12.5 <sup>bc</sup> ±3.5	15.0 <sup>bc</sup> ±2.1		
T <sub>8</sub> (dried)	$14.0^{bc} \pm 0.5$	$7.8^{\circ} \pm 3.1$		

Values are means of two replicates  $\pm$ standard deviation Values with different superscript letter (<sup>abc</sup>) in a row differ significantly (p<0.05)

Sample	Aerobic count		Total c	oliforms	Ye	east	Mould		
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	
T <sub>1</sub>	$6.4^{ab} \pm 0.0$	5.5 <sup>bc</sup> ±0.0	6.2 <sup>a</sup> ±0.0	3.9 bcd ±0.1	7.2 <sup>a</sup> ±0.1	5.7 bcd ±0.5	$5.8^{ab} \pm 0.7$	4.7 bcde ±0.5	
T <sub>2</sub>	5.2 bcd ±0.5	4.6 <sup>cd</sup> ±1.2	$4.5^{abcd} \pm 0.6$	2.7 <sup>cd</sup> ±0.0	4.7 def ±0.8	$4.5^{\text{def}} \pm 0.0$	4.7 bcde ±0.2	3.7 <sup>def</sup> ±0.5	
T <sub>3</sub>	4.8 <sup>cd</sup> ±0.2	4.5 <sup>cd</sup> ±0.1	5.0 <sup>ab</sup> ±1.6	4.4 abcd ±0.5	6.6 <sup>ab</sup> ±0.03	5.0 cde ±0.7	5.4 <sup>bc</sup> ±0.1	4.6 bcde ±0.4	
$T_4$	5.5 <sup>bc</sup> ±0.0	4.9 <sup>cd</sup> ±0.7	3.4 bcd ±0.6	$4.5^{abc} \pm 0.6$	$4.9^{cdef} \pm 1.5$	5.3 bcd ±0.0	4.4 <sup>cde</sup> ±1.7	5.2 <sup>bc</sup> ±0.3	
T <sub>5</sub>	4.8 <sup>cd</sup> ±0.0	4.6 <sup>cd</sup> ±0.8	4.3 abcd ±0.7	ND	ND	3.5 <sup>f</sup> ±0.9	5.0 bcd ±0.6	4.7 bcde ±0.7	
$T_6$	4.9 <sup>cd</sup> ±0.4	4.5 <sup>cd</sup> ±0.2	$4.1^{abcd} \pm 1.1$	$2.4^{d} \pm 0.0$	3.7 <sup>ef</sup> ±0.3	$4.9^{\text{cdef}} \pm 1.0$	4.3 <sup>cde</sup> ±0.0	3.6 <sup>ef</sup> ±0.2	
T <sub>6</sub> (fresh)	$4.6^{cd} \pm 1.2$	6.3 <sup>ab</sup> ±0.1	ND*	ND	ND	ND	$2.9^{\text{ f}} \pm 0.1$	$3.6^{ef} \pm 0.2$	
$T_7$	$6.2^{ab} \pm 0.2$	$5.2^{bcd} \pm 0.5$	$5.1^{ab} \pm 0.0$	$2.5^{d} \pm 2.0$	ND	$6.3^{abc} \pm 0.6$	$6.9^{a} \pm 0.5$	5.5 <sup>bc</sup> ±0.7	
$T_8$	$7.3^{a} \pm 0.1$	$4.0^{d} \pm 0.0$	3.6 <sup>bcd</sup> ±0.2	$4.3^{abcd} \pm 1.0$	ND	$6.4^{ab} \pm 0.8$	4.8 bcde ±0.1	4.8 bcde ±0.0	

 $T_1$ - $T_8$  = Truffles from different vendors

Values are mean of two replicates ±Standard deviation,

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

\*ND: Not Detected

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Table 3: Mycotoxins (µg/kg) in Kalahari truffles sold in Namibia

Sample	Deoxynivalenol		Fumonisin B <sub>1</sub>		Ochratoxin A		Total aflatoxin		Zearalenone	
-	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
T1	25462.2 ° ±109	26113.7 <sup>a</sup> ±30	17.4 <sup>b</sup> ±6.5	65.3 <sup>ab</sup> ±55.7	48.5 <sup>a</sup> ±9.2	0.1 ° ±0.1	26.5 <sup>a</sup> ±0.4	27.5 <sup>a</sup> ±1.3	59.0 <sup>b</sup> ±4.0	9680.0 <sup>a</sup> ±9438.0
T <sub>5</sub>	25887.7 <sup>b</sup> ±167	25130.3 <sup>d</sup> ±98	142.1 <sup>a</sup> ±115.6	23.9 <sup>ab</sup> ±2.4	1.7 ° ±2.2	19.1 <sup>b</sup> ±0.7	27.3 <sup>a</sup> ±1.1	26.5 <sup>a</sup> ±0.3	117.0 <sup>b</sup> ±10.0	63.0 <sup>b</sup> ±15.0
T <sub>6</sub>	25933.3 a ±55	25556.2 ° ±58	86.1 <sup>ab</sup> ±66	35.8 <sup>ab</sup> ±27.7	0.8 ° ±0.1	4.0 ° ±2.2	26.6 <sup>a</sup> ±0.2	26.7 <sup>a</sup> ±0.1	89.0 <sup>b</sup> ±3.0	59.0 <sup>b</sup> ±9.0
T <sub>7</sub>	24965.7 <sup>d</sup> ±101	25596.2 ° ±95	73.0 <sup>ab</sup> ±21.7	18.1 <sup>b</sup> ±4.0	0.6 ° ±0.7	3.6 ° ±2.0	27.7 <sup>a</sup> ±0.3	26.5 <sup>a</sup> ±0.0	271.0 <sup>b</sup> ±109.0	50.0 <sup>b</sup> ±5.0
T <sub>8</sub>	25882.2 <sup>b</sup> ±53	25552.3 ° ±73	140.4 <sup>ab</sup> ±13.1	49.2 <sup>ab</sup> ±48.9	0.2 <sup>c</sup> ±0.2	3.2 ° ±3.1	$26.8^{a} \pm 0.1$	26.3 <sup>a</sup> ±0.3	$99.0^{b} \pm 18.0$	45.0 <sup>b</sup> ±3.0
T1, T5, T6, T7, T8=Truffles from different vendors.										

Values are means of two replicates  $\pm$  Standard deviation, Values with different letter superscript ( $\frac{abc}{b}$ ) in columns per mycotoxin are significantly (p<0.05) different

Table 4: Heavy metals (mg/kg) in Kalahari truffles sold in Namibia

Sample	Cadmium (Ca)		Chromium (Cr)		Iron (Fe)		Manganese (Mn)		Zinc (Zn)	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
T <sub>1</sub>	$0.14^{t} \pm 0.01$	0.26 <sup>def</sup> ±0.06	10.1 <sup>de</sup> ±0.5	16.67 <sup>a</sup> ±1.3	521.04 <sup>b</sup> ± 36.4	746.72 <sup>a</sup> ±40.0	5.61° ±0.5	6.10 <sup>b</sup> ±0.1	153.24 <sup>a</sup> ±9.2	$103.61^{\circ} \pm 3.0$
T <sub>2</sub>	$0.81^{b} \pm 0.2$	$0.34^{de} \pm 0.1$	9.31 <sup>def</sup> ±1.1	5.75 <sup>g</sup> ±0.3	300.03° ±27.4	$166.64^{g} \pm 2.5$	3.15 <sup>f</sup> ±0.3	2.67 <sup>g</sup> ±0.01	67.93° ±3.3	65.02°±1.9
$T_3$	$0.28^{def} \pm 0.09$	$0.21^{ef} \pm 0.02$	10.74 <sup>cd</sup> ±0.3	$14.07^{b} \pm 0.4$	434.34°± 15.5	531.44 <sup>b</sup> ± 15.2	$4.75^{d} \pm 0.2$	5.57°±0.03	135.17 <sup>b</sup> ±7.1	$51.98^{gh} \pm 0.3$
$T_4$	0.39 <sup>cd</sup> ±0.0	$0.16^{t} \pm 0.08$	14.50 <sup>b</sup> ±0.5	$2.23^{h} \pm 0.2$	$404.30^{cd} \pm 17.8$	$120.03^{h} \pm 0.01$	$3.84^{e} \pm 0.1$	$1.50^{ij} \pm 0.01$	49.56 <sup>gh</sup> ±2.8	$61.79^{et} \pm 1.7$
T5	$0.27^{def} \pm 0.0$	ND	12.14°±1.3	$2.57^{h}\pm 0.05$	$259.21^{f} \pm 4.1$	147.62 <sup>gh</sup> ±15.1	2.79 <sup>fg</sup> ±0.03	2.76 <sup>fg</sup> ±0.04	$56.13^{tg} \pm 0.6$	46.26 <sup>h</sup> ±0.2
T <sub>6</sub>	$0.25^{def} \pm 0.02$	ND	$5.66^{g} \pm 0.5$	8.16 <sup>f</sup> ±1.6	108.96 <sup>h</sup> ± 22.5	377.33 <sup>d</sup> ±30.2	1.77 <sup>hi</sup> ±0.3	2.84 <sup>fg</sup> ±0.3	$45.33^{h} \pm 2.9$	46.43 <sup>h</sup> ±3.5
T6 (fresh)	$0.16^{ef} \pm 0.06$	ND	1.13 <sup>hi</sup> ±0.05	$0.28^{i} \pm 0.03$	42.66 <sup>i</sup> ± 3.7	$55.19^{i} \pm 1.8$	0.57 <sup>k</sup> ±0.03	$0.66^{k} \pm 0.0$	11.70 <sup>i</sup> ±1.0	$8.25^{i} \pm 0.2$
T <sub>7</sub>	0.53°±0.07	ND	4.59 <sup>g</sup> ±0.3	$2.09^{h} \pm 0.3$	$241.34^{f} \pm 11.1$	117.18 <sup>h</sup> ±2.2	$1.98^{h}\pm0.1$	$1.22^{j} \pm 0.1$	85.57 <sup>d</sup> ±4.8	65.09°±1.3
T <sub>8</sub>	$0.27^{\text{det}}{\pm}0.09$	$1.35^{a}{\pm}\ 0.06$	$8.62^{\text{et}}{\pm}0.6$	10.65 <sup>cd</sup> ±0.8	366.67 <sup>d</sup> ±14.5	444.27 <sup>c</sup> ± 12.9	5.20°±0.2	9.56 <sup>a</sup> ±0.01	$52.61^{gh} \pm 1.3$	136.63 <sup>b</sup> ± 0.4

TI-TB=Tnuffles from different vendors. Values are means of two replicates ± standard deviation Values with different superscript letters in rows per heavy metal differ significantly (*p*<0.05); ND: Not Detected

#### Discussion

The microbiological, mycotoxins, and selected metals were assessed in Kalahari truffles to generate baseline information on the quality and safety of these wild manifested food resources in Namibia. The fresh truffles moisture content was above the 68% reported by Wahiba et al. (2016) in truffles from Iran, but similar or lower than 73-77% moisture values reported by Yousif et al. (2020) and Behzadi et al. (2021) in truffles from Iraq and Iran, respectively. As can be seen in Figure 1, the truffles are kept in an open air at the open markets until they are sold. This exposure allows for evaporation and thus the relatively low moisture content found in this study than what is commonly reported for fresh truffles.

The aerobic counts detected in both unwashed and washed truffle samples in this study were less than those found in other truffle species in Spain (Rivera et al. (2010), Italy (Saltarelli et al., 2008), and Spain (Phong et al., 2022). The differences in the microbial counts could be linked to the heterogeneity of the sample, the harvesting season, mechanical damage or internal parasitisation (Rivera et al., 2010). The directive 2004/24/EC of the European Commission (2004) states that the acceptable total aerobic count limit is <5.7 log CFU/g. So, all the truffle samples of present study had counts below the acceptable limit except for sample T6 (fresh) that had counts above the acceptable limits based on European Commission (2004). Overall, the results indicated that the majority of truffle samples had suitable sanitary quality.

The total coliforms results were similar to those reported by Cirlincione et al. (2021) on black summer truffles in Italy. The unwashed truffle  $(T_1, T_2, T_3, and T_8)$ and the washed T<sub>4</sub> samples had higher total coliform counts than the total coliforms counts (4.0  $\log CFU/g$ ) that Reale et al. (2009) detected on fresh black truffles in Italy. These differences could be attributed to the type of species and the level of soil contamination from which the truffles were harvested. The presence of coliforms in foodstuff may indicate poor hygienic quality of truffles. This suggests that these truffles should be subjected to thorough cleaning and processing such as cooking. The acceptable limit for total coliforms based on European Commission (2004; directive 2004/24/EC) is <1 CFU/g. Based on these recommendations, only samples T<sub>6</sub>(fresh) and T<sub>5</sub> (washed) had total coliform counts below the acceptable limit.

The yeast counts were in the same range as those reported by Cirlincione et al. (2021) on black summer truffles in Italy. They were also similar or higher than the 4-5.5 log CFU/g reported in truffles from Spain by Phong et al. (2022) and were higher than the 3.4 log CFU/g yeast counts that Rivera et al. (2010) detected in dried Tuber aestivum and Tuber melanosporum truffles samples that were also from Spain. The presence of yeast in food products is not a hazard to health, but consumption of yeast contaminated food could lead to allergic reactions and food spoilage. The acceptable limit of yeast and mould count based on European Commission (2004; directive 2004/24/EC) is <log 3.7 CFU/g. Irrespective of washing of truffles, majority of the samples had yeast counts above the acceptable limits of the European Commission (2004; directive 2004/24/EC). This could indicate a shorter shelf life of truffles and thus, further preservation methods may be necessary.

Washing of truffles generally reduced mould counts. However, the mould counts between unwashed and washed truffles did not generally differ significantly. For most of the samples irrespective of washing, mould counts were similar or higher than the values reported by Cirlincione et al. (2021) on black summer truffles in Italy and by Tejedor-Calvo et al. (2020) on fresh truffles in Spain. Furthermore, the mould values in this study were higher than the 3.7 log CFU/g that Rivera et al. (2011) detected in fresh T. aestivum truffles from Spain. Regardless of washing of truffles, only three samples had mould counts below the acceptable limit of <3.7 log CFU/g based on the European Commission (2004; directive 2004/24/EC). This study did not detect Salmonella, which is in agreement with the findings of Reale et al. (2009) in fresh black truffles in Italy and those of Phong et al. (2022) in truffles from Spain.

There is little published studies in the literature regarding evaluation of mycotoxins in truffles. The discussion on mycotoxins results is therefore limited to comparison with other foods where a particular mycotoxin had been investigated and/or relating the findings to existing regulations such as those of the Food and Drug Administration (FDA). Although washing of truffles had significantly reduced the levels of deoxynivalenol, all the truffle samples had deoxynivalenol levels above the 500  $\mu$ g/kg in foodstuffs. Since mycotoxins are stable chemicals, decontamination of truffles should be carried out to reduce the levels of deoxynivalenol to safe levels.

Nevertheless, fumonisin  $B_1$  has been reported in some tubers. Amri and Lenoi (2016) reported the occurrence of fumonisin B1 (12.34 to 267.86 µg/kg) in dried sweet potato chips. The United States FDA has the regulatory levels for fumonisins (2,000-3,000 µg/kg) in foodstuffs (FAO, 2004). Based on FDA regulations, the levels of fumonisin B<sub>1</sub> detected in all the truffle samples 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  $B_1$ . The advisory limits of ochratoxin A in foodstuffs for European Union is set at 5 µg/kg (FAO, 1997). Based on the European Union regulations, only samples T<sub>1</sub> (unwashed) and T<sub>5</sub> (washed) had levels above the advisory limits, while 80% of the samples had ochratoxin A levels within the acceptable limits.

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  $B_1$  (1.93 µg/kg to 4.21 µg/kg) in dried and stored Nigerian Oyster mushrooms. 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. Deoxynivalenol and aflatoxin occurrence of up to 99% and 66%, respectively is reported in grains and grain-based food products. Also, other processing methods such as fermentation and heat treatments can significantly reduce the mycotoxin levels (Sarmast et al., 2021).

As stated, the occurrence of zearalenone in truffles has not been reported in literature. However, zearalenone occurrence in medicinal dried rhizomes (Acorus calamus, Bergenia ciliata, Curcuma longa, Zingiber officinale) and root tubers (Pueraria tuberosa) has been reported by Koul and Sumbali (2008) and were in the range of 520 to 14,510 µg/kg roots and tubers, which like truffles grow underground. The levels of zearalenone in truffles were less or similar to the levels detected by Koul and Sumbali (2008) in dried rhizomes and root tubers collected from India. Thailand established the maximum limits for zearalenone (30-1,000 µg/kg) in foodstuffs (Anukul et al., 2013). Based on zearalenone regulations for Thailand, almost all the truffle samples had zearalenone levels were within the acceptable limit. Ezekiel et al. (2013) did not detect any mycotoxin in the dried mushrooms from Nigeria. This study gives a preliminary basis upon which confirmation is warranted using advanced techniques such as Liquid Chromatography-Mass Spectrometry (LC-MS) to identify and quantify the specific mycotoxins and microorganisms.

The absence of Hg and Ni could be an indication that the fields from which the truffles were harvested are not contaminated with Hg and Ni. Hg was however found in some mushrooms. Fang et al. (2014) detected 0.02 mg/kg in dry mushrooms. Presence of low levels of Hg in foodstuffs can seemingly be allowed. The permissible levels for Hg in food is 0.6 mg/kg as per Codex Alimentarius Commission (2011) standard.

Except for three truffle samples, all others had lower levels of Cd that the 0.404 mg/kg reported by Xu et al. (2019) in truffles in China. Similarly, the levels of Cd in all truffles were lower than the 54.2 mg/kg Cd level that Sarikurkcu et al. (2011) detected in wild edible mushrooms from Soguksu National Park in Ankara in Turkey. They were also lower than the Cd levels of up to 148 mg/kg reported by Michelot et al. (1999) in most mushrooms' species collected from Latin America (French Guyana, Colombia, Costa Rica). These differences can be attributed to levels of Cd contamination of the fields where truffles were harvested. The permissible limits for Cd in food samples is 1.0 mg/kg (European Commission (2008) (directive 2008/629/EC). Based on this, all truffle samples in this study had Cd levels within permissible levels irrespective of washing.

The levels of Cr determined in this study are similar or lower that those reported by Sarikurkcu et al. (2011), who reported a range from not detected to 21.6 mg/kg in mushrooms originated from Soguksu National Park in Ankara in Turkey. They were also within the range of 4-22 mg/kg Cr amounts in truffles collected from Iraq reported by Qazmooz et al. (2020). On the other hand, they were higher than the 0.036-0.05 mg/kg Cr levels in truffles from Turkey reported by Akyüz and Kirbag (2018). The Fe levels were within the 10.4-4,900 mg/kg range reported by Michelot et al. (1999) in the majority of mushrooms species collected from French Guyana, Colombia, and Costa Rica. Moreover, this study Fe results were similar or higher than the Fe levels (400-500 mg/kg) detected in Amanita rubescens mushrooms from Poland by Rudawska and Leski (2005a, 2005b). Sarikurkcu et al. (2020) reported some mushroom samples from Turkey with higher Fe levels up to 1,580 mg/kg in mushrooms, which is more than double the levels found in this study.

Mn levels detected in this study were lower than the 10-77 mg/kg that Rudawska and Leski (2005a, 2005b) reported in mushroom species collected from the wild in Turkey. Michelot et al. (1999) found a wide range (4.1-400 mg/kg) of Mn amounts in many mushrooms species collected from French Guyana, Colombia, and Costa Rica, which encompasses the results of this study. The Mn results were, however, higher than the 0.02-0.112 mg/kg Mn levels in truffles from Turkey reported by Akyüz and Kirbag (2018).

Falandysz et al. (2001) detected Zn that was as high as 460 mg/kg in dried mushrooms from Poland. This was much higher than this study's findings. Similarly, the Zn results of this study were lower or within the 23.9-369 mg/kg of Zn found in mushrooms species collected from French Guyana, Colombia, and Costa Rica (Michelot et al., 1999). Some samples in this study had higher amounts of Zn than the 55.6-57.3 mg/kg that was determined in truffles from Iran (Behzadi et al., 2021). Zn is apparently an antagonist of other metals such as Cd, Pb, and Ni. Thus its presence in some mushrooms can potentially reduce the risks associated with other toxic metals at high concentrations (Codex Alimentarius Commission, 1995). The metals (Cd, Fe, Zn, and Mn) intake levels results, based on the maximum amounts quantified in this study, showed that all the truffle samples were within the respective metal consumption safety range, except for one sample. This is based on the reference daily intakes (µg/kg/day) of 0.5 for Cd, 300 for Fe, and Zn and 140 for Mn (Sarikurkcu et al., 2020). Using 70 kg as the average weight of an adult, the upper recommended daily intake reported in Marini et al. (2021) for Cd, Cr, and Zn were 0.36, 4.29 and 357.14  $\mu$ g/kg/day, respectively. The values for Cr in truffles used in this study were higher than the recommended daily intake. A survey on the human intake of Cd across European countries through food consumption found a higher intake than the reference daily intakes (EFSA, 2011). The upper daily Mn intake by an adult person recommended by the EFSA NDA (2013) is 42.86  $\mu$ g/kg/day. This was over 10 times more than what the studied truffles can deliver based on Sarikurkcu et al. (2020) formula. These results can be the basis to build and confirm the understanding of elemental composition of Kalahari truffles.

## Conclusion

The aerobic counts in 87.5% of the truffle samples were within the acceptable limits. Washing reduced the total coliforms, yeast, and mould counts in over 70% of all the truffle samples. *Salmonella*, Hg, and Ni were not detected in any of the truffle samples. Deoxynivalenol, fumonisin  $B_1$ , Ochratoxin A, total aflatoxin, and zearalenone were detected and further advanced quantification is warranted. Almost all the truffle samples had Cd, Cr, Fe, and Mn levels within the daily metal intake levels by an average adult regular consumer. The analyzed microorganisms, mycotoxins, and heavy metals in underprized Kalahari truffles may impair the safety, shelf life, and human health. Thus, they should be subjected to appropriate processing before consumption.

## Author contributions

T.A.H., W.E., K.K.M.N., and N.P.K. designed the experiment; W.E., K.K.M.N., and L.I. adapted the methods; T.A.H. did experimental work, analyzed data, and wrote the manuscript; W.E., K.K.M.N., N.P.K., and L.I. facilitated the resources, edited, and reviewed the manuscript. All authors read and approved the final manuscript.

#### **Conflicts of interest**

The authors have no competing interests.

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#### References

AACC International. (1999). Method 44-15.02: moisture-airoven methods. *Approved Methods of Analysis*.[DOI: 10.1094/ AACCIntMethod-44-15.02]

- Akyüz M., Kirbag S. (2018). Nutritive value of desert truffles species of genera *Terfezia* and *Picoa* (Ascomycetes) from Arid and semiarid regions of eastern Turkey. *International Journal of Medicinal Mushrooms*. 20: 1097-1106. [DOI: 10.1615/IntJMedMushrooms.2018028796]
- Álvarez-Lafuente A., Benito-Matías L.F., Peñuelas-Rubira J.L., Suz L.M. (2018). Multi-cropping edible truffles and sweet chestnuts: production of high-quality *Castanea sativa* seedlings inoculated with *Tuber aestivum*, its ecotype *T. uncinatum*, *T. brumale*, and *T. macrosporum*. *Mycorrhiza*. 28: 29-38. [DOI: 10.1007/s00572-017-0805-9]
- Amri E., Lenoi S.O. (2016). Aflatoxin and fumonisin contamination of sun-dried sweet potato (*Ipomoea batatas* L.) chips in Kahama District, Tanzania. *Journal of Applied and Environmental Microbiology*. 4: 55-62. [DOI: 10.12691/jaem-4-3-2]
- Anukul N., Vangnai K., Mahakarnchanakul W. (2013). Significance of regulation limits in mycotoxin contamination in Asia and risk management programs at the national level. *Journal of Food and Drug Analysis*. 21: 227-241. [DOI: 10.1016/j.jfda. 2013.07.009]
- AOAC International. (2016). Official Methods of Analysis. Method 995.20:2016. Approved Methods of Analysis.
- Behzadi A.A., Zareie M., Abbasi A., Masoomi B., Ashrafi-Dehkordi E., Morte A. (2021). Physicochemical properties, nutritional composition, and phylogenic analysis of black truffles grown in Fars Province, Iran. *International Journal of Nutrition Sciences*. 6: 45-51. [DOI: 10.30476/IJNS.2021. 90183.1128]
- Cirlincione F., Francesca N., Settanni L., Donnini D., Venturella G., Gargano M.L. (2021). Microbial safety of black summer truffle collected from Sicily and Umbria Regions, Italy. *Journal of Food Quality and Hazards Control.* 8: 13-20. [DOI: 10.18502/jfqhc.8.1.5458]
- Codex Alimentarius Commission. (1995). General standard for contaminants and toxins in food and feed. CXS 193-1995.
- Codex Alimentarius Commission. (2011). Joint FAO/WHO food standards programme codex committee on contaminants in foods. 15<sup>th</sup> Session. The Hague, The Netherlands.
- EFSA NDA (European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies). (2013). Scientific opinion on dietary reference values for manganese. *EFSA Journal*. 11: 3419. [DOI: 10.2903/j.efsa.2013.3419]
- EFSA (European Food Safety Authority). (2011). Statement on tolerable weekly intake for cadmium. *EFSA Journal*. 9: 1975. [DOI: 10.2903/j.efsa.2011.1975]
- European Commission. (2004). Directive 2004/24/EC of the European parliament and of the council of 31 March 2004 amending, as regards traditional herbal medicinal products, directive 2001/83/EC on the community code relating to medicinal products for human use. Official Journal of the European Union. L 136/47: 85-90.
- European Commission. (2008). Commission regulation (EC) No 629/2008 of 2 July 2008 amending regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union. L 173/6-9.
- Ezekiel C.N., Sulyok M., Frisvad J.C., Somorin Y.M., Warth B., Houbraken J., Samson R.A., Krska R., Odebode A.C. (2013). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *International Journal of Food Microbiology*. 162: 231-236. [DOI: 10.1016/j.ijfoodmicro.2013.01.025]
- Falandysz J., Szymczyk K., Ichihashi H., Bielawski L., Gucia M., Frankowska A., Yamasaki S.-I. (2001). ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland. *Food Additives and Contaminants*. 18: 503-513. [DOI: 10.1080/02652030119625]
- Fang Y., Sun X., Yang W., Ma N., Xin Z., Fu J., Liu X., Liu M., Mariga A.M., Zhu X., Hu Q. (2014). Concentrations and health risks of lead, cadmium, arsenic, and mercury in rice and edible mushrooms in China. *Food Chemistry*. 147: 147-151. [DOI: 10.1016/j.foodchem.2013.09.116]
- Feng P., Weagant S.D., Grant M.A., Burkhardt W., Shellfish M., Water B. (2002). Enumeration of *Escherichia coli* and the

coliform bacteria. *Bacteriological Analytical Manual*. 13: 1-13.

- Food and Agriculture Organization (FAO). (1997). Worldwide regulations for mycotoxins. FAO Food and Nutrition Paper 64.
- Food and Agriculture Organization (FAO). (2004). Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper 81.
- Giron H.C. (1973). Perkin elmer atomic spectrophotometer. Atomic absorption newsletter. 12: 28.
- Jonathan S.G., Esho E.O. (2010). Fungi and aflatoxin detection in two stored oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus pulmonarius*) from Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry*. 9: 1722-1730.
- Koul A., Sumbali G. (2008). Detection of zearalenone, zearalenol and deoxynivalenol from medicinally important dried rhizomes and root tubers. *African Journal of Biotechnology*. 7: 4136-4139.
- Marini M., Angouria-Tsorochidou E., Caro D., Thomsen M. (2021). Daily intake of heavy metals and minerals in food - a case study of four Danish dietary profiles. *Journal of Cleaner Production.* 280: 124279. [DOI: 10.1016/j.jclepro.2020. 124279]
- Maturin L., Peeler J.T. (2001). Aerobic plate count. Food and Drug Administration (FDA), Bacteriological Analytical Manual Online. 8<sup>th</sup> Edition. Silver Spring, Berlin.
- Michelot D., Poirier F., Melendez-Howell L.M. (1999). Metal content profiles in mushrooms collected in primary forests of Latin America. Archives of Environmental Contamination and Toxicology. 36: 256-263. [DOI: 10.1007/s002449900469]
- Paithankar J.G., Saini S., Dwivedi S., Sharma A., Chowdhuri D.K. (2021). Heavy metal associated health hazards: an interplay of oxidative stress and signal transduction. *Chemosphere*. 262: 128350. [DOI: 10.1016/j.chemosphere.2020.128350]
- Phong W.N., Chang S., Payne A.D., Dykes G.A., Coorey R. (2022). Microbiological evaluation of whole, sliced, and freeze-dried black truffles (*Tuber melanosporum*) under vacuum packaging and refrigerator storage. *JSFA Reports*. 2: 92-99. [DOI: 10.1002/jsf2.32]
- Qazmooz H.A., Guda M.A., Algburi J.B., Al-Zurfi S.K.L., Al-Graiti T.A. (2020). Determination of heavy metal in samples of *Tirmania nivea* fungi in different soils. *Plant Archives*. 20: 313-317.
- Reale A., Sorrentino E., Iacumin L., Tremonte P., Manzano M., Maiuro L., Comi G., Coppola R., Succi M. (2009). Irradiation treatments to improve the shelf life of fresh black truffles (truffles preservation by gamma-rays). *Journal of Food Science*. 74: M196-M200. [DOI: 10.1111/j.1750-3841.2009. 01142.x]
- Rivera C.S., Blanco D., Oria R., Venturini M.E. (2010). Diversity of culturable microorganisms and occurrence of *Listeria* monocytogenes and Salmonella spp. in Tuber aestivum and Tuber melanosporum ascocarps. Food Microbiology. 27: 286-293. [DOI: 10.1016/j.fm.2009.11.001]
- Rivera C.S., Venturini M.E., Oria R., Blanco D. (2011). Selection of a decontamination treatment for fresh *Tuber aestivum* and *Tuber melanosporum* truffles packaged in modified atmospheres. *Food Control.* 22: 626-632. [DOI: 10.1016/j. foodcont.2010.10.015]
- Rudawska M., Leski T. (2005a). Macro- and microelement contents in fruiting bodies of wild mushrooms from the Notecka forest in West-central Poland. *Food Chemistry*. 92: 499-506. [DOI: 10.1016/j.foodchem.2004.08.017]
- Rudawska M., Leski T. (2005b). Trace elements in fruiting bodies of ectomycorrhizal fungi growing in Scots pine (*Pinus* sylvestris L.) stands in Poland. Science of the Total Environment. 339: 103-115. [DOI: 10.1016/j.scitotenv.2004. 08.002]
- Saltarelli R., Ceccaroli P., Cesari P., Barbieri E., Stocchi V. (2008). Effect of storage on biochemical and microbiological parameters of edible truffle species. *Food Chemistry*. 109: 8-16. [DOI: 10.1016/j.foodchem.2007.11.075]

DOI: 10.18502/jfqhc.9.1.9687

- Sarikurkcu C., Copur M., Yildiz D., Akata I. (2011). Metal concentration of wild edible mushrooms in Soguksu National Park in Turkey. *Food Chemistry*. 128: 731-734. [DOI: 10. 1016/j.foodchem.2011.03.097]
- Sarikurkcu C., Popović-Djordjević J., Solak M.H. (2020). Wild edible mushrooms from Mediterranean Region: metal concentrations and health risk assessment. *Ecotoxicology and Environmental Safety*. 190: 110058. [DOI: 10.1016/j.ecoenv. 2019.110058]
- Sarmast E., Fallah A.A., Jafari T., Khaneghah A.M. (2021). Occurrence and fate of mycotoxins in cereals and cereal-based products: a narrative review of systematic reviews and meta-analyses studies. *Current Opinion in Food Science*. 39: 68-75. [DOI: 10.1016/j.cofs.2020.12.013]
- Tejedor-Calvo E., Morales D., García-Barreda S., Sánchez S., Venturini M.E., Blanco D., Soler-Rivas C., Marco P. (2020). Effects of gamma irradiation on the shelf-life and bioactive compounds of *Tuber aestivum* truffles packaged in passive modified atmosphere. *International Journal of Food Microbiology*. 332: 108774. [DOI: 10.1016/j.ijfoodmicro. 2020.108774]

- Trappe J.M., Claridge A.W., Arora D., Smit W.A. (2008). Desert truffles of the African Kalahari: ecology, ethnomycology, and taxonomy. *Economic Botany*. 62: 521-529. [DOI: 10.1007 /s12231-008-9027-6]
- Ünüsan N. (2019). Systematic review of mycotoxins in food and feeds in Turkey. *Food Control.* 97: 1-14. [DOI: 10.1016/j. foodcont.2018.10.015]
- Venturini M.E., Reyes J.E., Rivera C.S., Oria R., Blanco D. (2011). Microbiological quality and safety of fresh cultivated and wild mushrooms commercialized in Spain. *Food Microbiology*. 28: 1492-1498. [DOI: 10.1016/j.fm.2011.08.007]
- Wahiba B., Wafaà T., Asmaà K., Bouziane A., Mohammed B. (2016). Nutritional and antioxidant profile of red truffles (*Terfezia claveryi*) and white truffle (*Tirmania nivea*) from southwestern of Algeria. *Der Pharmacia Letter*. 8: 134-141.
- Xu Y., Xing Y., Li C., Zhao X. (2019). Analysis of various heavy metals and different forms of arsenic and mercury in truffles. *Journal of Food Safety and Quality*. 10: 510-514.
- Yousif P.A., Jalal A.F., Faraj K.A. (2020). Essential constituents of truffle in Kurdistan Region. Zanco Journal of Pure and Applied Sciences. 32: 158-166. [DOI: 10.21271/ZJPAS.32.5.15]