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Proximate and Mineral Contents in Fruiting Bodies of Edible Wild Mushroom from Al-Jabal Alakhdar Province/Libya

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ABSTRACT

In order to promote the use of wild edible mushrooms as source of nutrients and environmental marker, several experiments were performed in fruiting bodies (cap and stipe) of wild edible mushrooms which were collected from different regions of Al-Jabal Alakhdar province / Libya, including Alabraq, Alosita, Asalpiea, Ashnishen, Balanaje, Faidiyah, Marawah, Omar Mukhtar, Salantah, Sidihamri, and Werdama regions. The collections were done from September to November during 2012 and 2013. The analysis of nutrients included determination of proteins, fats, ash, and carbohydrates, while, macro- (K, Na, and Ca) and microelement (Fe, Zn, Cu, Cd, Pb) trace metal levels were determined by a flame photometer and atomic absorption spectrometry. The macronutrient profiles, in general, revealed that the wild mushrooms were rich sources of protein and carbohydrates and had a low amount of fat. In fruiting bodies, the highest mean concentration of macro elements (dry weight basis) was found for K (26612.4), followed by Ca (3049.80) and Na (2836.85). K and Na were preferably translocated into the cap rather than the stipes. Calcium, however, was often found in higher concentration in stipes than in caps. The mean microelement concentrations, across all tested fungi, were in the following order, Fe > Zn > Cu > Pb > Cd. Microelements showed different distributions, depending on the part of the fruiting body. Some were more concentrated in the caps and some in stipes and distributions varied among regions. This study proves that wild edible fungi which consumed traditionally in Al-Jabal Alakhdar province be used in well-balanced diets due to their high contents of functional minerals. Also, their low contents of heavy metals (Pb and Cd) shows that collection areas are not polluted, therefore all collected edible mushroom species can be unreservedly consumed without any health risk.

1. INTRODUCTION

Worldwide the nutritive and medicinal values of mushrooms have long been recognized. Many studies have indicated that edible mushrooms species are highly nutritious, their nutritional value comparing favorably with meat, egg, and milk [1]. Fruit body of mushrooms is appreciated, not only for texture and flavor but also for its chemical and nutritional properties.

On a dry weight basis, they are considered to be good sources of digestible proteins (10–40%), carbohydrates (3–21%) and dietary fiber (3–35%). Mushrooms contain all the essential amino acids and are limiting in the sulfur-containing amino acids, cysteine, and methionine [2]. Although mushrooms contain all the main classes of lipids, including free fatty acids, mono-, di- and triglycerides, sterol esters and phospholipids, their levels are low at approximately 2–8% (on dry weight basis). Mushrooms are excellent sources of thiamine (vitamin B1), riboflavin (vitamin B2), nicotinic acid (vitamin B3), biotin and ascorbic acid (vitamin C) [3]. Edible mushrooms have also been reported as therapeutic foods, useful in preventing diseases such as hypertension, and cancer. These functional characteristics are mainly due to their chemical composition [4].

Pollution of the environmental with heavy metals is a serious problem which is recognized in most countries of the world [5]. Trace metals have been introduced to the environmental both through natural processes and as a consequence of human activities such as industrialization or fuel combustion [6]. Growing up on a substrate with a high concentration of various heavy metals, edible wild mushrooms can become toxic, accumulating a lot of heavy metals. Until now, various studies have shown that accumulation of heavy metals in mushrooms is dependent on the species of mushrooms, the mushrooms age, the source of pollution with heavy metals and distance to this source [7]. Many wild edible mushroom species are known to accumulate high levels of heavy metals and mainly cadmium (Cd) and lead (Pb) [8, 9]. The factors governing the accumulation of metals in mushrooms are not well-known. The principal factors influencing the accumulation of heavy metals in macro-fungi are environmental ones (metal concentration in soil, pH, and contamination by atmospheric deposition) and fungal ones (fungal structure, morphological portion, development stages, biochemical composition, and decomposition activities). The determination of heavy metal concentration in the fruiting bodies of mushrooms is essential in dietary intake studies. Different heavy metals are toxic, such as Pb, Cd, and mercury (Hg); on the other hand, many elements are essential for the human metabolism, such as iron (Fe), zinc (Zn), and copper (Cu) [7].

Despite the nutritional, medicinal and physiological properties, wild mushrooms growing in East of Libya have not been investigated for their major components or for their trace metal and antioxidant composition that would be a reliable indicator of the nutritional value. People living in Al-Jabal Alakhdar region of Libya consume wild edible mushrooms because of their abundance during the rainy season. Therefore, this study was performed to study, the chemical composition included the determination of proteins, fats, ash, and carbohydrates in wild edible mushrooms growing in the different area of Al-Jabal Alakhdar province of Libya.The levels of heavy metal and to report possible contamination that would represent a health hazard. However, there has been no report, to our knowledge, on the heavy metal levels in wild mushroom samples in Al-Jabal Alakhdar province.

2. MATERIALS AND METHODS

2.1. Mushrooms:

Fruiting bodies (Cap and stipe) of wild edible mushrooms (*Agaricus bisporus)* were harvested from different regions of Al-Jabal Alakhdar province/Libya, including Alabraq, Alosita, Asalpiea, Ashnishen, Balanaje, Faidiyah, Marawah, Omar Mukhtar, Salantah, Sidihamri, and Werdama region. The areas of study included forests and lawns, which possess favorable environmental conditions for the growth of mushrooms. The collections were done from September to November; this period corresponds to the rainy season in Al-Jabal Alakhdar province. The habitat and morphological characteristics of mushroom found in the localities were recorded and photographed for diagnosis. At first, the collected fresh samples were freed from foreign materials. Damaged or soiled (muddy) parts were trimmed off with a plastic knife, and smaller particles were removed with a fine brush.

Approximately 10 gm of each sample were taken immediately for moisture determination. The collected samples were cut into small pieces and dried at 105^0C for 24 hrs; dried samples were ground to a fine powder using an agate homogenizer and stored in pre-cleaned polyethylene bottles until the analysis started.

2.2. Dry Matter Contents:

The water content was determined by weighing, after drying, 10 gm of each sample in an oven at 105±2 ^{0}C, the loss in weight obtained after drying was regarded as the dry matter content according to AOAC [10].

2.3. Crude Protein Content:

The crude protein content (N X 4.38) of the samples was estimated by the macro-Kjeldahl method according to AOAC [10].

2.4. Crude Fat Content:

Two grams (2.0 gm) of powdered sample was extracted with 30 ml of petroleum ether by using Soxhlet extractor for 4 hrs. The extract was evaporated to dryness in a weighed flask using a vacuum evaporator. The weighed flask was dried in the oven at 80^{0}C for 2 hrs, allowed to cool and reweighed. The difference between the initial and final weights was regarded as the lipid content of the sample [10].

2.5. Ash Content:

The powdered mushroom sample (3.0 gm) was ashed in a muffle furnace (Carbolite S 336 RB) in previously ignited and cooled crucible of known weight at 550^{0}C for 6 hrs. Fairly cooled crucibles were put in desiccators and weighed [10].

2.6. Total Carbohydrates:

Total carbohydrates were calculated by difference.

2.7. Metal Content:

2.7.1. Reagents:

Nitric acid 69% (analytical grade, BDH Ltd. Pool England) and perchloric acid 60% (analytical grade, Riedel- de Haen AG Germany) were used without additional purification. Deionized water from a Milli-Q water purification (Millipore, Bedford, MA, USA) was used for the preparation of samples and standards. The element standard solutions of iron, copper, zinc, cadmium and lead used for calibration were prepared by diluting stock solutions of 1000 mg/l of each element supplied from BDH. All containers and glassware were soaked in 20% nitric acid for at least 16 hours and rinsed with distilled and deionized water before use.

2.7.2. Methods:

A dried powdered form of each sample (0.5 ± 0.0005 g) was weighed into 150 ml pyrex beaker. 10 ml HNO_3 and 3 ml 60% $HClO_4$ were added and heated on a hot plate, slowly at first, until frothing ceases. Samples were heated to white fumes of $HClO_4$. After cooling 10 ml HCl (1+1) were added and transfer quantitatively to 50 ml volumetric [11]. Fe, Cu, Zn, Pb, and Cd concentration were determined as three replicates by M6 Graphite furnace atomic absorption spectrometer with Zeeman background, Thermal. K, Ca and Na were determined by a flame photometer (JENWAY PFP 7 Flame Photometer). The recovery of metals was studied by adding known amounts of standard solution to samples. All of the obtained results were converted according to the recovery percentage.

2.8. Statistical Analyses:

The experiment was conducted with 3 replicates in a randomized complete design. Values are expressed as means ± standard error and the differences between groups were evaluated using one-way analysis of variance (ANOVA) at ($P \leq 0.05$). Means were separated using Duncan's multiple range test with $\alpha = 0.05$. Pearson correlation coefficients were calculated between the results of total phenolic, total flavonoid, and different antioxidant assays, and between different antioxidant assays. These statistical analyses were carried out using Microsoft Excel 2007.

3. RESULTS AND DISCUSSION

3.1. Dry Matter:

The percentage content of dry matter in the analyzed wild mushrooms is summarized in Table (1), these results clearly indicate that the high content of dry matter was found in high levels and varied between 7.12% in cap (Alpine region) and 15.94% in cap (Balanaje region), while, dry matter content in stipe ranged from 5.70% to 16.26% in Marawah and Balanaje regions respectively.

Agrahar-Murugkar and Subbulakshmi [12] reported that the dry matter of seven species of Indian wild mushrooms was ranged from 4.7% in *C. gigantea* to 15.9% in *C. cibarius*. Dry matter of mushrooms is very low, usually in the range of 6-14%. Commonly, dry matter content of 10% has been used for calculation if the factual is unknown. Dry matter proportion increases during mushroom cooking due to water loss. For instance, boiling, for 10 min, of the cultivated species *Agaricus bisporus* (portabella) decreases the weight by 30%, but only 11-16% in *Grifola frondosa* (maitake) and *Flammulina velutipes* (enokitake) [13]. Environmental factors can have an impact on the abundance of certain compounds in mushrooms, but the reasons for the variations in the composition of mushrooms species collected from background areas remains unclear [14].

3.2. Proteins:

Edible mushrooms are highly valued as a good source of protein and their protein contents usually, range from 28.93% to 39.1% of dry weight [15]. The obtained results in Table (2) revealed that among the different regions, crude protein content of cap was significantly higher ($P \leq 0.05$) in Balanaje and Alpine regions (43.28% and 42.49% d.w respectively), than crude protein content of cap in Alosita region (29.64% d.w).

Stipe protein content also was higher in Asalpiea and Balanaje regions (39.99% and 39.94% d.w respectively), on the other hand, the lowest value of only 30.26% d.w Marawah region (Table 2). The results revealed that the crude protein content remained significantly higher in cap than in stipe.

Protein contents of mushrooms were reported to vary according to the genetic structure of species and physical and chemical differences in growing medium [12, 16]. The obtained values were almost similar to crude protein contents of edible mushrooms reported by Bauer Petrovska [17]. She determined the mean crude content of 32.6% in a dry matter of 47 species of Macedonian edible mushrooms, however, wide variations occurred. The highest content of 48.8% in *C. gambosa*, while the lowest value was 16.2% in the widely consumed in *C. cibarins*, however, Khatun, *et al.* [18] reported that the highest protein content of edible Indian mushrooms (*pleuratus florida*) was found 22-23% dw.

Table (1): Dry Matter content of mushroom samples in different regions

Region	Dry Matter (%)*	
	Cap	Stipe
Alabraq	7.73[de] + 0.07	7.81[de] + 0.83
Alosita	11.79[b] + 0.63	9.03[cd] + 0.17
Asalpiea	7.12[efg] + 0.09	6.00[fg] + 0.21
Ashnishen	12.43[b] + 0.83	7.65[def] + 0.39
Balanaje	15.94[a] + 1.09	16.26[a] + 1.03
Faidiyah	11.60[b] + 0.72	8.88[cde] + 0.52
Marawah	8.85[cde] + 0.72	5.70[g] + 0.55
Omar Mukhtar	8.91[cde] + 0.46	15.18[a] + 0.24
Salantah	9.93[c] + 0.09	12.55[b] + 0.62
Sidihamri	8.58[cde] + 0.35	5.26[g] + 0.13
Werdama	11.85[b] + 0.42	12.01[b] + 0.5

[a-e] Means with different letter are significantly different ($P \leq 0.05$). *Means (% on dry weight basis) ± standard error of triplicate.

Table (2): Crude protein content of mushroom samples in different regions

Region	Protein (%)*	
	Cap	Stipe
Alabraq	37.53[ef] + 0.02	34.59[hij] + 0.06
Alosita	29.64[l] + 0.08	32.63[k] + 0.17
Asalpiea	42.49[ab] + 0.07	39.99[d] + 0.11
Ashnishen	34.45[ij] + 0.04	33.38[jk] + 0.06
Balanaje	43.28[a] + 0.30	39.94[d] + 0.28
Faidiyah	39.04[de] + 0.08	38.72[de] + 0.07
Marawah	32.70[k] + 0.13	30.26[l] + 0.04
Omar Mukhtar	36.82[fg] + 0.83	35.68[ghi] + 0.18
Salantah	41.55[bc] + 0.44	37.56[ef] + 0.08
Sidihamri	40.28[cd] + 2.04	34.83[hij] + 0.07
Werdama	36.10[fgh] + 0.32	32.75[k] + 0.04

[a-e] Means with different letter are significantly different ($P \leq 0.05$).

[*]Means (% on dry weight basis) ± standard error of triplicate.

It is known that the protein content of mushrooms is affected by a number of factors, namely the type of mushrooms, the stage of development, the part sampled, the level of nitrogen available and location [19].

4.3. Lipids:

Data presented in Table (3) indicate that the mean concentration of crude fat content in the cap and stipe of wild mushrooms (*Agaricus bisporus*) collected from eleven regions of Al-Jabal Alakhdar Provence. All concentrations were determined on a dry weight basis.

The highest crude fat concentration of cap was detected in Omar Mukhtar region while the lowest was detected in Sidihamri region. The results showed that Alabraq region had the highest crude fat concentration in stipe which was recorded as 2.35%, on the other hand, the lowest crude fat concentration of stipe was detected in Salantah region and there was no significant difference ($P > 0.05$) between Salantah and Alosita region which was recorded as 1.16% and 1.17% respectively. It was surprising that crude fat content in the stipe from Alabraq region (2.35%) and Sidihamri region (2.24%) was higher than that obtained from the cap in the same region (1.77% and 1.02% respectively).

In the literature crude fat concentration in wild mushrooms samples have been reported in the range of 0.92 to 4.88% in Portugal [20], 0.5 to 4.7% in India [21], 1.0 to 6.7% in China [14].

Table (3): Fat content of mushroom samples in different regions

Region	Fat (%)*	
	Cap	Stipe
Alabraq	1.77^{ij} + 0.09	2.35^{cd} + 0.03
Alosita	1.73^{j} + 0.03	1.17^{k} + 0.02
Asalpiea	2.64^{b} + 0.06	1.91^{ghij} + 0.06
Ashnishen	2.05^{fg} + 0.1	1.97^{fgh} + 0.06
Balanaje	1.93^{fghi} + 0.03	1.73^{j} + 0.06
Faidiyah	2.61^{b} + 0.06	2.11^{ef} + 0.0
Marawah	2.62^{b} + 0.09	2.03^{fg} + 0.01
Omar Mukhtar	2.82^{a} + 0.01	1.83^{hij} + 0.08
Salantah	2.38^{cd} + 0.0	1.16^{k} + 0.03
Sidihamri	1.02^{k} + 0.01	2.24^{de} + 0.14
Werdama	2.43^{c} + 0.01	1.94^{fghi} + 0.01

$^{a-e}$ Means with different letter are significantly different ($P \leq 0.05$).

*Means (% on dry weight basis) ± standard error of triplicate.

In general, wild mushrooms were a richer source of protein and had a lower amount of crude fat than commercial mushrooms making it an ideal snack material [12].

4.4. Carbohydrates:

Edible mushrooms are highly valued as a good source of carbohydrates and their contents usually, range from 40.6% to 53.3% of dry weight [15]. Data in Table (4) proved that the highest carbohydrate content in the cap, calculated by difference, was detected in Alosita and Marawah regions which were recorded as 53.21% and 45.24% respectively; on the other hand, the lowest concentration was observed in Asalpiea which was 33.86%.

Maximum carbohydrate content in stipe was obtained in Alosita region (48.42%), also as indicate in Table (4) the results showed no significant difference ($P > 0.05$) between Alosita, Ashnishen, Omar Mukhtar, Werdama, and Marawah. Sidihamri region ranked second from point view carbohydrate content.

The current data showed that the content of carbohydrate in both cap and stipe of wild Libyan mushrooms were higher than those reported by [20], on the other hand, similar results were already reported [4, 22].

Table (4): Carbohydrates content of mushroom samples in different regions

Region	Carbohydrates (%)*	
	Cap	Stipe
Alabraq	40.59^{g} + 0.13	42.56^{f} + 0.17
Alosita	53.21^{a} + 0.17	48.42^{b} + 0.13
Asalpiea	33.86^{j} + 1.86	39.04^{h} + 0.15
Ashnishen	44.64^{cd} + 0.17	48.38^{b} + 0.83
Balanaje	41.02^{g} + 0.16	43.08^{ef} + 0.08
Faidiyah	43.92^{def} + 0.18	41.12^{g} + 0.18
Marawah	45.24^{cd} + 0.49	47.67^{b} + 0.33
Omar Mukhtar	44.14^{de} + 0.07	47.93^{b} + 0.07
Salantah	37.41^{i} + 0.02	39.20^{h} + 0.15
Sidihamri	38.99^{h} + 0.13	45.77^{c} + 0.14
Werdama	44.59^{cd} + 0.14	48.75^{b} + 0.41

$^{a\text{-}e}$ Means with different letter are significantly different ($P \leq 0.05$).

*Means (% on dry weight basis) ± standard error of triplicate.

4.5. Mineral Composition:

4.5.1. Ash:

The analysis of ash content in Libyan wild mushrooms (*Agaricus bisporus*) is shown in Table (5). It can be seen that stipe samples from Salantah region and cap samples from Asalpiea region generally have higher ash contents which were 22.08% and 21.01% respectively, followed by 20.49% (stipe/Alabraq), 20.11% (cap/Alabraq), and 20.03% (stipe /Marawah), while, cap samples of mushroom which collected from Balanaje region showed average low ash value 13.77%.

Agrahar-Murugkar and Subbulakshmi [12] reported that ash content varied between 6.3% to 13.9% in seven wild mushroom species, which regularly consumed by the Khasis of Megha layek-India.

Barros, *et al.* [20] recorded ash content of 14.93 and 16.48 g/100g in the wild edible mushrooms such as *A. silicola* and *A. silvaticus* respectively, which variably seems to be lower than that of crude protein. The variation in ash content may be attributed to geographical area and degree of ripeness as well as the climate of region.

Table (5): Ash content of mushroom samples in different regions

Region	Ash (%)*	
	Cap	Stipe
Alabraq	20.11^{d} + 0.04	20.49^{c} + 0.12
Alosita	15.41^{m} + 0.05	17.78^{h} + 0.12
Asalpiea	21.01^{b} + 0.06	19.06^{f} + 0.05
Ashnishen	18.87^{fg} + 0.11	16.27^{l} + 0.06
Balanaje	13.77^{o} + 0.04	15.25^{m} + 0.06
Faidiyah	14.43^{n} + 0.07	18.06^{h} + 0.09
Marawah	19.44^{e} + 0.18	20.03^{d} + 0.04
Omar Mukhtar	16.22^{l} + 0.05	14.56^{n} + 0.05
Salantah	18.66^{g} + 0.05	22.08^{a} + 0.11
Sidihamri	19.72^{e} + 0.19	17.17^{i} + 0.08
Werdama	16.88^{j} + 0.1	16.56^{k} + 0.18

$^{a-e}$ Means with different letter are significantly different ($P \leq 0.05$).

*Means (% on dry weight basis) ± standard error of triplicate.

4.5.2. Major Elements:

4.5.2.1. Potassium:

The amount of potassium in cap and stipe of wild Mushroom in different regions of Al-Jabl Alkhader province is presented in Table (6).

The twenty-two samples analyzed, the highest amount (26612.4 mg/kg dw) were found in stipe mushroom samples which collected from Asalpiea region, while, the lowest amount (17336.6 mg/kg dw) was found in cap mushroom samples obtained from Faidiyah region.

The range of potassium level varied among different categories of wild mushroom samples, potassium in cap and stipe ranged from 17336.6 mg/kg dw to 26311.0 mg/kg dw and 20216.7 mg/kg dw to 26612.4 mg/kg dw respectively. Studies from other geographical location also revealed potassium to be the most abundant element [23-25].

Table (6): Potassium content of mushroom samples in different regions

Region	Potassium (ppm)*	
	Cap	Stipe
Alabraq	26311.0^{b} + 2.01	24251.3^{f} + 3.32
Alosita	19296.8^{u} + 3.17	20994.5^{q} + 3.34
Asalpiea	26236.4^{c} + 0.81	26612.4^{a} + 5.94
Ashnishen	25182.3^{e} + 2.17	21454.2^{o} + 2.38
Balanaje	19696.5^{t} + 2.77	22607.8^{j} + 2.21
Faidiyah	17336.6^{v} + 2.58	22878.0^{i} + 1.83
Marawah	21870.8^{m} + 2.92	20216.7^{s} + 6.08
Omar Mukhtar	23238.6^{h} + 1.48	21781.9^{n} + 2.00
Salantah	23308.0^{g} + 2.73	21268.9^{p} + 1.07
Sidihamri	25415.0^{d} + 2.40	20420.7^{r} + 2.6
Werdama	22579.2^{k} + 1.63	22442.4^{l} + 3.77

$^{a-e}$ Means with different letter are significantly different ($P \leq 0.05$).

*Means (ppm on dry weight basis) ± standard error of triplicate.

4.5.2.2. Calcium:

Although mean calcium values varied from 797.044 mg/kg dw (cap samples of wild mushroom from Balanaje region) to 3049.80 mg/kg dw (cap samples of wild mushroom from Werdama region), significant differences ($P \leq 0.05$) were detected among cap samples (Table 7). Stipe samples of wild mushroom from Marawah region had the highest calcium content (2608.25 mg/kg dw) whereas Asalpiea region had the lowest calcium content (1018.28 mg/kg dw).

Values for calcium, a mineral receiving a great deal of attention in nutrition, are somewhat higher than 741 mg/kg dw reported for wild edible mushroom (*T. ganhajun*) by Wu, *et al.* [26]. However, almost similar contents to the present study for calcium (2004 mg/kg dw in *L. amethystea*) presented by Liu, *et al.* [27].

4.5.2.3. Sodium:

Table (8) shows the means, standards errors and ranges of sodium contents in cap and stipe of wild mushrooms obtained from different regions of Al-Jabal Alakhdar province.

The maximum sodium contents detected in stipe samples (Ashnishen region) followed by cap samples (Alosita region). The total sodium content of both cap and stipe samples vary between 194.13 mg/kg dw to 2836.85 mg/kg dw.

Sodium content could be depending on the regional and climatic conditions. Fruiting bodies of cultivated Agaricus bisporus white button mushrooms from Australia had sodium contents ranged from 840 to 1640 mg dw [28]. It can be seen that sodium content in presented study generally higher than those reported by Liu, *et al.* [27] for China wild mushrooms.

The observation of different results can be attributed that the major element (potassium, calcium, and sodium) profile of mushrooms has been affected by environmental factors such as climate, growing conditions, region and soil content [29]. Moreover, most of the metals are distributed unevenly within a fruiting body, with the highest contents usually found in the cap, particularly in the hymenophore, lower in the spores and in the rest of cap and even lower in the stipe [30].

Table (7): Calcium content of mushroom samples in different regions

region	Calcium (mg/kg)	
	Cap	Stem
Al Abraq	1155.40[o] + 2.24	1512.65[g] + 4.0
Alosita	2373.36[c] + 4.51	1357.02[j] + 3.1
Asalpiea	1015.96[r] + 2.22	1018.28[r] + 3.74
Ashnishen	1270.29[l] + 1.81	1438.19[h] + 1.58
Balanaje	797.044[t] + 4.66	1524.16[f] + 2.56
Faidiyah	830.28[s] + 3.38	1128.36[p] + 2.16
Marawah	2151.74[d] + 4.83	2608.25[b] + 2.28
Omar Mukhtar	1089.17[q] + 5.57	1170.74[n] + 2.84
Salantah	1014.07[r] + 4.04	1817.33[e] + 4.72
Sidihamri	1231.77[m] + 2.75	1377.89[i] + 5.31
Werdama	3049.80[a] + 3.13	1285.44[k] + 1.63

[a-e] Means with different letter are significantly different ($P \leq 0.05$).

[*]Means (mg/kg on dry weight basis) ± standard error of triplicate.

Table (8): Sodium content of mushroom samples in different regions

Region	Sodium (ppm)[*]	
	Cap	Stipe
Alabraq	728.36[n] + 1.56	886.83[k] + 2.28
Alosita	1797.31[b] + 2.56	1150.48[e] + 1.19
Asalpiea	804.50[m] + 3.21	829.56[l] + 1.71
Ashnishen	702.46[p] + 1.00	2836.85[a] + 0.99
Balanaje	293.38[s] + 0.47	663.42[q] + 1.98
Faidiyah	194.13[t] + 0.27	532.27[r] + 1.87
Marawah	951.36[i] + 1.76	833.71[l] + 1.88
Omar Mukhtar	963.71[h] + 1.9	897.32[j] + 1.92
Salantah	720.91[o] + 1.81	988.49[g] + 4.01
Sidihamri	726.02[no] + 1.81	1174.91[d] + 1.05
Werdama	1412.73[c] + 1.66	1035.63[f] + 3.53

[a-e] Means with different letter are significantly different ($P \leq 0.05$).

*Means (ppm on dry weight basis) ± standard error of triplicate.

4.5.3. Minor Elements:

4.5.3.1. Iron:

Iron is vital for almost all living organisms, participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. It is known that adequate iron in a diet is very important for decreasing the incidence of anemia. Iron deficiency occurs when the demand for iron is high, e.g., in growth, high menstrual loss, and pregnancy, and the intake is quantitatively inadequate or contains elements that render the iron unavailable for absorption [31]. High concentrations of iron may lead to tissue damage, as a result of the formation of free radicals.

The iron contents of the wild edible mushrooms (*Agaricus bisporus*) samples are given in Table (9), the iron content of cap of *A. bisporus* ranged from 498.82 to 1566.88 mg/kg dw obtained from Alosita region Sidihamri region respectively, whereas iron contents of stipe samples of *A. bisporus* were higher than cap samples which ranged from 1170.06 to 1743.47 mg/kg dw.

The reported iron values for mushroom samples were 31.3–1190 mg/kg [8], 30–150 mg/kg [32], 180–407 mg/kg [33], 146–835 mg/kg [34], 56.1–7162 mg/kg [35], 568–3904 mg/kg [36], 102–1580 mg/kg [37], 211–628 mg/kg [38], 110–11460 mg/kg [39], and 150–1741 mg/kg [40]. The iron values in the present study are in agreement with reported value in the literature.

4.5.3.2. Zinc:

Zinc is an integral component of a wide variety of different enzymes in which it plays catalytic, structural, and regulatory roles. Zinc deficiency which can result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism. The deficiency of zinc, particularly in children, can lead to loss of appetite, growth retardation, weakness, low-spirited, stagnation in sexual growth.

Mushrooms are known as zinc accumulators and the sporophore: substrate ratio for zinc ranges from 1 to 10 mg/kg [33]. Table (4.9) showed that the minimum and maximum values of zinc in analyzed samples were 87.65 and 151.94 mg/kg dw in the cap of wild edible mushroom obtained from Marawah region and stipe of wild edible mushroom obtained from Omar Mukhtar region respectively. The WHO permissible limit of zinc in foods is 60 mg/kg [41]. The values for zinc in investigated mushroom samples were above the WHO's values. Zinc concentrations of mushroom samples in the literature have been reported to be in the ranges: 25–122 mg/kg [36], 35.9–96.6 mg/kg [42], 45.2–173.8 mg/kg [39], 43.5–205 mg/kg [40] and 51.5-162 mg/kg [38], respectively. Zinc values presented in this study are in agreement with literature values.

Table (4.8): Iron content of mushroom samples in different regions

Region	Iron (ppm)*	
	Cap	**Stipe**
Alabraq	1281.49^{i}+0.86	1743.47^{a}+3.69
Alosita	498.82^{r}+2.31	1325.21^{g}+2.42
Asalpiea	1234.51^{k}+2.98	1361.04^{ef}+3.26
Ashnishen	1096.94^{p}+1.8	1248.78^{j}+4.29
Balanaje	1118.15^{o}+2.11	1365.14^{e}+1.56
Faidiyah	1137.25^{n}+2.47	1184.43^{l}+0.58
Marawah	1355.94^{f}+0.64	1559.70^{d}+0.67
Omar Mukhtar	1170.39^{m}+1.66	1173.81^{m}+0.51
Salantah	1354.94^{f}+2.57	1702.87^{b}+1.85
Sidihamri	1566.88^{c}+2.37	1170.06^{m}+2.05
Werdama	1086.77^{q}+1.45	1296.49^{h}+3.09

a-e Means with different letter are significantly different (P ≤ 0.05).

*Means (ppm on dry weight basis) ± standard error of triplicate.

Table (4.9): Zinc content of mushroom samples in different regions

Region	Zinc (ppm)*	
	Cap	**Stipe**
Alabraq	107.67gh + 1.39	105.19hi + 0.69
Alosita	128.88b + 1.93	97.17k + 1.45
Asalpiea	115.40de + 1.74	108.07fgh + 0.35
Ashnishen	131.38b + 0.85	128.49b + 1.49
Balanaje	121.61c + 0.84	101.28ij + 0.6
Faidiyah	116.23d + 0.59	111.56efg + 1.36
Marawah	151.94a + 1.72	118.77cd + 0.74
Omar Mukhtar	105.03hi + 1.35	87.65l + 1.37
Salantah	101.60i + 1.29	91.86l + 0.81
Sidihamri	112.02ef + 0.87	110.71fg + 1.3
Werdama	97.72jk + 1.9	97.40k + 1.82

a-e Means with different letter are significantly different ($P \leq 0.05$).

*Means (ppm on dry weight basis) ± standard error of triplicate.

4.5.3.3. Copper:

Copper is the third most abundant trace element in the human body, with vitamin-like impact on living systems. A small amount of copper is found in the human body (50-120 mg), but it plays a critical role in a variety of biochemical processes [43]. It is known that copper may be toxic to both humans and animals when its concentration exceeds the safe limits [44]. Among wild-edible mushroom (*Agaricus bisporus*) obtained from different areas of Al-Jabal Alkahder province, the copper content of the samples ranged from 32.12 to 63.32 mg/kg dw, stipe of wild edible mushroom obtained from Ashnishen region had the lowest copper concentration whereas Werdama region had the highest (Table 4.10). Copper concentrations, accumulated in mushroom species, are usually 100–300 mg/kg, which is not considered a health risk [37]. These levels are below the WHO permissible limits in foods, which is 40 mg/kg [45]. Copper contents of mushroom samples in the literature have been reported to be in the ranges: 11.4-15.8 mg/kg [46], 10.3-145 mg/kg [8], 12-181 mg/kg [34], 13.4- 50.6 mg/kg [37], 10.6-144.2 mg/kg [39], and 15-73 mg/kg [40], respectively. Our copper levels were found to be lower than those reported in the literature. In general, copper contents in mushrooms are higher than those in green plant and vegetables and should be considered as a nutritional source of this element [33].

4.5.3.4. Cadmium:

Cadmium is considered to be one of the most toxic metals. In addition, it may accumulate in the human body and may induce kidney dysfunction, skeletal damage, reproduction deficiencies, prostate cancer, mutations, and fetal (embryonic) death [47].

It was reported that cadmium is accumulated mainly in kidneys, spleen, and liver, and its blood serum level increases considerably following mushroom consumption[32]. Mushroom, in particular, can be very rich in cadmium. Cadmium accumulation has been demonstrated in the literature [48].

Data presented in Table (4.11) indicate the mean concentration of cadmium in the cap and stipe of wild edible mushrooms (*Agaricus bisporus*) collected from eleven regions of Al-Jabal Alakhader province. Cadmium concentrations were determined on a dry weight basis.

The cadmium content in cap samples was found to be varied between 0.02 to 0.39 mg/kg dw, the highest cadmium concentration was detected in Asalpiea region while the lowest was detected in Omar Mukhtar region, Of 22 samples, maximum cadmium content was recorded in stipe obtained from Ashnishen region (0.44 mg/kg dw) also as indicate in Table (4.11) the results showed no significant difference between stipe samples obtained from Balanaje, Omar Mukhtar, and Salantah regions which ranked first from point view lowest cadmium content. It was surprising that no significant differences ($P > 0.05$) of cadmium contents in both cap and stipe samples obtained from Alabraq, Alosita, Balanaje, Omar Mukhtar, and Salantah regions.

Table (4.10): Copper content of mushroom samples in different regions

Region	Copper (ppm)*	
	Cap	Stipe
Alabraq	58.37^{b} + 1.48	52.10^{de} + 0.66
Alosita	51.10^{ef} + 0.62	44.32^{gh} + 1.15
Asalpiea	36.69^{i} + 0.97	46.25^{gh} + 1.27
Ashnishen	54.85^{bcde} + 1.57	32.12^{j} + 1.08
Balanaje	55.77^{bcd} + 1.39	65.74^{a} + 1.72
Faidiyah	47.27^{gh} + 1.02	51.71^{def} + 1.25
Marawah	53.98^{cde} + 0.76	48.04^{fg} + 1.71
Omar Mukhtar	65.15^{a} + 1.78	51.99^{de} + 1.15
Salantah	56.98^{bc} + 1.56	37.02^{i} + 0.8
Sidihamri	39.60^{i} + 0.91	43.88^{h} + 1.29
Werdama	52.75^{de} + 0.95	63.32^{a} + 1.41

a-e Means with different letter are significantly different ($P \leq 0.05$).

*Means (ppm on dry weight basis) ± standard error of triplicate.

Table (4.12): Cadmium content of mushroom samples in different regions

Region	Cadmium (mg/kg)*	
	Cap	Stem
Alabraq	0.06[i] + 0.07	0.05[i] + 0.08
Alosita	0.21[cd] + 0.01	0.20[de] + 0.02
Asalpiea	0.39[b]+ 0.02	0.14[g] + 0.04
Ashnishen	0.23[c] + 0.02	0.44[a] + 0.12
Balanaje	0.04[j] + 0.09	0.01[j] + 0.07
Faidiyah	0.11[h] + 0.1	0.15[g] + 0.23
Marawah	0.14[g] + 0.08	0.06[i] + 0.09
Omar Mukhtar	0.02[j] + 0.03	0.01[j] + 0.05
Salantah	0.06[i] + 0.11	0.01[j] + 0.04
Sidihamri	0.17[ef] + 0.01	0.21[cd] + 0.01
Werdama	0.06[i] + 0.11	0.16[fg] + 0.08

a-e Means with different letter are significantly different ($P \leq 0.05$).

*Means (mg/kg on dry weight basis) ± standard error of triplicate.

Certain countries have established statutory limits for Cd and Pb in edible mushrooms. In the Czech Republic, limits of 2.0 and 10.0 mg/ kg dry matter have been established for Cd and Pb, respectively. In Poland, recommendations concerning the concentrations of Cd and Pb in dried mushrooms are slightly different. The tolerance limits set for Cd and Pb are 1.0 and 2.0 mg / kg dry weight, respectively [49].

The maximum level for certain contaminants in foodstuffs established by the Commission of the European Communities [50][51] (European Commission Regulations, 2001) is set at about 0.2 and 0.3 mg/kg wet weight for Cd and Pb, respectively, in cultivated fungi. Assuming that the dry matter content of mushrooms is 10% [32], these same limits for the dry material will be ten times higher and approach 2.0 and 3.0 mg / kg dry weight for Cd and Pb, respectively.

The levels of cadmium in both stipe and cap samples were lower than the statutory limits of Poland and the European Community. The determined values were generally comparable to the values reported by other authors. It can be seen that wild edible mushrooms obtained from Al-

Jabal Alakhdar province generally have somewhat lower cadmium contents than those reported from the central Anatolia, Turkey by Yamaç, *et al.* [39] and almost similar than those reported from the Yunnan Province, China [48].

4.5.3.5. Lead:

Lead has been introduced to the environmental both through natural processes and as a consequence of human activities such as industrialization or fuel combustion [6]. The toxicity of lead is attributed to the fact that it interferes with the normal function of enzymes. Bipolar lead forms strong bonds with enzymes bearing sulfhydryl groups thus inhibiting their action. Lead is toxic to the blood and the nervous, urinary, gastric and genital systipe. Furthermore, it is also implicated in causing carcinogenesis, mutagenesis, and teratogenesis in experimental animals [11].

The average lead contents (mg/kg dw) of wild edible mushroom (*Agaricus bisporus*) obtained from different areas of Al-Jabal Alakhdar province are given in Table (4.13). In the cap samples, the highest lead content was 0.92 mg/kg dw obtained from Sidihamri region, while, the lowest concentration of lead was 0.33 mg/kg dw in the cap obtained from Werdama region.

The results showed that all samples (cap and stipe) of *A. bisporus* from the Al-Jabel Alakhdar province had Pb concentrations lower than both the Polish and European tolerance limits set for dried mushrooms (3 mg/kg).

As shown in Table (4.13) the results showed no significant difference between the cap and stipe samples obtained from Al Abraq, Alosita, Ashnishen, Marawah, and Omar Mukhtar from point of view of lead content. The current data show the concentration of lead in cap and stipe samples of wild edible mushrooms (*A. bisporus*) obtained from different areas of Al-Jabel Alakhdar province were higher than those reported from Dambovita County, Romania by Stihi, *et al.* [7], western Black Sea region, Turkey by Konuk, *et al.* [51], and Island of Lesvos, Greece by Aloupi, *et al.* [30] on the other hand similar results were reported from Balikasir, Turkey by Işıloğlu, *et al.* [33], and Ordu, Turkey by Mendil, *et al.* [38].

Table (4.13): Lead content of mushroom samples in different regions

Region	Lead (mg/kg)*	
	Cap	Stem
Alabraq	0.80^{defg} + 0.05	0.84^{bcd} + 0.09
Alosita	0.87^{ab} + 0.18	0.86^{bc} + 0.1
Asalpiea	0.85^{bc} + 0.08	0.78^{efgh} + 0.19
Ashnishen	0.87^{ab} + 0.06	0.82^{bcde} + 0.09
Balanaje	0.57^{k} + 0.06	0.68^{i} + 0.08
Faidiyah	0.63^{j} + 0.16	0.76^{gh} + 0.15
Marawah	0.77^{fgh} + 0.05	0.82^{cdef} + 0.1
Omar Mukhtar	0.44^{l} + 0.10	0.40^{l} + 0.46
Salantah	0.69^{i} + 0.05	0.74^{h} + 0.14
Sidihamri	0.92^{a} + 0.14	0.80^{defg} + 0.21
Werdama	0.33^{m} + 0.12	0.44^{l} + 0.21

a-e Means with different letter are significantly different ($P \leq 0.05$).

*Means (mg/kg on dry weight basis) ± standard error of triplicate.

The trace metal content of mushrooms is related to species of mushroom, collecting site of the sample, the age of fruiting bodies and mycelium, distance from sources of pollution and mainly affected by acidic and organic matter content of the soil. Metal ion uptake of mushrooms is considerably higher than plants because of their effective take-up mechanism.

5. CONCLUSION

It is shown that the investigated wild edible mushrooms obtained from Al-Jabal Alakhdar province were rich sources of protein and carbohydrates and had low amounts of fat. The obtained results were closely similar to each other and agreed well with the previous data. The studied fruiting bodies (Cap and stipe) contain minerals required in the human diet, such as Fe, Zn, and Cu. The results make these wild edible mushrooms popular to consume as good mineral sources.

This first survey of heavy metal contents in cap and stipe of wild edible mushroom samples from different regions of Al-Jabal Alakhdar province, Libya. The levels of the heavy metals Cd and Pb in all studied samples can be considered sufficiently low and therefore pose no health risk, it is appropriate to analyze concentrations of these metals in mushrooms to test contamination of the environment by heavy metals.

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