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


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## ORIGINAL RESEARCH

# Heavy metals accumulation in the oyster mushroom basidiomes cultivated on different substrates

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## Abstract

The aim of this study was to assess the ability of oyster mushrooms (*Pleurotus ostreatus* (Jacq.) P. Kumm.) to absorb heavy metals (Fe, Zn, Cu, Co, Mn, Ni, Cr, Cd, and Pb) from different plant-based substrates and to determine the bioaccumulation factor of the aforementioned heavy metals from the substrate to the oyster mushroom basidiomes. The substrate used in this study were: maize straw, beech sawdust supplemented with wheat bran at a rate of 20%, a mixture of maize straw and spent coffee grounds in a ratio of 70:30, and a mixture of maize straw and spent coffee grounds in a ratio of 50:50. Heavy metal contents in substrate and mushroom samples were analyzed by atomic absorption spectroscopy using the Shimadzu AA-7000 device, while the bioaccumulation factors of oyster mushroom for each investigated heavy metal were calculated from the heavy metal content in mushrooms divided by that found in substrates. The study showed that oyster mushrooms have a high capacity to absorb Zn and Cd from the growing medium and bioaccumulation factor values for Zn and Cd determined in this study strongly support this observation. On the other hand, bioaccumulation factor values for Ni, Fe, and Mn were less than 0.3 and ranged from 0.04 to 0.05, from 0.09 to 0.12, and from 0.10 to 0.25, respectively. In general, the results of this study lead to the conclusion that substrate chemical composition strongly affects the heavy metal accumulation in oyster mushroom basidiomes. The results of this study also showed that oyster mushrooms can be considered a promising species for Cd and Zn bioremediation.

## Keywords

cultivation; translocation; biomass; *Pleurotus ostreatus*

## 1. Introduction

Edible mushrooms are generally recognized as a healthy food source due to their high content of nutrients, including carbohydrates, proteins, fatty acids, vitamins, and minerals. They also have numerous health-beneficial properties, such as antioxidative, antibacterial, and anti-inflammatory activities, leading to their increasing production and consumption worldwide (Assemie & Abaya, 2022).

On the other hand, it is well known that mushrooms have the ability to absorb high concentrations of toxic heavy metals from the soil or substrate in which they grow. In this light, numerous studies have revealed that the consumption of mushrooms contaminated with toxic heavy metals such as Cr, Cd, and Pb causes intense toxicological damage to human health (Govorushko et al., 2019; Orywal et al., 2021). Adverse health effects associated with exposure to Cr include, among others, respiratory distress, skin irritation, and the risk of lung cancer (Shin et al., 2023), Cd affects the kidneys, liver,

bone, and heart (Fatima et al., 2019), while the Pb exposure mainly causes anemia, kidney dysfunction, hypertension, loss of appetite and behavioral changes (Collin et al., 2022). This problem is especially severe in areas with a considerable degree of industrialization, where the levels of toxic heavy metals in the environment, and consequently in the food chain, are constantly increasing. It is not surprising, therefore, that in recent years considerable attention has been focused on assessing the health risks associated with mushroom consumption.

The accumulation of heavy metals in mushrooms is a complex process influenced by numerous factors, including mushroom species, mushroom growth stage, and substrate composition. However, without any doubt, substrate composition and its chemical properties are the key factors that determine the heavy metal availability to mushrooms (Jasinska et al., 2022).

*Pleurotus* is a genus of edible mushrooms widely cultivated throughout the world. It consists of a number of different species, including *Pleurotus ostreatus* (Jacq.) P. Kumm., *Pleurotus sajor-caju* (Fr.) Sing., *Pleurotus cystidiosus* O.K. Miller and *Pleurotus tuber-regium* (Fr.) Sing. The most important commercial species among them is *Pleurotus ostreatus* (Jacq.) P. Kumm. It has the ability to grow in a wide variety of agricultural wastes/residues such as sawdust, wood chips, rice husk, and straw. A mixture of various agricultural wastes in a different ratio can also be an excellent growing medium for oyster mushroom cultivation. In addition, the utilization of agricultural residues as sources of mushroom substrates can be an efficient instrument for promoting a circular economy (Ab Rhaman et al., 2021). However, all the mushroom substrates derived from agricultural wastes may potentially contain heavy metals in large quantities, mainly due to agricultural activities. Among agricultural sources of heavy metals in plant-based wastes, inorganic fertilizers, pesticides, and sewage sludge are the most common (Rashid et al., 2023).

Given the fact that mushrooms have the ability to absorb heavy metals from the growing media, it is clear that the content of heavy metals in the mushroom substrate determines the content of heavy metals in mushroom basidiomes and, thus their quality and safety. This means that most of the heavy metals from the mushroom substrate can easily enter the food chain by consuming mushrooms grown on that substrate. From this point of view, the selection of suitable plant-based substrates for mushroom production is crucial to avoid the accumulation of heavy metals in mushroom basidiomes and, thus, in the food chain.

Among the various edible mushroom species, oyster mushrooms are one of the most widely consumed and cultivated mushrooms in the world (Ejigu et al., 2022). In addition, numerous studies have shown that oyster mushrooms have a higher resistance to heavy metals, i.e. Cu, Cd, Zn, Ni, and Co, compared to other mushroom species, including *Hypsizygus ulmarius* (Bull.) Redhead, *Agaricus bisporus* (J.E. Lange) Imbach and *Lentinus arcularius* (Batsch) Zmitr. (Sanglimsuwan et al., 1993; Sithole et al., 2022). However, the differences in accumulation potential for different heavy metals may be ascribed to the different types of growth substrates in which mushrooms grow. In light of this fact, the aim of this study was to assess the ability of oyster mushrooms (*Pleurotus ostreatus* (Jacq.) P. Kumm.) to absorb heavy metals (Fe, Zn, Cu, Co, Mn, Ni, Cr, Cd and Pb)

from different plant-based substrates and to determine the bioaccumulation factor of the aforementioned heavy metals from the substrate to the mushrooms. We hypothesized that heavy metal contents in oyster mushroom basidiomes are strongly associated with heavy metal contents in the substrate in which they grow.

## 2. Material and methods

The experiment was carried out at the experimental station of the Faculty of Agriculture and Food Science in Sarajevo from November 2023 to March 2024 in order to assess the ability of oyster mushrooms to absorb heavy metals from different substrates. The experiment included four substrate treatments with three replicates for each substrate. The substrate treatments were: (1) maize straw, (2) beech sawdust supplemented with wheat bran at a rate of 20% (3) a mixture of maize straw and spent coffee grounds in a ratio of 70:30, (4) a mixture of maize straw and spent coffee grounds in a ratio of 50:50. The experiment was set up with four mushroom substrate types in each of three replications.

Maize straw was collected right after harvest from a farmer's field in the Ilidža municipality, approximately 10 km east of Sarajevo town. After being collected, the maize straw was dried for 2–3 weeks on mats in the sun and then stored in a dry place until the next use. Beech sawdust was purchased from the local wood processing industry, wheat bran from the local supermarket, and spent coffee grounds from the local coffee bar.

The maize straw-based mushroom substrate was prepared as follows: dry maize straw was chopped into small pieces around 2–5 cm long. Approximately 10 kg of maize straw in a high-density polyethylene barrel was soaked for eight hours in 20 liters of hot water. The maize straw was then removed and strained using raffia baskets to remove excess water. The maize straw substrate obtained in this way was also used to make a substrate based on spent coffee grounds by mixing maize straw substrate with spent coffee grounds in a ratio of 70:30 and 50:50.

The mushroom substrate containing beech sawdust and wheat bran in a ratio of 80:20 was prepared as follows: 10 kg of beech sawdust together with 2 kg of wheat bran were placed in a styrofoam container and mixed well. After adding 25 l of water, the substrate components were mixed again until there was no more water at the bottom of the container.

Following preparation, the substrates with a moisture content of approximately 70% were packed in polypropylene bags (20.32 × 30.48 cm) with a total weight of 1.5 kg in each bag, on a wet weight basis. The bags were pasteurized at 65 °C for 8 h and oyster mushroom mother spawn in a concentration of 10% was added to each bag when the substrate had cooled down. The oyster mushroom mother spawn used in this study was donated by Urban Farm Mikić (Orašje, Bosnia and Herzegovina), and was prepared using the method of spawn preparation previously described by Stamets and Chilton (1983).

The bags were then incubated in a climate growth chamber at 22 °C in darkness with a relative humidity of 80% for 20 days, by which time the substrate was entirely colonized with mycelium. During the mycelium colonization period, each bag was shaken at 4-day intervals to further distribute

**Table 1** Basic chemical properties of the mushroom substrate.

Chemical properties	Value	Substrate 1*	Substrate 2	Substrate 3	Substrate 4
pH (H <sub>2</sub> O)	pH unit	6.7	6.9	6.6	6.5
pH (KCl)	pH unit	6.3	6.4	6.1	6.0
Organic matter	%	73.1	78.7	69.7	67.4
P <sub>2</sub> O <sub>5</sub>	mg 100 g <sup>-1</sup>	112.6	80	204.5	242.6
K <sub>2</sub> O	mg 100 g <sup>-1</sup>	246	230	178.6	138.4

\* Substrate 1: maize straw, Substrate 2: beech sawdust + wheat bran in a ratio of 80:20, Substrate 3: maize straw + spent coffee grounds in a ratio of 70:30, Substrate 4: maize straw + spent coffee grounds in a ratio of 50:50.

the mycelium through the substrate. Thereafter, the bags were sliced (width 1 cm) at six positions, and incubated at 16 °C for a further three days to stimulate fructification. Thereafter, the conditions in a climate growth chamber were modified to 18 ± 2 °C, 85% relative humidity, and incandescent light (400 lux) for 12 h on/off cycle until harvest. Irrigation in the period of the colonization and harvest phase was carried out with tap water when a reduction in the humidity of the casing was observed. Days from the mycelial stage to pin head formation ranged from 10 to 13 days. Oyster mushroom basidiomes were harvested after five days of appearing pinhead. A total of three flushes were harvested with an interval of seven days, and the yield of each flush was recorded in weight (g) for each flush.

After harvesting, the fresh basidiomes in each bag were dried to constant weight in an oven at 40 °C. Dried mushroom samples were then ground into a fine powder with a blender and stored in paper bags until further use.

Extraction of heavy metals from mushroom sample was done by acid digestion (HNO<sub>3</sub> (65%; Merck, Darmstadt, Germany)–H<sub>2</sub>SO<sub>4</sub> (96%; Merck, Darmstadt, Germany) in a ratio of 2.5:1) as follows: 3 g of dried mushroom sample was added in a 100 ml round bottom flask, followed by addition of 10 ml HNO<sub>3</sub> and 4 ml H<sub>2</sub>SO<sub>4</sub>. Approximately sixteen hours later, the solution in the flask was heated to its boiling temperature on a hot plate in a lab fume hood for one hour and then cooled to room temperature. Finally, the extracts were filtered through quantitative filter paper in a 50 ml flask and then diluted with deionized water to the mark (Lisjak et al., 2009).

Preparation of substrate samples for chemical analysis was done as follows: mushroom substrates were dried to constant weight in an oven at 40 °C, ground to a fine powder using a mortar/pestle, passed through a 2 mm sieve and then subjected to laboratory analysis in order to evaluate basic substrate chemical properties i.e. soil acidity (pH), organic matter content and content of heavy metals (Zn, Cu, Fe Co, Mn, Ni, Cr, Cd and Pb). Substrate pH was determined using a glass electrode in a 1:5 (V/V) suspension of soil in water and soil in 1 M KCl (ISO 10390, 2021), organic matter content by oxidation with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in the presence of H<sub>2</sub>SO<sub>4</sub> (ISO 14235, 1998), and heavy metal contents were determined by atomic absorption spectroscopy (ISO 11047, 1998) after an aqua regia (a mixture of HCl (37%; Merck, Darmstadt, Germany) and HNO<sub>3</sub> in a ratio of 3:1) extraction.

Extraction of heavy metals from the mushroom substrate sample was done by aqua regia solution as follows: 3 g of dried

substrate sample was added in a 250 ml round bottom flask, followed by the addition of 28 ml of aqua regia. Approximately sixteen hours later, the flasks were heated for 2 h under reflux and then cooled to room temperature. The extracts were filtered through quantitative filter paper in a 100 ml flask and then diluted to the mark with deionized water (ISO 11466, 1995).

Heavy metal contents (Zn, Cu, Fe Co, Mn, Ni, Cr, Cd, and Pb) in substrate and mushroom samples were analyzed by atomic absorption spectroscopy. Working solutions for each investigated heavy metal were prepared by diluting standard stock solutions (Merck AAS solutions) with deionized water. The flame atomic absorption spectrometer Shimadzu AA-7000 Model (Shimadzu Instruments, Tokyo, Japan) was used for Zn, Cu, Fe Co, Mn, Ni, Cr, Cd, and Pb determination in all solutions.

The substrate-to-mushroom transfer factor or bioaccumulation factor (BAF) is defined as the ratio of the heavy metal content in oyster mushroom basidiomes to that in the substrate and was calculated using the following formula:

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

where  $C_{\text{mushroom}}$  and  $C_{\text{substrate}}$  represent the heavy metal content in the mushroom and substrate on a dry mass basis, respectively (Olowoyo et al., 2010). The substrate was analyzed before mycelium inoculation.

Higher BAF values ( $\geq 1$ ) indicate higher heavy metal absorption from the substrate and higher suitability of the mushroom for myco-remediation.

All assays were performed in triplicates, and the results were expressed as mean ± standard error mean. Data were analyzed using one-way analysis of variance (ANOVA), followed by a least significance difference (LSD) test at  $p < 0.05$ . All statistics were performed using the computer software Statistical Package for the Social Sciences (SPSS) program (Version 12.0, SPSS Inc., Chicago, USA).

### 3. Results

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

All mushroom substrates used in this study were slightly acidic and, therefore, suitable for oyster mushroom cultivation. The distinguishing property of all mushroom substrates was a substantial organic matter, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O content, making them an excellent medium for mushroom growth.

**Table 2** Heavy metal contents in mushroom substrate samples.

Heavy metals	Value	Substrate 1 <sup>*</sup>	Substrate 2	Substrate 3	Substrate 4	LSD <sub>0.05</sub>
Zn	mg kg <sup>-1</sup>	10.4 ± 0.6 <sup>b**</sup>	39.6 ± 6.2 <sup>a</sup>	10.7 ± 1.4 <sup>b</sup>	10.8 ± 2.5 <sup>b</sup>	3.23
Cu	mg kg <sup>-1</sup>	3.8 ± 0.7 <sup>c</sup>	8.6 ± 1.4 <sup>ab</sup>	7.5 ± 2.5 <sup>b</sup>	10.0 ± 2.1 <sup>a</sup>	1.74
Co	mg kg <sup>-1</sup>	n.d.	n.d.	n.d.	n.d.	-
Mn	mg kg <sup>-1</sup>	20.2 ± 1.4 <sup>b</sup>	92.3 ± 3.1 <sup>a</sup>	19.1 ± 2.1 <sup>b</sup>	18.3 ± 1.5 <sup>b</sup>	2.01
Ni	mg kg <sup>-1</sup>	3.3 ± 0.8	3.0 ± 0.8	2.7 ± 1.2	2.4 ± 0.6	-
Fe	mg kg <sup>-1</sup>	341.7 ± 9.1 <sup>a</sup>	230.5 ± 9.9 <sup>b</sup>	349.8 ± 8.2 <sup>a</sup>	355.2 ± 11.1 <sup>a</sup>	9.51
Cr	mg kg <sup>-1</sup>	1.67 ± 0.18 <sup>a</sup>	1.09 ± 0.44 <sup>b</sup>	1.34 ± 0.11 <sup>b</sup>	1.13 ± 0.23 <sup>b</sup>	0.28
Cd	mg kg <sup>-1</sup>	0.01 ± 0.01 <sup>c</sup>	0.17 ± 0.08 <sup>a</sup>	0.04 ± 0.02 <sup>bc</sup>	0.06 ± 0.02 <sup>b</sup>	0.04
Pb	mg kg <sup>-1</sup>	n.d.	n.d.	n.d.	n.d.	-

\* Substrate 1: maize straw, Substrate 2: beech sawdust + wheat bran in a ratio of 80:20, Substrate 3: maize straw + spent coffee grounds in a ratio of 70:30, Substrate 4: maize straw + spent coffee grounds in a ratio of 50:50.

\*\* Averages denoted by the same letter in the same column indicate no significant difference ( $p < 0.05$ ).

**Table 3** Heavy metal contents in basidiomes of oyster mushrooms on different substrates.

Substrate treatment <sup>*</sup>	Zn	Cu	Mn	Fe	Cd	Ni	Cr
1	33.5 ± 9.2 <sup>b**</sup>	2.6 ± 1.1 <sup>c</sup>	5.1 ± 1.6 <sup>b</sup>	38.9 ± 3.9 <sup>a</sup>	0.03 ± 0.05 <sup>b</sup>	0.13 ± 0.08	0.77 ± 0.11 <sup>a</sup>
2	80.2 ± 6.1 <sup>a</sup>	6.3 ± 2.4 <sup>b</sup>	9.2 ± 1.8 <sup>a</sup>	20.2 ± 5.6 <sup>b</sup>	0.44 ± 0.09 <sup>a</sup>	0.14 ± 0.12	0.61 ± 0.19 <sup>b</sup>
3	33.8 ± 9.8 <sup>b</sup>	6.4 ± 1.6 <sup>b</sup>	4.3 ± 1.2 <sup>b</sup>	40.5 ± 4.1 <sup>a</sup>	0.06 ± 0.03 <sup>b</sup>	0.11 ± 0.08	0.66 ± 0.13 <sup>b</sup>
4	32.8 ± 7.8 <sup>b</sup>	8.5 ± 2.7 <sup>a</sup>	4.3 ± 1.1 <sup>b</sup>	36.2 ± 5.2 <sup>a</sup>	0.07 ± 0.02 <sup>b</sup>	0.13 ± 0.09	0.65 ± 0.15 <sup>b</sup>
LSD <sub>0.05</sub>	8.63	2.07	1.39	4.13	0.06	-	0.09

\* Substrate 1: maize straw, Substrate 2: beech sawdust + wheat bran in a ratio of 80:20, Substrate 3: maize straw + spent coffee grounds in a ratio of 70:30, Substrate 4: maize straw + spent coffee grounds in a ratio of 50:50.

\*\* Averages denoted by the same letter in the same column indicate no significant difference ( $p < 0.05$ ).

Levels of investigated heavy metals in mushroom substrate samples are presented in Table 2. All results are expressed on a dry mass basis.

The most abundant heavy metals in the tested mushroom substrate samples were Fe, followed by Mn and Zn. The levels of Ni, Cr, and Cd were relatively low, while Co and Pb were not found in substrate samples.

Levels of investigated heavy metals (Zn, Cu, Mn, Fe, Cd, Ni, and Cr) in oyster mushroom basidiomes grown on different substrates, expressed as mg kg<sup>-1</sup> on a dry-mass basis, are given in Table 3.

The most abundant heavy metals in the tested oyster mushroom basidiomes were Zn and Fe, ranging between 32.8–80.2 mg kg<sup>-1</sup> and 20.2–40.5 mg kg<sup>-1</sup> dry mass, respectively. In general, the quantities of all tested heavy metals were within the limits proposed by the World Health Organization (WHO/FAO, 2007). Accordingly, the limit values of Pb, Cd, Zn, Cr, Ni, Fe, and Cu in the food crops proposed are 2, 0.2, 60, 2.3, 4, 48, and 40 mg kg<sup>-1</sup>, respectively. The only exception to this general trend was the Cd and Zn content in oyster mushrooms grown on substrate 2 (beech sawdust supplemented with wheat bran at a rate of 20%), which was higher than the limit value.

Bioaccumulation factor values (BAF) for Zn, Cu, Mn, Fe, Cd, Ni and, Cr are given in Table 4.

The highest BAF value (higher than 1) was observed for Zn and Cd, regardless of the substrate in which oyster mushrooms

grew. The lowest BAF value was observed for Ni, followed by Mn and Fe.

#### 4. Discussion

Mushrooms are known to accumulate heavy metals in large quantities, which vary more or less depending on mushroom species, mushroom morphological parts, substrate i.e., growing medium, and heavy metal mobility and availability. However, given the fact that heavy metals in mushrooms originate from their growing medium, it is evident that substrate chemical composition is the main factor affecting the heavy metal accumulation in mushroom basidiomes (Alaimo & Varrica, 2023). From a health point of view, it is, therefore, imperative to determine the heavy metals composition of the mushroom substrates. This opinion is further supported by the fact that some toxic metals, such as Cd or Zn, can be easily transferred from the substrates to mushroom basidiomes during cultivation (Pei et al., 2015).

In this study, the levels of all tested heavy metals (Zn, Cu, Mn, Fe, Cd, Ni, and Cr) in all substrates used for the oyster mushroom cultivation were lower than the threshold limits recommended by the European Commission on Environment (EU, 2002). Accordingly, the threshold values of Cd, Mn, Pb, Ni, Zn, and Cr in agricultural soil or substrates are 3, 2000, 100, 50, 300, and 100 mg kg<sup>-1</sup>, respectively. In this study, Co and Pb were below the method detection limit in any of the tested mushroom samples.

**Table 4** Bioaccumulation factor values for tested heavy metals.

Heavy metal	Substrate treatment			
	Substrate 1	Substrate 2	Substrate 3	Substrate 4
Zn	4.18 ± 0.71	2.03 ± 0.18	3.15 ± 0.61	3.03 ± 0.44
Cu	0.68 ± 0.21	0.72 ± 0.16	0.87 ± 0.09	0.85 ± 0.11
Mn	0.25 ± 0.07	0.10 ± 0.02	0.22 ± 0.05	0.24 ± 0.04
Fe	0.11 ± 0.01	0.09 ± 0.01	0.12 ± 0.02	0.10 ± 0.01
Cd	2.67 ± 1.99	2.64 ± 0.54	1.51 ± 0.45	1.18 ± 0.21
Ni	0.04 ± 0.02	0.05 ± 0.03	0.04 ± 0.01	0.05 ± 0.01
Cr	0.49 ± 0.07	0.57 ± 0.08	0.52 ± 0.09	0.52 ± 0.04

\* Substrate 1: maize straw, Substrate 2: beech sawdust + wheat bran in a ratio of 80:20, Substrate 3: maize straw + spent coffee grounds in a ratio of 70:30, Substrate 4: maize straw + spent coffee grounds in a ratio of 50:50.

The above-mentioned results suggest that agriculture biomass used as a substrate for mushroom cultivation in this study was not contaminated with tested heavy metals. However, this does not mean that mushrooms grown on these substrates do not pose a risk to human health, and the results of this research confirm this assumption from the point of view of the accumulation of Zn and Cd in mushroom basidiomes. Namely, the content of Zn and Cd in oyster mushrooms cultivated on substrate 2 (beech sawdust supplemented with wheat bran at a rate of 20%) was higher than the permissible value prescribed by WHO/FAO (2007), which is 60 mg kg<sup>-1</sup> for Zn and 0.2 mg kg<sup>-1</sup> for Cd. These results suggest that oyster mushrooms have a high capacity to absorb Zn and Cd from growing medium, and BAF values for Zn and Cd determined in this study strongly support this observation.

In this study, oyster mushrooms had a BAF value for Zn higher than 2, regardless of the substrates in which they grew. These findings are not surprising because Zn participates in various physiological processes and metabolic pathways, including cell signaling, cell growth promotion and enzymes activation, and therefore mushrooms tend to absorb as much Zn as possible from growing medium (Jarosz et al., 2017).

The current study also showed that oyster mushrooms had a BAF value for Cd higher than 1, regardless of the substrate in which they were grown. This finding suggests that oyster mushrooms do not possess effective mechanisms to prevent the uptake of Cd from growing medium. Numerous studies have also revealed that oyster mushrooms have the ability to accumulate large amounts of Cd in basidiomes (Golian et al., 2021; Kapahi & Sachdeva, 2017). Given the fact that Cd may cause liver disease, cardiovascular disease, osteoporosis, or brain damage (Genchi et al., 2020), this finding is highly undesirable from a health point of view.

However, although mushrooms do not possess effective mechanisms for blocking or avoiding heavy metal uptake, especially for Zn and Cd, they have developed numerous cellular mechanisms for the detoxification of heavy metal ions after their uptake into the body, including heavy metal sequestration and vacuolar compartmentalization. Damodaran et al. (2013) reported that mushrooms have a high ability to produce specific organic compounds, i.e., metallothioneins, which participate in the compartmentalization of heavy metals in cell vacuoles, thereby reducing heavy metal toxicity

in the cytoplasm where cell division, respiration, and other physiological processes occur.

In this study, oyster mushroom BAF values for Cr were relatively high and ranged from 0.49 to 0.57, suggesting that oyster mushrooms have effective mechanisms that allow them to transfer Cr from the substrate to their fruiting bodies. A number of recent studies have also demonstrated that oyster mushrooms have a high Cr accumulation ability (Mohamadhasani & Rahimi, 2022; Sithole et al., 2022). From the consumer's point of view, these results are undesirable because it is well known that Cr is harmful to human health.

Oyster mushrooms also had a high BAF value for Cu, ranging from 0.68 to 0.87, depending on the substrate in which they grew. This finding is not surprising, given the fact that Cu is an essential heavy metal participating in many physiological processes in mushrooms (Festa & Thiele, 2011). However, excess Cu in mushrooms is toxic to human health, and the permissible value for Cu content in foodstuffs prescribed by WHO/FAO is 40 mg kg<sup>-1</sup>. The Cu content in oyster mushrooms in the present study ranged from 2.6 to 8.5 mg kg<sup>-1</sup>, and this is several times lower than the permissible value. From this point of view, the consumption of oyster mushroom basidiomes from this study can be considered safe for human health.

In this study, the Fe content ranged from 20.2 ± 5.6 in oyster mushrooms grown on substrate 2 (beech sawdust supplemented with wheat bran) to 40.5 ± 4.1 mg kg<sup>-1</sup> in oyster mushrooms grown on substrate 3 (mixture of maize straw and spent coffee grounds in a ratio of 50:50). The obtained Fe levels in the studied mushrooms were below the safe limits set by WHO/FAO (2007) which is 48 mg kg<sup>-1</sup>. Interestingly, the BAF values of oyster mushrooms for Fe were relatively low and ranged from 0.09 to 0.12, indicating that the oyster mushrooms cannot be classified as Fe hyperaccumulators.

The current study also showed very low BAF values of oyster mushrooms for Mn (ranging from 0.10 to 0.25). Interestingly, BAF values for Mn in mushrooms are generally lower than in plants, and this can obviously be associated with the role of Mn in plants. Namely, Mn is an essential heavy metal for plants, and they need it in large quantities. Many physiological processes depend on Mn, including photosynthesis and various oxidation-reduction reactions, among others, and therefore plants have the tendency to absorb more Mn from grow-

ing medium compared with mushrooms that cannot perform photosynthesis (Alejandro et al., 2020).

The lowest BAF value in this study was recorded for Ni, suggesting that among studied heavy metals, Ni showed the lowest affinity to translocate from substrate to oyster mushroom basidiomes. This can, among others, be explained by the substrate pH value (ranging from 6.5 to 6.9 in H<sub>2</sub>O), which was not favorable for Ni uptake by mushrooms. Similar findings were found by Širić et al. (2023).

In this study, the content of the toxic heavy metals Pb and Co in the oyster mushrooms grown on different substrates was too low to be detected by the available equipment and analytical techniques.

## 5. Conclusion

In this study, oyster mushrooms had a BAF value for Zn and Cd higher than 1, regardless of the substrates in which they were grown, indicating that this mushroom species has the ability to transfer Zn and Cr very efficiently from growing medium to fruiting bodies. BAF values of oyster mushrooms for Cu and Cr were also high (ranging from 0.68 to 0.87 and from 0.49 to 0.57, respectively), suggesting that oyster mushrooms have effective mechanisms that allow them to easily take up Cu and Cr from the substrate. The lowest BAF value in this study was recorded for Ni, suggesting that among studied heavy metals, Ni showed the lowest affinity to translocate from substrate to oyster mushroom fruiting bodies. In general, the results of this study lead to the conclusion that substrate chemical composition strongly affects the Zn, Cu, Mn, Fe, Cd, Ni, and Cr accumulation in oyster mushroom basidiomes. The results of this study also showed that oyster mushrooms can be considered a promising species for Cd and Zn bioremediation. Further studies are needed to confirm this.

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