

## Comparative study of the mineral composition of selected cultivated mushrooms

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### Abstract

The present study reports a comparison of the mineral composition of four selected cultivated mushrooms: lion's mane (*Hericiuma erinaceus*), reishi (*Ganoderma lucidum*), pink oyster mushroom (*Pleurotus djamor*) and oyster mushroom (*Pleurotus ostreatus*). The levels of studied mineral elements (K, Ca, Zn, Cu, Co, Mn, Ni, Cr, Cd and Pb) in the substrate and mushroom samples were determined by atomic absorption spectroscopy using the Shimadzu AA-7000 device. The most abundant mineral elements in the analyzed mushrooms were K and Ca, ranging between 23703.2-36721.8 mg kg<sup>-1</sup> and 105.3-123.1 mg kg<sup>-1</sup> dry mass, respectively. The content of heavy metals (Ni, Cr and Cd) was very low, while Pb and Co were below detectable levels. The study findings suggest that the translocation of mineral elements from substrate to mushroom fruiting bodies is strongly species-dependent.

**Key words:** *Hericiuma erinaceus*, *Ganoderma lucidum*, *Pleurotus djamor*, *Pleurotus ostreatus*, nutrients, substrate, translocation

### Introduction

Mushrooms are a well-known source of various biomolecules with high nutritional value and bioactive properties, mostly related to their anti-inflammatory, antiproliferative and antioxidant activity (Łysakowska *et al.*, 2023). Due to these properties, edible mushrooms have been recognized and accepted as both functional foods, and natural health products.

Some of the most popular mushrooms among producers and consumers are oyster mushroom (*Pleurotus ostreatus*), pink oyster mushroom (*Pleurotus djamor*), lion's mane (*Hericium erinaceus*) and reishi (*Ganoderma lucidum*). These mushrooms are a good source of vitamins, antioxidants and some important minerals such as selenium and potassium. They are also rich in fiber and high-quality protein (Dimopoulou *et al.*, 2022).

However, edible mushrooms may also contain substances that can have a negative impact on human health, including, among others, toxic heavy metals. Gebrelibanos *et al.* (2016) reported that the heavy metal content of mushrooms is always considerably higher than that of plants growing in similar localities. This finding suggests that mushrooms do not possess effective mechanisms for blocking or avoiding heavy metal uptake.

In view of this, this study aimed to evaluate heavy metals contents (Zn, Cu, Co, Mn, Ni, Cr, Cd and Pb) in four selected edible mushrooms: oyster mushroom (*Pleurotus ostreatus*), pink oyster mushroom (*Pleurotus djamor*), lion's mane (*Hericium erinaceus*) and reishi (*G. lucidum*), grown on a substrate obtained by mixing beech sawdust with wheat bran in a ratio of 80:20. An additional aim of this study was to evaluate K and Ca content, in mushrooms mentioned above. We hypothesized that there are

significant differences in mineral composition among the selected mushrooms.

### Material and methods

**Location of study site:** The experiment was carried out at the experimental station of the Faculty of Agriculture and Food Science in Sarajevo (43°49'34.41" N and 18°19'18.47" E) during October 2023 to mid-January 2024.

**Substrate collection and preparation:** Sawdust was purchased from the wood market, while the wheat brans were collected from a farmer's field from around Sarajevo town. Substrate containing beech sawdust and wheat bran was prepared as follows: beech sawdust with the wheat brain was manually mixed at ratio 80:20 and then water [70% (v/w)] was added to the mixture after passing it off through a sieve with a pore size of 5 mm. Thereafter, 1.5 kg of sawdust-wheat bran mixtures was filled in polypropylene bags (20.32 × 30.48 cm) and pasteurized at 65°C for 8 h.

**Substrate inoculation and incubation:** After pasteurization, filled bags were cooled at room temperature and then inoculated with mushroom mother spawn in a concentration of 10%. Mushroom mother spawn was generously donated by Urban Farm Mikić (Orašje, Bosnia and Herzegovina), and was prepared using the method of spawn preparation outlined by Stamets and Chilton (1983). Following inoculation, the bags were incubated in a climate chamber at 22°C and humidity of 80% for 20 days under dark conditions, at which point the substrates were fully colonized with mycelium. The bags were then sliced (width 1 cm) at six positions and then exposed at 16°C to temperature shock for three days to stimulate fructification, followed by incubation at 18±2°C at 85% humidity until harvest. Air relative

humidity was maintained at 85% using a fogging system, and light exposure at 2000 lux set on a 12 h on/off cycle. This study included four treatments (four different mushroom species) and three replications so that a total of 12 bags were used.

**Collection and preparation of mushroom samples for analysis:** Samples of complete fruiting bodies from each substrate were dried in an oven at 40 °C to a constant weight. Then, dried fruiting bodies were ground into a fine powder using an electric blender and stored in paper bags until analysis was performed. Preparation and extraction of mushroom samples for mineral elements determination were performed as follows: 3 g of mushroom powder was placed into a 100 mL round bottom flask, then 10 mL HNO<sub>3</sub> and 4 mL H<sub>2</sub>SO<sub>4</sub> were added. The flasks were kept overnight, and the next day, all the samples were heated on a hot plate at 120°C until complete digestion. After cooling to room temperature, the solution in the flask was filtered through quantitative filter paper into a 50 mL flask and diluted with deionized water to the mark (Lisjak *et al.*, 2009).

**Preparation of substrate samples for analysis:** The following chemical properties of substrate samples were the subject of analysis: soil acidity (pH), organic matter content and content of mineral elements (K, Ca, Zn, Cu, Co, Mn, Ni, Cr, Cd and Pb). pH in H<sub>2</sub>O and 1M KCl was determined using a glass electrode in a 1:5 (V/V) suspension of soil in water/1M KCl (ISO, 2005) and organic matter by oxidation with potassium dichromate in the presence of sulphuric acid (ISO, 1998).

Extraction of mineral elements from the mushroom substrate was carried out with aqua regia solution (a mixture of HCl and HNO<sub>3</sub> in a ratio of 3:1) as follows: 3 g of the air-dried substrate sample was transferred into a 250 mL round bottom flask, and then 28 mL of aqua regia was added. The flask was kept overnight, and the next day, the mixture was heated on a hot plate under reflux for 2 h. After cooling to room temperature, the mixture was filtered through quantitative filter paper into a 100 mL Erlenmeyer flask and diluted to the mark with deionized water (ISO, 1995).

**Determination of mineral elements in substrate and mushroom samples:** The levels of mineral elements in substrate and mushroom samples were analyzed by atomic emission spectrophotometry using the Shimadzu AA-7000 device. The standard solutions of examined mineral elements were prepared by dilution of standard stock solutions (Merck AAS solutions) with deionized water (ISO, 1998).

**Determination of the substrate-to-mushroom transfer factor:** The substrate-to-mushroom transfer factor (TF) is defined as the ratio of the contents of mineral elements in mushroom to that in the substrate and was calculated using Eq. 1 (Olowoyo *et al.*, 2010).

$$TF = C_{\text{mushroom}} / C_{\text{substrate}}$$

where C mushroom and C substrate represent the mineral element content in the mushroom and substrate on a dry mass basis, respectively. Higher TF values ( $\geq 1$ ) indicate higher element absorption from the substrate by mushrooms.

**Statistical analysis:** All assays were performed in triplicates and the results were expressed as means $\pm$ standard deviation. One-way analysis of variance (ANOVA) was conducted using computer software SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA) and

differences between means were tested using the least significance difference (LSD) test at  $p < 0.05$ .

## Results and discussion

Results of the chemical analysis of the mushroom growing media, *i.e.*, the substrate used in the study, are presented in Table 1. All results are expressed on a dry mass basis.

Table 1. Basic chemical characteristics of the mushroom substrate

Parameter	Value
pH (H <sub>2</sub> O)	6.9
pH (H <sub>2</sub> O)	6.4
organic matter	78.7
K (mg kg <sup>-1</sup> )	14198.32 $\pm$ 401.5
Ca (mg kg <sup>-1</sup> )	365.02 $\pm$ 45.1
Zn (mg kg <sup>-1</sup> )	49.6 $\pm$ 11.5
Cu (mg kg <sup>-1</sup> )	8.63 $\pm$ 2.1
Co (mg kg <sup>-1</sup> )	n.d.
Mn (mg kg <sup>-1</sup> )	92.3 $\pm$ 9.2
Ni (mg kg <sup>-1</sup> )	3.01 $\pm$ 0.7
Cr (mg kg <sup>-1</sup> )	1.09 $\pm$ 0.3
Cd (mg kg <sup>-1</sup> )	0.15 $\pm$ 0.05
Pb (mg kg <sup>-1</sup> )	n.d.*

\* n.d. - not detected

The mushroom substrate used in this study was slightly acidic and had a very high level of organic matter. The most abundant mineral elements in the analyzed mushroom substrate samples were K and Ca. The levels of Zn, Cu, Mn, Ni, Cr and Cd were relatively low, while Co and Pb were not even detected in substrate samples.

The levels of studied mineral elements (K, Ca, Zn, Cu, Co, Mn, Ni, Cr, Cd and Pb) in the fruiting bodies of four investigated mushroom species are given in Table 2.

Table 2. The levels of mineral elements in mushroom fruiting bodies

Element	Mushroom species				LSD <sub>0.05</sub>
	Oyster	Pink Oyster	Lion's Mane	Reishi	
K	38718.1 $\pm$ 400.0 <sup>a*</sup>	32194.2 $\pm$ 1432.1 <sup>c</sup>	36721.8 $\pm$ 1160.4 <sup>b</sup>	23703.2 $\pm$ 699.1 <sup>d</sup>	987.4
Ca	88.5 $\pm$ 17.4 <sup>c</sup>	111.8 $\pm$ 24.2 <sup>ab</sup>	123.1 $\pm$ 15.1 <sup>a</sup>	105.3 $\pm$ 12.3 <sup>b</sup>	15.1
Zn	72.9 $\pm$ 6.2 <sup>b</sup>	89.9 $\pm$ 8.4 <sup>a</sup>	71.6 $\pm$ 10.7 <sup>b</sup>	45.5 $\pm$ 11.2 <sup>c</sup>	8.9
Cu	5.9 $\pm$ 0.7 <sup>d</sup>	8.9 $\pm$ 2.5 <sup>c</sup>	20.6 $\pm$ 3.0 <sup>b</sup>	37.9 $\pm$ 3.9 <sup>a</sup>	2.7
Co	n.d.	n.d.	n.d.	n.d.	-
Mn	9.2 $\pm$ 3.2 <sup>b</sup>	13.4 $\pm$ 4.2 <sup>b</sup>	24.2 $\pm$ 5.3 <sup>a</sup>	9.6 $\pm$ 4.8 <sup>b</sup>	4.5
Ni	0.45 $\pm$ 0.63	0.91 $\pm$ 0.76	0.57 $\pm$ 0.53	0.82 $\pm$ 0.52	-
Cr	0.61 $\pm$ 0.24	0.51 $\pm$ 0.27	0.56 $\pm$ 0.34	0.71 $\pm$ 0.42	-
Cd	0.43 $\pm$ 0.31 <sup>b</sup>	0.87 $\pm$ 0.28 <sup>a</sup>	0.30 $\pm$ 0.23 <sup>b</sup>	0.17 $\pm$ 0.16 <sup>bc</sup>	0.2
Pb	n.d.**	n.d.	n.d.	n.d.	-

\*Averages denoted by the same letter in the same column indicate no significant difference ( $p < 0.05$ ). \* n.d. - not detected

All results are expressed in mg kg<sup>-1</sup> on a dry-mass basis. The most abundant mineral elements in the analyzed mushroom fruiting bodies were K and Ca, ranging between 23703.2-36721.8

mg kg<sup>-1</sup> and 105.3-123.1 mg kg<sup>-1</sup> dry mass, respectively. The contents of heavy metals Ni, Cr and Cd were low, while Co and Pb were not even detected in mushroom samples.

Transfer substrate-mushroom factor values of mineral elements are given in Table 3. It was observed that K and Cd have the highest TF value, followed by Zn and Cu, and Mn has the lowest TF value, regardless of mushroom species.

Table 3. Transfer substrate-mushroom factor values of mineral elements

Element	Mushroom species			
	Oyster	Pink Oyster	Lion's Mane	Reishi
K	2.73	2.27	2.59	1.67
Ca	0.24	0.31	0.34	0.29
Zn	1.47	1.81	1.44	0.92
Cu	0.68	1.03	2.39	4.39
Mn	0.09	0.14	0.26	0.10
Ni	0.15	0.29	0.19	0.27
Cr	0.56	0.47	0.51	0.65
Cd	2.87	5.80	2.0	1.13

In this study, K and Ca were the major mineral elements found in the mushroom substrate and in the fruiting bodies of all examined mushroom species. The levels of K in the mushroom fruiting bodies ranged from 2.37 to 3.87%, depending on the mushroom species. The highest K content was determined in oyster mushrooms, followed by lion's mane and pink oyster. All mushroom species had the substrate-to-mushroom transfer factor (TF) for K higher than 1, indicating that mushrooms can transfer K very efficiently from growing media to the fruiting body. These findings are in line with previous studies (Vinichuk *et al.*, 2010; Malinowski *et al.*, 2021).

Ca content in mushroom fruiting bodies ranged from 88.5 in oyster mushrooms to 123.1 mg kg<sup>-1</sup> in lion's mane mushrooms. However, the TF values for Ca were below 0.4 for all mushroom species, suggesting that the mushrooms do not have a high capacity to absorb Ca from growing media. Similar findings have been reported by Lee *et al.* (2009). Low TF values were also determined for Ni and Mn, regardless of mushroom species.

In this study, the Ni content in pink oyster mushrooms was the highest (0.91±0.76 mg kg<sup>-1</sup>) and least in oyster mushroom (0.45±0.63 mg kg<sup>-1</sup>). However, there was no significant difference in Ni content between mushroom species in this study. Ważny *et al.* (2021) reported that Ni is an essential element required for a number of physiological functions in mushroom bodies, including proper enzyme activity and cell wall formation. Hence, it is expected that mushrooms can absorb Ni very efficiently from growing media, but the results of this study did not confirm this hypothesis. In this study, the TF value for Ni was low (below 0.3) for all investigated mushroom species. Many scientists agree that the uptake of Ni by mushrooms is a very complex process that depends on several factors, including substrate/soil composition and pH value, among others (Congeevaram *et al.*, 2007; Tel *et al.*, 2014; Kokkoris *et al.*, 2019). The excerpt describes the effect of the pH and potassium (K) concentration of a growth medium on nickel (Ni) absorption in mushrooms. It shows how these factors play in concert to regulate Ni uptake, and thereby the nutritional and chemical composition of mushrooms. It suggests that mushrooms tend to absorb more nickel when grown in environments with low pH or low potassium levels. In this particular study, the mushrooms were grown in a slightly acidic

substrate with a high potassium content, which might explain the observed low translocation factor (TF) for nickel.

TF values for Mn in this study were low and ranged from 0.09 to 0.26, depending on the investigated mushroom species. Interestingly, TF values for Mn in plants are usually several times higher than in mushrooms, which can be related to the role of Mn in plants. Mn is an essential element for photosynthesis, particularly for the structure of photosynthetic proteins (Messant *et al.*, 2023). Therefore, plants generally have a higher capacity to accumulate Mn than mushrooms that cannot perform photosynthesis.

On the other hand, the content of heavy metals such as Cr, Zn, Cu, and especially Cd is likely to be higher in mushrooms than in plants. The results of this study support this hypothesis. The TF values for Zn, Cu and Cd were generally higher than one for all investigated mushroom species, suggesting that mushrooms have effective mechanisms that allow them to easily take up Zn, Cu, Cd and some other heavy metals from the substrate on which they grow.

Although mushrooms do not possess effective mechanisms to prevent the uptake of heavy metals, they have developed various mechanisms to neutralize heavy metals within their body. Some of these mechanisms include vacuolar sequestration and the synthesis of various biochemical compounds that have the potential to immobilize heavy metals (Robinson *et al.*, 2021).

In this study, Zn was found in all the investigated mushroom species with content ranging from 45.5±11.2 mg kg<sup>-1</sup> in reishi to 89.9±8.4 mg kg<sup>-1</sup> in pink oyster mushrooms. This mineral element plays a crucial role in enzyme function in various metabolic pathways, and therefore, mushrooms tend to absorb as much Zn as possible from the growing media (Ma *et al.*, 2021). High TF values for Zn observed in this study confirm this.

Cu, like Zn, is an essential element for mushroom growth and development. It serves as an activator of numerous enzymes involved in various physiological processes in mushrooms (Bell *et al.*, 2022). All investigated mushroom species in this study had a high TF value (from 0.68 in oyster mushroom to 4.39 in reishi), indicating that mushrooms can transfer Cu very efficiently from the substrate to the mushroom fruiting bodies. These findings are in line with previous studies (Smith *et al.*, 2017; Akpasi *et al.*, 2023).

In this study, the Cr content was found to vary from 0.51±0.27 mg kg<sup>-1</sup> in pink oyster mushroom to 0.71±0.42 mg kg<sup>-1</sup> in reishi. Cr content values in mushroom samples have been reported to be in the ranges 0.87-2.66 mg kg<sup>-1</sup> (Tüzen 2003), 0.77-80.3 mg kg<sup>-1</sup> (Kaya *et al.*, 2011), and 0.74-3.51 mg kg<sup>-1</sup> (Nambafu *et al.*, 2023), respectively. The Cr content of the mushrooms studied in the present study was found to be lower than those reported earlier. From this point of view, the consumption of mushroom-fruiting bodies from this study cannot be considered a toxicological risk.

The analysis of Cd content in the investigated mushroom species revealed that the highest content was found in pink oyster mushroom (0.87±0.28 mg kg<sup>-1</sup> dry mass) and the lowest in reishi (0.17±0.16 mg kg<sup>-1</sup> dry mass). The Cd content of mushroom species usually varies in the range of <0.5 to 1 mg kg<sup>-1</sup> (Melgar *et al.*, 2016; Širić *et al.*, 2016; Nowakowski *et al.*, 2021), indicating



that the Cd content values determined in this study are consistent with those reported in the scientific literature.

The results of this study also showed that TF value for Cd was very high (higher than 1), regardless of mushroom species. This finding strongly suggests that all of the studied mushrooms have a strong ability to transfer Cd from substrate to fruiting bodies. Numerous studies have also revealed that mushrooms have a good Cd accumulation potential (Muszyńska *et al.*, 2018; Gałgowska and Pietrzak-Fiećko, 2021). From the consumer's point of view, these findings are very undesirable because Cd is one of the most dangerous elements for human health.

In this study, the content of the toxic heavy metal Pb and Co in the investigated mushroom species were too low to be detected by the analytical technique used in this study.

The most abundant mineral elements in the analyzed mushrooms were K, Ca, and Zn. The content of heavy metals (Ni, Cr and Cd) was very low, while Pb and Co were below detectable levels. The study findings suggest that the translocation of mineral elements from the substrate to mushroom fruiting bodies is strongly species-dependent.

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