



# The importance of Cu × Pb interactions to *Lentinula edodes* yield, major/trace elements accumulation and antioxidants

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## Abstract

Due to the use of various substrates in the production of edible mushrooms which may contain metals, including Cu and Pb, it is important to understand the influence of mutual interactions between them in the process of their accumulation in fruit bodies. For this reason, the effects of Cu, Pb, and Cu × Pb on yield, accumulation of five major elements (Ca, K, Mg, Na and P), trace elements (Cu, Pb and Fe) and some bioactive compounds in *Lentinula edodes* fruit bodies were studied. Both the metals were added in doses of 0.1 and 0.5 mM (Cu<sub>0.1</sub>, Cu<sub>0.5</sub>, Pb<sub>0.1</sub>, Pb<sub>0.5</sub> and their combinations). The addition of the metals resulted in a reduction in size, amount and finally yield of fruit bodies. Depending on the presence of Cu and/or Pb and their concentration in the substrate, both antagonism and synergism may occur. The influence on the accumulation of other determining elements was also recorded. Among phenolic compounds, phenolic acids and flavonoids were detected. 2,5-Dihydroxybenzoic acid dominated in fruit bodies in the control variant, Pb<sub>0.1</sub>, Pb<sub>0.5</sub> and all experimental variants enriched with Cu + Pb, while gallic acid was the major phenolic after Cu<sub>0.1</sub> and Cu<sub>0.5</sub> addition. Only protocatechuic acid content increased in all combinations. A significant decrease of all aliphatic acid contents in comparison to the control variant was observed in the Cu<sub>0.1</sub> and Pb<sub>0.1</sub> variants. Significant stimulation of aliphatic acid synthesis was recorded in Cu<sub>0.5</sub> and Pb<sub>0.5</sub> variants and in the mixture of both the metals. The additions pointed to the possible role of the determined molecules in detoxification mechanisms.

**Keywords** Shiitake · Copper · Lead · Interaction · Mineral composition · Phenolic compounds

## Introduction

Historically, the greatest production of *Lentinula edodes* (shiitake) with evergreen *Castanopsis cuspidata* was in Japan until the mid 1980s [1]. To reduce the time of the crop cycle and increase yield, the growth process was changed into cultivation using sawdust. Owing to the similarity of *C. cuspidata* to *Fagus* (beech) and *Quercus* (oak), the use of sawdust from these tree species has become common [2, 3]. Unfortunately, the wood of various tree species may contain a high number of toxic elements. According to Kovacs et al. [4], *Q. robur* L. efficiently accumulates Cd, Co, Cu, Cr, Ni, Pb and Zn. Stojnić et al. [5] described notably high contents of Cu, Fe, Mn and Zn in *Q. petraea* (Matt.) Liebl. and in *Q. robur* L. Likewise in *Fagus sylvatica* L., Breckle and Kahle [6] reported significant Cd and Pb accumulation. Furthermore, the variability of Al, Cd, Cr, Cu, Fe, Mn and Pb content in the wood of healthy and dying *F. sylvatica* was shown by Nicewicz and Szczepkowski [7]. The presence of major

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and trace elements in wild-growing mushroom species is a consequence of their accumulation from the soil, as well as in cultivated species, where the efficiency of their accumulation depends on the chemical composition of the substrate [8]. Too high a concentration of bioavailable elements in the substrate may lead to a potential risk for consumers [9, 10]. On the other hand, the occurrence of individual elements in the substrate in low concentration may be modulated in the presence of other element(s) due to synergistic or antagonistic interactions [11, 12]. Despite the role of interactions between elements (especially toxic heavy metals) in this environment, our knowledge is still highly limited [13, 14]. Addition to the substrate of components containing toxic elements influences the profile of, e.g. amino acids, which may interfere with the accumulation of other elements and biomass yield [15].

According to Kalač [16], the content of Cu in cultivated mushroom species is up to 30 mg kg<sup>-1</sup> dry weight (DW), while that of Pb is up to 5 mg kg<sup>-1</sup> DW. Based on unpublished data, the authors indicate that the problem of excessive amounts of Cu and/or lead in Poland is sporadic and in relation to the annual production of cultivated mushrooms (especially *A. bisporus* and *P. ostreatus*), it accounts for less than 1%. However, the possibility of the substrate being polluted with Cu and/or Pb components remains and may exist to a much greater extent in other countries. In Europe, the majority of dried shiitake mushrooms are imported from Asian countries. Hardwood sawdust and organic additives as agricultural and food wastes are used to grow this fungus. In our previous research, we found that the content of some elements considered toxic was higher in fruit bodies of various species imported from this region compared to those grown in Europe [17]. Therefore, it is assumed that the organic additives to the substrates of these mushrooms have contained intolerable amounts of heavy metals, including Cu and Pb [18].

Thus, in relation to the above information, the aim of this study was to determine the effects of Pb, Cu and Cu×Pb interaction on yield, the accumulation of selected major and trace elements (Ca, Cu, Fe, K, Mg, Na, P and Pb) in *Lentinula edodes* fruit bodies to underline the importance of the proper selection of components used in the cultivation of this mushroom species. Additionally, the effect of Cu and Pb addition on selected bioactive compounds was also studied.

## Materials and methods

### Experimental materials and experiment design

In the experiment, *L. edodes* (Berk.) Pegler M3790 strain was used. The mycelium of this strain originated from Mycelia BVBA in Belgium. The substrate was prepared

from a mixture of beech and oak sawdust (1:1 vol.). The mixture was supplemented with wheat bran to the amount of 25%, corn flour 5%, soy meal 5% and gypsum 1% in relation to the substrate dry matter and moistened with the investigated salt solutions to a moisture content of 60%. The following salts were used in the experiment: copper(II) nitrate hemi(pentahydrate) Cu(NO<sub>3</sub>)<sub>2</sub>×2.5H<sub>2</sub>O and lead(II) nitrate Pb(NO<sub>3</sub>)<sub>2</sub>. These salts were dissolved in such an amount of distilled water sufficient to obtain appropriate concentrations of 0.1 (Cu<sub>0.1</sub>, Pb<sub>0.1</sub>, Cu<sub>0.1</sub>+Pb<sub>0.1</sub>), 0.5 (Cu<sub>0.5</sub>, Pb<sub>0.5</sub>, Cu<sub>0.5</sub>+Pb<sub>0.5</sub>) or 0.1 and 0.5 mM (Cu<sub>0.1</sub>+Pb<sub>0.5</sub>, Cu<sub>0.5</sub>+Pb<sub>0.1</sub>). Overall, nine experimental variants were prepared; a control and eight supplemented with metals. The substrates were then placed in polypropylene bags with a micro-filter (1 kg per bag and 10 bags per variant) and sterilized at a temperature of 121 °C for 1 h. After sterilization, the substrates were cooled down to a temperature of 25 °C and inoculated with spawn to the amount of 2% of substrate weight.

Incubation was conducted at a temperature of 23 ± 1 °C and 80–85% air humidity until the substrate became overgrown with mycelium. Next, the temperature was reduced to 19 ± 1 °C and cultivation was lit by a fluorescent lamp (day-light) for 10 h (50 lx intensity of irradiation) daily. The mycelium was matured under these conditions for 70 days. The bags were then placed in the cultivation room and the foil was removed from the substrate. Air humidity 85 ± 2%, temperature 15 ± 1 °C, and CO<sub>2</sub> concentration below 1000 ppm were maintained in the growth facility. The cultivation was lit with a fluorescent lamp (day light) for 12 h (500 lx intensity of irradiation) daily. Whole fruit bodies (caps and stipes) were harvested and dried at a temperature of 40 °C to a constant weight.

### Sample preparation

Representative fruit bodies were dried at 45 ± 1 °C for 96 h in an electric oven (SLW 53 STD, Pol-Eko, Poland) and ground in a laboratory Cutting Boll Mill PM 200 (Retsch, Germany). For sample digestion, the microwave preparation system Mars 6 (CEM, Matthews, USA) was used. Accurately weighed 0.500 ± 0.001 g of a dry sample was digested by 5 mL of concentrated nitric acid in closed Teflon containers in the microwave sample preparation system (180 °C, 20 min ramp time, 20 min heating time, 20 min cooling). After digestion, the samples were filtered and diluted with deionized water to a final volume of 10.0 mL. Each of the samples was analyzed in triplicate using the whole sample preparation procedure.

### Instrument and analytical method validation

The inductively coupled plasma optical emission spectrometer Agilent 5110 ICP-OES (Agilent, USA) was used for Ca,

Cu, Fe, K, Mg, Na, P and Pb determination. A synchronous vertical dual view (SVDV) of the plasma was accomplished with dichroic spectral combiner (DSC) technology, allowing the axial and radial view to be analyzed simultaneously. The common instrumental conditions were used: radio frequency (RF) power 1.2 kW, nebulizer gas flow 0.7 L min<sup>-1</sup>, auxiliary gas flow 1.0 L min<sup>-1</sup>, plasma gas flow 12.0 L min<sup>-1</sup>, charge-coupled device (CCD) temperature – 40 °C, viewing height for radial plasma observation 8 mm, accusation time 5 s, three replicates. The wavelengths were: 327.395 nm for Cu (axial view), 220.353 nm for Pb (axial view), 253.561 nm for P (axial view), 238.204 nm for Fe (axial view) 422.673 nm for Ca (radial view), 285.213 nm for Mg (axial view), 766.491 nm for K (radial view), 589.592 nm for Na (radial view), respectively. The detection limits were determined as 3-sigma criteria and were at the level of 0.0X mg kg<sup>-1</sup> dry weight (DW) for all elements determined (0.01 mg kg<sup>-1</sup> for Cu, 0.04 mg kg<sup>-1</sup> for Pb, 0.03 mg kg<sup>-1</sup> for P, 0.02 mg kg<sup>-1</sup> for Fe, 0.03 mg kg<sup>-1</sup> for Ca; 0.03 mg kg<sup>-1</sup> for K; 0.01 mg kg<sup>-1</sup> for Mg and 0.03 mg kg<sup>-1</sup> for Na). Traceability was checked using standard addition methods, and recoveries at the level 80–120% were found as satisfactory.

### Determination of phenolic compounds and aliphatic acids as bioactive compounds

#### Total phenolic content

Total phenolic content (TP) was determined according to the procedure of Singleton et al. [19] with some modifications. Briefly, 1 mL of mushroom extract, 1 mL of Folin–Ciocalteu phenol reagent 1:1 (v/v) and 3 mL of 20% Na<sub>2</sub>CO<sub>3</sub> were mixed and kept in darkness for 30 min at room temperature. Absorbance of the samples was then measured at 765 nm. The results were expressed as mg of gallic acid equivalent (GAE) per g.

#### Determination of phenolic compounds and aliphatic acids

The extraction of phenolic compounds was carried out from dried and powdered fruit bodies with 80% (v/v) methanol as previously described [20], while aliphatic acids were extracted with water. First, the samples were sonicated at 40 °C for 15 min and then shaken for 8 h. The mixtures were centrifuged (3600 rpm/min for 15 min at 25 °C) and evaporated to dryness under vacuum. The dried extracts were dissolved in 1 mL of 80% methanol or in 1 mL of water, and then analyzed using the ACQUITY UPLC H-Class System (Waters Corp., Milford, MA, USA) equipped with an Acquity UPLC HSS T3 C18 column (150 mm × 2.1 mm, particle size 1.8 μm) (Waters, Ireland) and a photodiode array detector (PDA) eλ (Waters Corporation, Milford, MA, USA). The mobile phase was composed of components A

(water, containing 0.10% formic acid) and B (acetonitrile, containing 0.10% formic acid). The gradient program was applied. The concentration of phenolic compounds was determined using an external standard at wavelengths λ = 280 nm and λ = 320 nm [21].

### Determination of antioxidant activity

Scavenging activity was measured towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals according to Dong et al. [22] with modifications. One mL of the mushroom extract was mixed with 2.7 mL of 6 μM methanolic solution of DPPH, shaken and kept in the dark for 1 h. Absorption at 517 nm was then measured. The radical scavenging activity was calculated as the percentage of DPPH discoloration as below:

$$\% = A - A_c / A \times 100,$$

A<sub>c</sub> absorbance of methanolic extract of mushroom; A absorbance of control (DPPH solution without extract).

### Statistical analysis

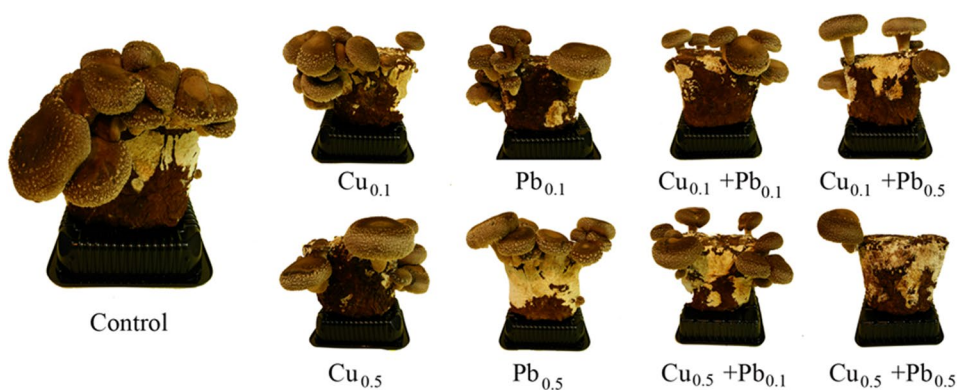
Statistical analyses were performed using the Agricolle package (R). One-way ANOVA with the post hoc Tukey's HSD (statistically significant difference) test was used to compare yield, content of elements and bioactive compound content of *L. edodes* growing under substrates enriched with particular Cu and/or Pb additions. Moreover, a general distribution of *L. edodes* exposed to particular substrates was performed using a Principal Component Analysis (PCA) as a useful graphical presentation of the obtained results [23, 24]. A Heatmap with a cluster analysis was performed for additional visualization of multidimensional data (comparison of *L. edodes* fruit bodies growing in particular experimental variants with regard to the content of other elements jointly). Additionally, to compare the content of Ca, Fe, K, Mg, Na and P in particular experimental variants, the rank sum was performed.

## Results

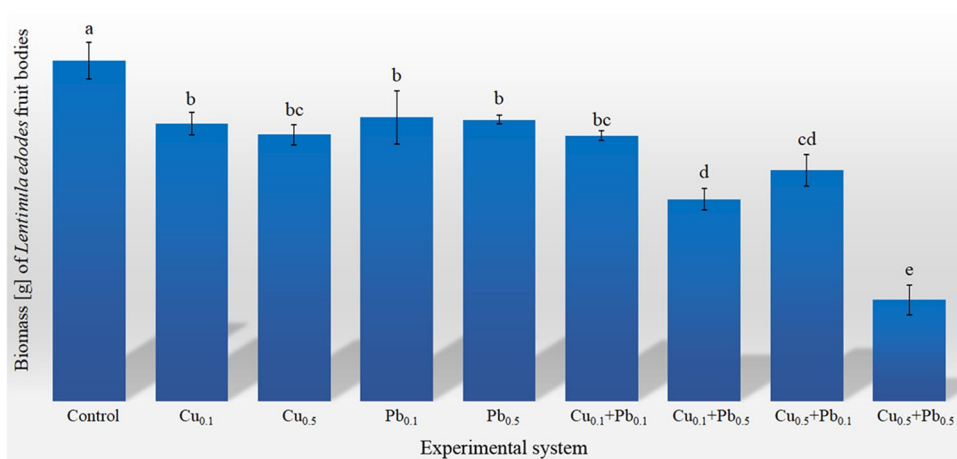
### Mushroom morphology and yield

Fruit bodies of *L. edodes* differed with respect to the amount of Cu, or especially Pb added to the substrate (Fig. 1). There were no significant differences in colour or shape of fruit bodies but clear differences in their amount, size and yield. The highest yield was observed in the control variant (319 ± 17 g) (Fig. 2). There were no significant differences in the yield of mushrooms growing under Cu<sub>0,1</sub>, Cu<sub>0,5</sub>, Pb<sub>0,1</sub>,

**Fig. 1** Macroscopic shape of *Lentinula edodes* growing on substrates with different Cu and/or Pb supplementation



**Fig. 2** Yield (g) of *Lentinula edodes* growing on substrates with different Cu and/or Pb supplementation



Pb<sub>0.5</sub> and Cu<sub>0.1</sub> + Pb<sub>0.1</sub> (from 266 ± 25 g for Pb<sub>0.1</sub> to 249 ± 5 g for Cu<sub>0.1</sub> + Pb<sub>0.1</sub>).

The addition of higher amount of Pb to substrate containing Cu (Cu<sub>0.1</sub> + Pb<sub>0.5</sub>) caused a significantly lower yield than for the Pb<sub>0.5</sub> variant (189 and 264 g, respectively). The lowest yield of *L. edodes* growing on substrate enriched with the maximal concentration of both Cu and Pb (Cu<sub>0.5</sub> + Pb<sub>0.5</sub>) was barely 95.0 g, which was over three times lower than for the control variant. This confirms the toxic effect of metals in given concentrations on the development of fruit bodies.

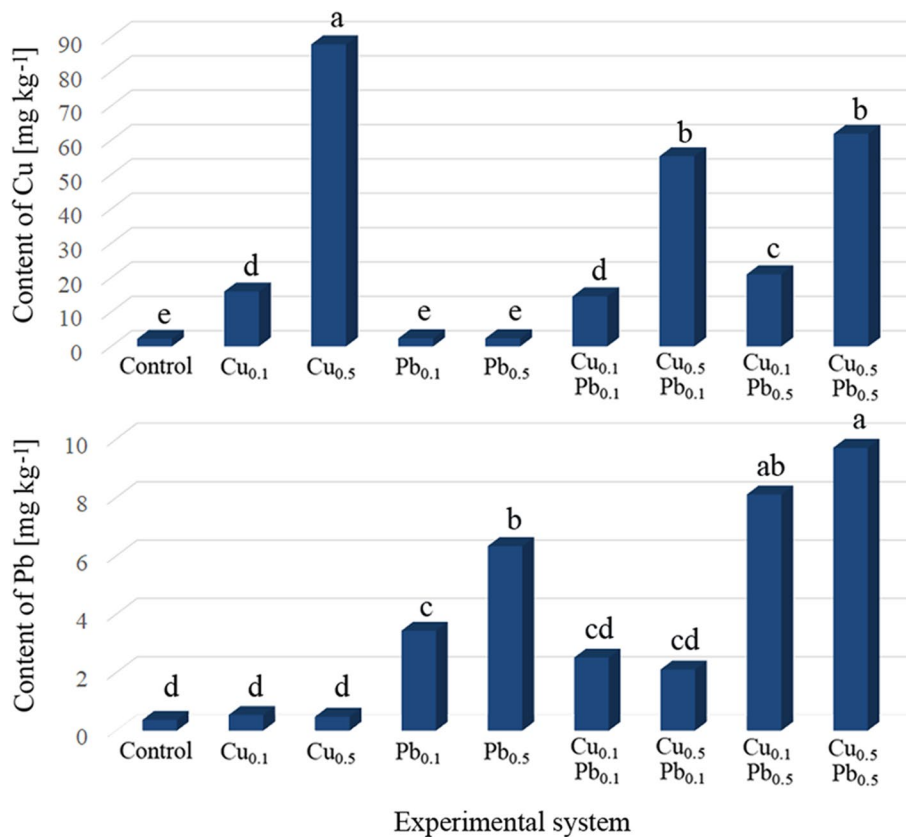
### Content of Cu and Pb in mushroom fruit bodies

The content of Cu in *L. edodes* significantly increased in fruit bodies growing in substrates Cu<sub>0.1</sub> and Cu<sub>0.5</sub> (16.0 and 87.9 mg kg<sup>-1</sup>, respectively) in relation to the control variant (Fig. 3). In the variant combining lower additions of both the metals (Cu<sub>0.1</sub> + Pb<sub>0.1</sub>), Cu content was 14.6 mg kg<sup>-1</sup>, which suggested no significant influence of Pb on Cu accumulation. The opposite situation was observed for the Cu<sub>0.1</sub> + Pb<sub>0.5</sub> variant, where the higher concentration of Pb caused a significantly higher content of Cu (21.0 mg kg<sup>-1</sup>) in fruit bodies. In the case of substrates enriched with a higher concentration

of Cu (0.5 mM), i.e. in the Cu<sub>0.5</sub> + Pb<sub>0.1</sub> and Cu<sub>0.5</sub> + Pb<sub>0.5</sub> variants, Cu content in fruit bodies was significantly lower (55.4 and 61.9 mg kg<sup>-1</sup>, respectively) than observed for the Cu<sub>0.5</sub> variant (87.9 mg kg<sup>-1</sup>). The obtained results show that lower Cu concentration (0.1 mM) in the substrate in combination with only higher Pb concentration (0.5 mM) can stimulate (synergism) the accumulation of Cu by *L. edodes*. On the other hand, a higher Cu concentration (0.5 mM) in the substrate combined with Pb (both 0.1 and 0.5 mM), may result in the reduction (antagonism) of copper accumulation in the fruit bodies of this mushroom species.

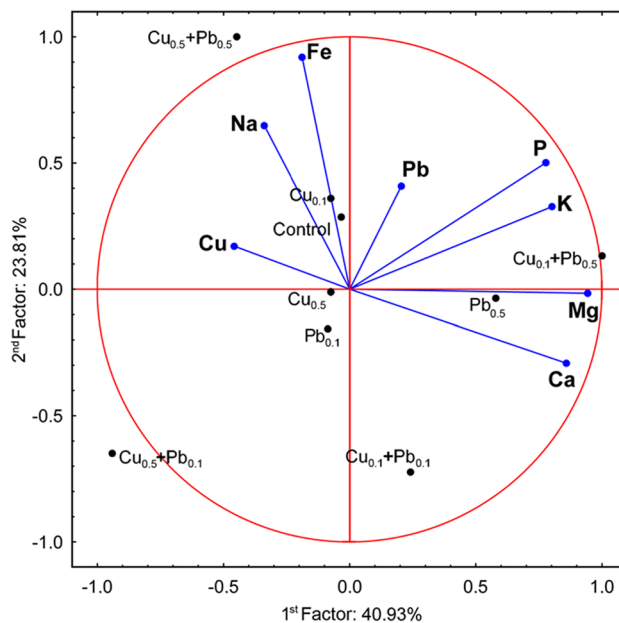
The increased concentration of Pb in experimental variants Pb<sub>0.1</sub> and Pb<sub>0.5</sub> led to a significantly higher content of this metal (3.43 and 6.33 mg kg<sup>-1</sup>, respectively) in *L. edodes* fruit bodies than in the control variant (add content). Copper addition at a lower concentration (0.1 mM) in variant Cu<sub>0.1</sub> + Pb<sub>0.1</sub> caused a 2.50 mg kg<sup>-1</sup> content of Pb, which was almost the same level as for fruit bodies under the Pb<sub>0.1</sub> variant. The same situation was observed in the case of the Cu<sub>0.5</sub> + Pb<sub>0.1</sub> combination with a Pb content of 2.10 mg kg<sup>-1</sup>. It is worth underlining that Cu addition to the substrate in both concentrations (0.1 and 0.5 mM) in combination with a higher concentration of Pb (0.5 mM) significantly increased

**Fig. 3** Content of Cu and Pb (mg kg<sup>-1</sup> DW) in fruit bodies



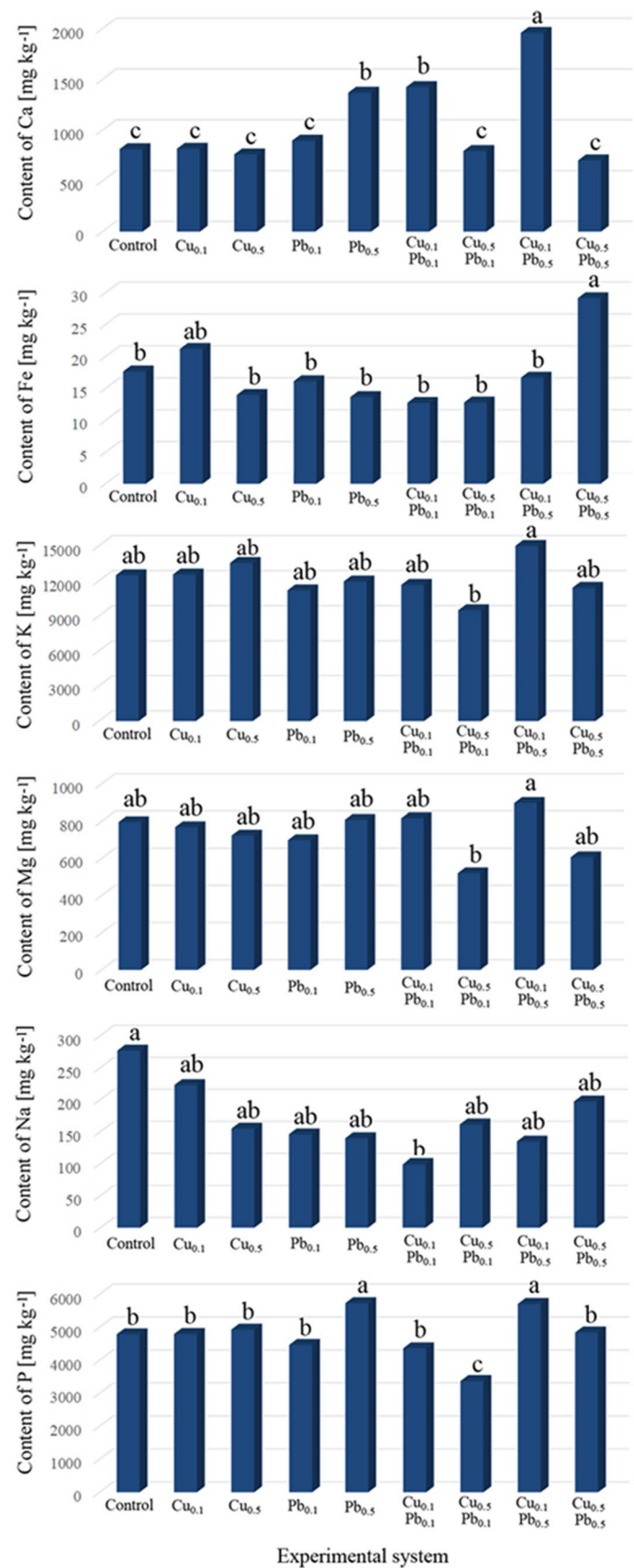
Pb content in *L. edodes* (8.10 and 9.70 mg kg<sup>-1</sup>, respectively). The obtained results show that the presence of Cu in substrate containing a low amount of Pb is not a factor stimulating the accumulation of toxic Pb in mushrooms, while the opposite situation is possible when higher concentrations of both Cu and Pb are present in the substrate.

PCA explained 64.74% (40.93 + 23.81) of total variability, which reliably reflects the relationships between element contents in fruit bodies growing in particular experimental variants (Fig. 4). The highest Cu content in *L. edodes* growing under Cu<sub>0.5</sub> variant was placed near the central point of this figure, while one of the highest Pb contents (Pb<sub>0.5</sub>) was placed at a greater distance from this point, similarly to the contents of Cu and Pb growing under Cu<sub>0.1</sub> and Pb<sub>0.1</sub>. This was due to the significantly higher content of Cu in *L. edodes* fruit bodies growing in substrates enriched with Pb than Cu. It is worth noting that the location of points characterizing Cu<sub>0.1</sub> + Pb<sub>0.1</sub> was opposite to those of Cu<sub>0.5</sub> + Pb<sub>0.5</sub>, the same as Cu<sub>0.1</sub> + Pb<sub>0.5</sub> in relation to Cu<sub>0.5</sub> + Pb<sub>0.1</sub>. Such a specific location is evidence of the mutual relation between these two metals. Moreover, the mutual quantitative relations of both the metals in the substrate caused particular changes in the content of other elements (Fig. 5). This fact clearly



**Fig. 4** Principal component analysis for all studied experimental variants concerning the content of all determined elements

**Fig. 5** Content of other elements ( $\text{mg kg}^{-1}$  DW) in fruit bodies



indicates that the concentration of both the elements influences the mineral composition of the final product available for potential consumers.

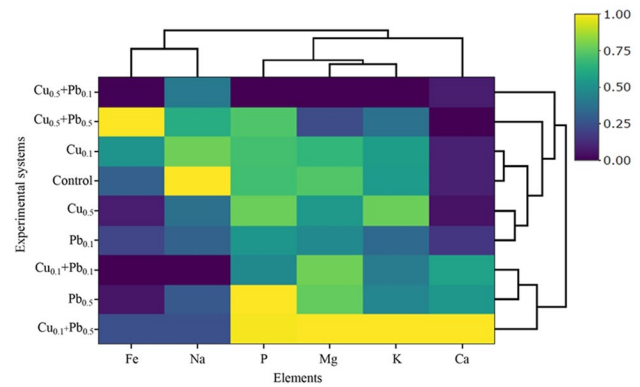
### Content of the other elements in mushroom fruit bodies

Differences in the contents of Cu and Pb in *L. edodes* fruit bodies growing in particular experimental variants were not the only ones recorded. Analysis of other selected elements showed that the addition of Cu, Pb or Cu + Pb to growing substrates may cause changes in major (Ca, K, Mg, Na and P) and essential trace element (Fe) contents of fruit bodies (Fig. 5).

An increase in Cu concentration in substrates ( $\text{Cu}_{0.1}$  and  $\text{Cu}_{0.5}$ ) did not result in any significant change of Ca content in *L. edodes* fruit bodies, the same as in the case of mushrooms in selected substrates enriched with Cu and/or Pb ( $\text{Cu}_{0.5} + \text{Pb}_{0.1}$ ,  $\text{Cu}_{0.5} + \text{Pb}_{0.5}$  and  $\text{Pb}_{0.1}$ ). Significantly higher contents of Ca than in the control variant ( $814 \text{ mg kg}^{-1}$ ) were observed in fruit bodies growing in the  $\text{Pb}_{0.5}$ ,  $\text{Cu}_{0.1} + \text{Pb}_{0.1}$  and  $\text{Cu}_{0.1} + \text{Pb}_{0.5}$  experimental variants ( $1370$ ,  $1420$  and  $1960 \text{ mg kg}^{-1}$ , respectively). This suggests that changes in Ca content in *L. edodes* fruit bodies are dependent on mutual quantitative relations between Cu and Pb in growing substrates.

The addition of Cu and/or Pb to the substrates did not cause significant changes in Fe content (from  $17.6 \text{ mg kg}^{-1}$  in the control variant to  $21.1 \text{ mg kg}^{-1}$  for  $\text{Cu}_{0.1}$ ), except for fruit bodies growing in the  $\text{Cu}_{0.5} + \text{Pb}_{0.5}$  variant, where the highest Fe content was observed ( $29.1 \text{ mg kg}^{-1}$ ). A high similarity was also observed for K and Mg contents. In the case of both these metals, the lowest contents were recorded in fruit bodies growing in the  $\text{Cu}_{0.5} + \text{Pb}_{0.1}$  variant ( $9100$  and  $521 \text{ mg kg}^{-1}$ , respectively), and the highest levels in the  $\text{Cu}_{0.1} + \text{Pb}_{0.5}$  variant ( $15,300$  and  $900 \text{ mg kg}^{-1}$ , respectively). The obtained results suggest that a mutual quantitative relation may stimulate or inhibit the accumulation of K and Mg from the substrate, which is important for the nutritional value of *L. edodes* fruit bodies.

The highest sodium content  $269 \text{ mg kg}^{-1}$  was determined in control fruit bodies. A significantly lower content of this metal ( $95 \text{ mg kg}^{-1}$ ) was observed only in fruit bodies growing in the  $\text{Cu}_{0.1} + \text{Pb}_{0.1}$  variant. Despite the visible diversity in Na content (between  $135$  and  $259 \text{ mg kg}^{-1}$ ) shown in Fig. 5, no significant differences were found between the other variants. Phosphorus contents were similar in *L. edodes* fruit bodies growing in the majority of experimental variants (from  $4360$  to  $4930 \text{ mg kg}^{-1}$ ). The highest contents were determined in the  $\text{Pb}_{0.5}$  and  $\text{Cu}_{0.1} + \text{Pb}_{0.5}$  variants ( $5630$  and  $5650 \text{ mg kg}^{-1}$ , respectively), the lowest in fruit bodies growing in the  $\text{Cu}_{0.5} + \text{Pb}_{0.1}$  variant ( $3190 \text{ mg kg}^{-1}$ ).



**Fig. 6** Correlation between particular experimental variants with respect to the content of all determined elements jointly (Heatmap) in mean values with the presentation of a hierarchical tree plot

A Heatmap with a cluster analysis revealed the high similarity of mushrooms from the control variant and those growing under the  $\text{Cu}_{0.1}$  variant, as well as mushrooms growing under  $\text{Pb}_{0.1}$  and  $\text{Cu}_{0.5}$  with respect to the content of Ca, Fe, K, Mg, Na and P jointly (Fig. 6). This group also includes fruit bodies growing under the  $\text{Cu}_{0.5} + \text{Pb}_{0.5}$  and  $\text{Cu}_{0.5} + \text{Pb}_{0.1}$  experimental variants. A separate group of mushrooms was these growing under the remaining experimental variants:  $\text{Pb}_{0.5}$ ,  $\text{Cu}_{0.1} + \text{Pb}_{0.1}$  and  $\text{Cu}_{0.1} + \text{Pb}_{0.5}$ .

The presence of mushrooms growing under the  $\text{Cu}_{0.1}$ ,  $\text{Cu}_{0.5}$  and  $\text{Pb}_{0.1}$  variant in the first group and  $\text{Pb}_{0.5}$  in the second one suggests that a higher Pb content in the substrate may stimulate changes in the content of other elements in fruit bodies. It is also worth noting that smaller changes in all the contents of other elements in fruit bodies were observed if Cu was present in a higher ( $\text{Cu}_{0.5} + \text{Pb}_{0.1}$  or  $\text{Cu}_{0.5} + \text{Pb}_{0.5}$ ) rather than in a lower amount ( $\text{Cu}_{0.1} + \text{Pb}_{0.1}$  or  $\text{Cu}_{0.1} + \text{Pb}_{0.5}$ ) in the substrates.

The rank sum test showed that fruit bodies growing in particular experimental variants were diverse as regards the content of Ca, Fe, K, Mg, Na and P jointly:  $\text{Cu}_{0.1} + \text{Pb}_{0.5} > \text{Cu}_{0.1} > \text{Control} > \text{Pb}_{0.5} > \text{Cu}_{0.5} > \text{Cu}_{0.5} + \text{Pb}_{0.5} > \text{Cu}_{0.1} + \text{Pb}_{0.1} > \text{Pb}_{0.1} > \text{Cu}_{0.5} + \text{Pb}_{0.1}$ . Interestingly, for the majority of variants, the content of all elements jointly was lower than for the control. A higher content of the mentioned elements was only recorded if Cu was added at a lower concentration ( $\text{Cu}_{0.1}$ ) or in combination with Pb at a higher concentration ( $\text{Cu}_{0.1} + \text{Pb}_{0.5}$ ).

### Phenolic compounds and aliphatic acid profile and antioxidant activity

Various groups of phenolics were quantified, mainly hydroxybenzoic acids (C6–C1), hydroxycinnamic acids (C6–C3) and different groups of flavonoids (C6–C3–C6) (Table 1). 2,5-Dihydroxybenzoic acid (2,5-DHBA) was

**Table 1** Content of phenolic compounds of mushrooms ( $\mu\text{g g}^{-1}$  DW) and antioxidant activity

Phenolic compounds	Structure	Control	Cu <sub>0,1</sub>	Cu <sub>0,5</sub>	Pb <sub>0,1</sub>	Pb <sub>0,5</sub>	Cu <sub>0,1</sub> + Pb <sub>0,1</sub>	Cu <sub>0,1</sub> + Pb <sub>0,5</sub>	Cu <sub>0,5</sub> + Pb <sub>0,1</sub>	Cu <sub>0,5</sub> + Pb <sub>0,5</sub>
2,5-DHBA	C6–C1	4.96 <sup>d</sup>	1.30 <sup>e</sup>	0.45 <sup>e</sup>	8.99 <sup>b</sup>	13.01 <sup>a</sup>	7.40 <sup>c</sup>	4.38 <sup>d</sup>	9.24 <sup>b</sup>	6.90 <sup>c</sup>
Gallic acid		2.66 <sup>b</sup>	3.08 <sup>ab</sup>	3.50 <sup>a</sup>	3.08 <sup>ab</sup>	1.66 <sup>c</sup>	1.15 <sup>c</sup>	1.54 <sup>c</sup>	1.23 <sup>c</sup>	1.28 <sup>c</sup>
<i>p</i> -HBA		1.36 <sup>d</sup>	0.39 <sup>e</sup>	0.55 <sup>e</sup>	1.94 <sup>a</sup>	1.77 <sup>ab</sup>	1.49 <sup>bc</sup>	0.53 <sup>e</sup>	1.63 <sup>abc</sup>	1.46 <sup>bc</sup>
3,4-DHBA		0.20 <sup>e</sup>	0.42 <sup>d</sup>	0.45 <sup>d</sup>	0.31 <sup>d</sup>	0.78 <sup>b</sup>	0.44 <sup>d</sup>	1.15 <sup>a</sup>	0.56 <sup>c</sup>	0.45 <sup>d</sup>
Syringic acid		1.37 <sup>a</sup>	0.79 <sup>b</sup>	bDL	0.34 <sup>cd</sup>	0.76 <sup>bc</sup>	1.25 <sup>a</sup>	0.43 <sup>bcd</sup>	1.22 <sup>a</sup>	1.56 <sup>a</sup>
Vanillic acid		0.73 <sup>a</sup>	0.26 <sup>c</sup>	0.45 <sup>b</sup>	0.90 <sup>a</sup>	0.77 <sup>a</sup>	0.49 <sup>b</sup>	0.36 <sup>bc</sup>	0.52 <sup>b</sup>	0.52 <sup>b</sup>
Caffeic acid	C6–C3	1.19 <sup>abc</sup>	1.47 <sup>a</sup>	0.47 <sup>ef</sup>	1.26 <sup>ab</sup>	1.00 <sup>bcd</sup>	0.70 <sup>de</sup>	0.22 <sup>f</sup>	0.46 <sup>ef</sup>	0.74 <sup>de</sup>
Chlorogenic acid		0.51 <sup>c</sup>	0.76 <sup>ab</sup>	bDL	0.82 <sup>a</sup>	0.37 <sup>d</sup>	0.68 <sup>b</sup>	bDL	bDL	0.66 <sup>b</sup>
<i>p</i> -Coumaric acid		0.85 <sup>bc</sup>	0.93 <sup>b</sup>	2.03 <sup>a</sup>	0.66 <sup>cd</sup>	0.49 <sup>d</sup>	0.14 <sup>e</sup>	0.19 <sup>e</sup>	0.12 <sup>e</sup>	0.23 <sup>e</sup>
Ferulic acid		bDL	bDL	0.17 <sup>b</sup>	0.11 <sup>d</sup>	0.36 <sup>a</sup>	0.15 <sup>bc</sup>	bDL	bDL	0.12 <sup>bc</sup>
Sinapic acid		0.13 <sup>e</sup>	0.63 <sup>de</sup>	0.35 <sup>e</sup>	1.07 <sup>d</sup>	4.73 <sup>a</sup>	0.90 <sup>d</sup>	1.74 <sup>c</sup>	4.08 <sup>b</sup>	1.78 <sup>c</sup>
Quercetin	C6–C1–C6	0.33 <sup>e</sup>	0.17 <sup>e</sup>	0.29 <sup>e</sup>	0.11 <sup>e</sup>	3.38 <sup>a</sup>	1.85 <sup>d</sup>	1.27 <sup>c</sup>	2.31 <sup>b</sup>	1.69 <sup>d</sup>
Rutin		2.36 <sup>b</sup>	0.31 <sup>d</sup>	0.25 <sup>d</sup>	3.19 <sup>a</sup>	1.60 <sup>c</sup>	1.57 <sup>c</sup>	0.55 <sup>d</sup>	0.23 <sup>d</sup>	1.33 <sup>c</sup>
Catechin		0.86 <sup>a</sup>	0.25 <sup>cde</sup>	0.15 <sup>e</sup>	0.54 <sup>b</sup>	0.36 <sup>bcd</sup>	0.43 <sup>bcd</sup>	0.22 <sup>de</sup>	0.39 <sup>bcd</sup>	0.45 <sup>bc</sup>

Identical superscripts in a row denote no significant differences between means according to a post hoc Tukey's HSD test at  $\alpha=95\%$  following one-way ANOVA

*bDL* below detection limit

the main phenolic acid in the control variant. The content of 2,5-DHBA was significantly reduced in the Cu<sub>0,1</sub> and Cu<sub>0,5</sub> variants, while the addition of Pb and mixtures of Cu and Pb significantly stimulated the synthesis of the acid. The addition of Cu and Pb also had a significant effect on *p*-hydroxybenzoic acid (*p*-HBA) and the content of other hydroxybenzoic acids (C6–C1): gallic, protocatechuic (3, 4-dihydroxybenzoic acid, 3, 4-DHBA), syringic and vanillic. The content of protocatechuic acid was significantly higher in mushrooms growing in all contaminated substrates, whereas both inhibition and enhancement of gallic acid and *p*-HBA synthesis were confirmed in substrates with Cu and Pb. For syringic and vanillic acids only inhibition of biosynthesis and non-changes in content were observed in some contaminated substrates.

Among hydroxycinnamic acids (C6–C3), caffeic acid dominated in the control variant, synthesis of which was enhanced in Cu<sub>0,5</sub> and in all combinations with the addition of Cu and Pb. The addition of Cu<sub>0,1</sub>, Pb<sub>0,1</sub>, Cu<sub>0,1</sub> + Pb<sub>0,1</sub>, Cu<sub>0,5</sub> + Pb<sub>0,5</sub> resulted in an increase of chlorogenic acid content. For *p*-coumaric acid, mainly the reduction of synthesis was confirmed in the contaminated substrates (besides Cu<sub>0,5</sub>). Ferulic acid was synthesized for Cu<sub>0,5</sub>, Pb<sub>0,1</sub>, Pb<sub>0,5</sub>, Cu<sub>0,1</sub> + Pb<sub>0,1</sub>, Cu<sub>0,5</sub> + Pb<sub>0,5</sub> variants. The synthesis of sinapic acid was stimulated in substrates enriched with Pb and in all combinations of Cu and Pb.

Rutin was the main flavonoid in the control variant, the content of which was generally reduced in fruit bodies growing in the substrate variants with the addition of both Cu and Pb (besides Pb<sub>0,5</sub>) and Cu + Pb. Quercetin (flavonol) content increased by addition to the substrate Pb<sub>0,5</sub>, and mixtures

of Cu and Pb. Catechin content was significantly reduced by Cu and Pb supplementation in all combinations in comparison to the control variant. The changes in the content of different phenolic compounds were reflected in the sum of the phenolic compound content, i.e., the addition of Cu resulted in a drop of the sum, while Pb elevated the sum of phenolic compounds. Moreover, the addition of both the elements had a significant effect on the sum. In comparison to the control variant and Cu-enriched mushrooms, the value was higher. Generally, in comparison to Pb-enriched mushrooms the sum of phenolic compounds was significantly lower (Fig. 7a).

The lowest TP content in fruit bodies was determined in the control variant and it increased in mushrooms growing in substrates with Cu and Pb addition in all combinations. The supplementation of substrates with both the elements caused a further increase of TP, with the highest level for the Cu<sub>0,5</sub> + Pb<sub>0,5</sub> variant. Moreover, the synergic effect of both the elements for TP value was confirmed for the experimental variants Cu<sub>0,1</sub> and Cu<sub>0,1</sub> + Pb<sub>0,5</sub>, Cu<sub>0,5</sub> and Cu<sub>0,5</sub> + Pb<sub>0,5</sub> (Fig. 7b).

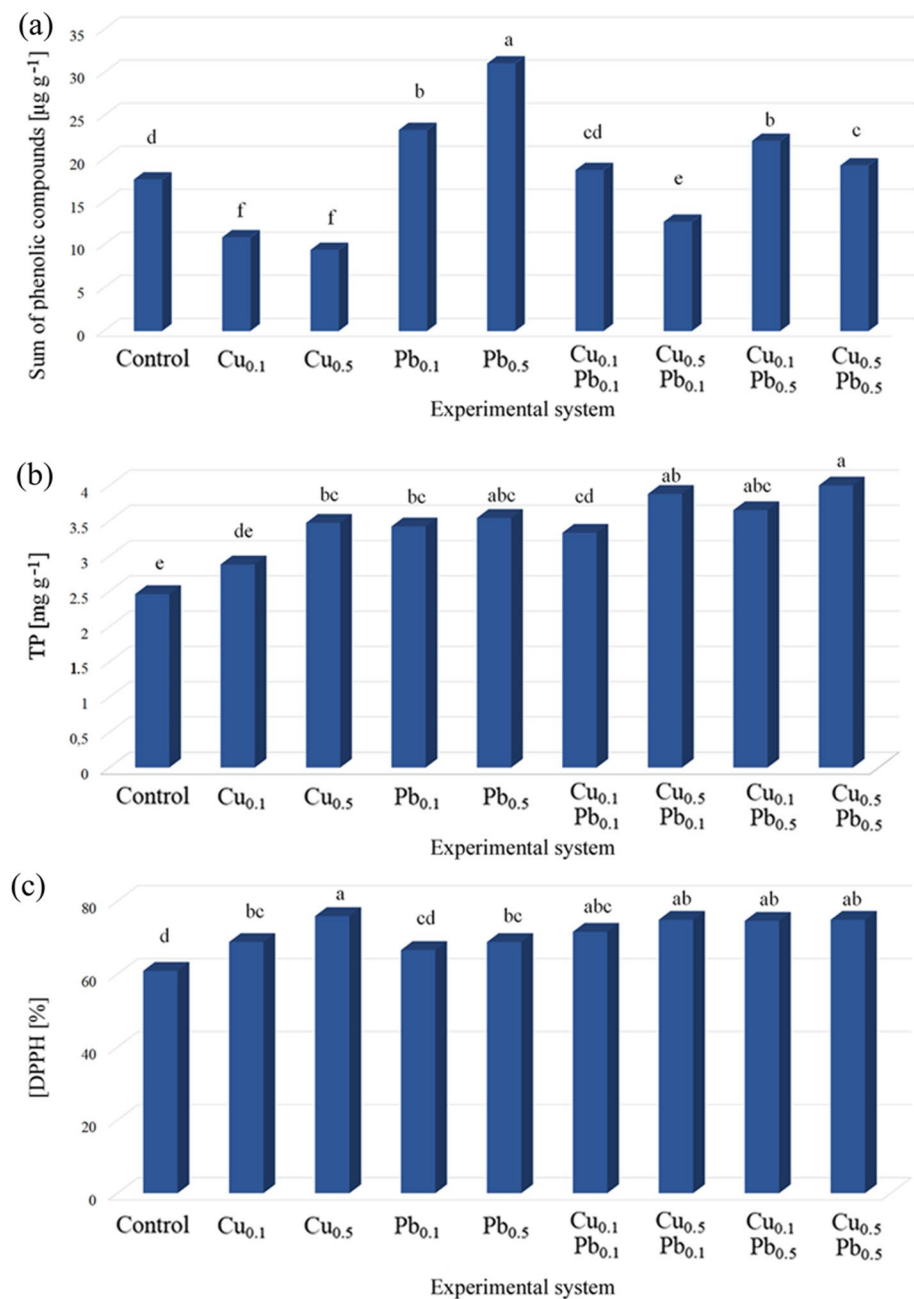
The antioxidant activity of fruit bodies towards DPPH radicals depended on the composition of the substrates. The lowest value was observed in the control variant. Cu and Pb addition significantly improved the antioxidant properties from 60.9 (control) to 74.9% (for Cu<sub>0,5</sub> + Pb<sub>0,5</sub>) (Fig. 7c).

### Aliphatic acid profile

The content and profile of aliphatic acids in *L. edodes* growing in nine different variants were analyzed and significant



**Fig. 7** Characteristics of (a) sum of phenolic compounds in mushrooms ( $\mu\text{g g}^{-1}$  DW), (b) total phenolic content in mushrooms ( $\text{mg g}^{-1}$  DW) and (c) scavenging effect of mushrooms on DPPH radicals



differences were found between them (Table 2). Three of the eight determined acids occurred in the control variant. Succinic, acetic and citric acids were determined, while the remaining acids were below the limit of detection (bLD). The addition of Cu<sub>0.1</sub> and Pb<sub>0.1</sub> caused a strong inhibition of the synthesis of aliphatic acids in fruit bodies. Acetic acid was only identified in mushrooms growing with Cu<sub>0.1</sub>, while in the Pb<sub>0.1</sub> variant only acetic and succinic were recorded. A significant decrease of succinic acid content was observed in both the variants, which consequently had an influence on the lowest content of the sum of all acids in fruit bodies.

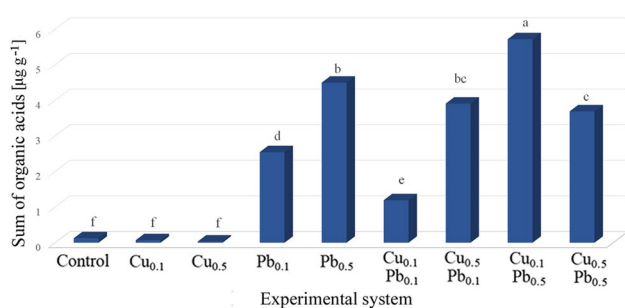
For Cu<sub>0.5</sub> and Pb<sub>0.5</sub> and for the mixture of both the elements (Cu<sub>0.5</sub> + Pb<sub>0.5</sub>), significant stimulation of aliphatic acid synthesis was observed (especially for quinic, malic, acetic, fumaric and succinic acids). These acids dominated in all these variants. The Cu<sub>0.5</sub> addition caused a significant increase of quinic, malic, malonic, citric, acetic and succinic acids, while in the Pb<sub>0.5</sub> variant the above-mentioned acids were also present with the exception of fumaric, malonic and citric acids, which were bDL. The sum of determined acids in Pb<sub>0.5</sub> was ~twofold higher than in Cu<sub>0.5</sub>, which is related to the highest content of quinic acid among all the analyzed variants.

**Table 2** Content ( $\mu\text{g g}^{-1}$  DW) of aliphatic acids in mushroom bodies

Aliphatic acid	Control	Cu <sub>0,1</sub>	Cu <sub>0,5</sub>	Pb <sub>0,1</sub>	Pb <sub>0,5</sub>	Cu <sub>0,1</sub> + Pb <sub>0,1</sub>	Cu <sub>0,5</sub> + Pb <sub>0,1</sub>	Cu <sub>0,1</sub> + Pb <sub>0,5</sub>	Cu <sub>0,5</sub> + Pb <sub>0,5</sub>
Oxalic	bDL	bDL	0.03 <sup>c</sup>	bDL	bDL	0.01 <sup>cd</sup>	0.22 <sup>a</sup>	0.14 <sup>b</sup>	0.03 <sup>c</sup>
Quinic	bDL	bDL	0.62 <sup>c</sup>	bDL	3.2 <sup>a</sup>	0.60 <sup>c</sup>	2.2 <sup>b</sup>	2.4 <sup>b</sup>	0.06 <sup>d</sup>
Malic	bDL	bDL	0.30 <sup>c</sup>	bDL	0.13 <sup>c</sup>	0.03 <sup>c</sup>	0.21 <sup>c</sup>	0.74 <sup>b</sup>	2.2 <sup>a</sup>
Malonic	bDL	bDL	0.15 <sup>a</sup>	bDL	bDL	bDL	bDL	0.13 <sup>a</sup>	0.12 <sup>a</sup>
Citric	0.02 <sup>c</sup>	bDL	0.12 <sup>d</sup>	bDL	bDL	bDL	0.76 <sup>a</sup>	0.54 <sup>b</sup>	0.21 <sup>c</sup>
Acetic	0.03 <sup>e</sup>	0.03 <sup>e</sup>	0.62 <sup>c</sup>	0.02 <sup>e</sup>	1.0 <sup>b</sup>	0.48 <sup>c</sup>	0.14 <sup>de</sup>	1.5 <sup>a</sup>	0.40 <sup>d</sup>
Fumaric	bDL	bDL	0.03 <sup>bc</sup>	bDL	bDL	bDL	0.03 <sup>b</sup>	0.01 <sup>bcd</sup>	0.08 <sup>a</sup>
Succinic	0.07 <sup>c</sup>	0.03 <sup>c</sup>	0.69 <sup>a</sup>	0.01 <sup>c</sup>	0.10 <sup>c</sup>	0.07 <sup>c</sup>	0.31 <sup>b</sup>	0.30 <sup>b</sup>	0.62 <sup>a</sup>

Identical superscripts in a row denote no significant differences between means according to a post hoc Tukey's HSD test at  $\alpha = 95\%$  following one-way ANOVA

bDL below detection limit



**Fig. 8** Sum of aliphatic acids ( $\mu\text{g g}^{-1}$  DW) in mushrooms growing on substrates with different Cu and/or Pb supplementation

The addition of all mixtures of Cu and Pb increased the content of all acids in comparison to the control; quinic, then malic and acetic acids were observed as dominating. The highest content of quinic acid was observed in Cu<sub>0,5</sub> + Pb<sub>0,1</sub> and Cu<sub>0,1</sub> + Pb<sub>0,5</sub>, while in the Cu<sub>0,1</sub> + Pb<sub>0,1</sub> and Cu<sub>0,5</sub> + Pb<sub>0,5</sub> variants the content was much lower. This resulted in an increase of the sum of studied acids, likewise, observed in the Pb<sub>0,5</sub> variant, where a higher content of quinic acid was also present. Malic acid content especially increased in mixtures with Pb<sub>0,5</sub>, while acetic acid synthesis increased for all the variants with Pb<sub>0,5</sub> (alone and in mixtures) and also for Cu<sub>0,1</sub> + Pb<sub>0,1</sub>.

In the experiment, the stimulation of aliphatic acid synthesis was determined in variants where lead was present individually and, in all Cu, and Pb mixtures simultaneously. For these variants, the sum of all determined acids was the highest in comparison to the control. Moreover, the obtained results showed that the presence of Cu (especially Cu<sub>0,1</sub>) in the substrate in combination with the presence of Pb (Pb<sub>0,1</sub> and Pb<sub>0,5</sub>) inhibited the production of aliphatic acids in *L. edodes* fruit bodies as compared to the variants where Pb was present alone, which is especially

evident in the sum of the studied aliphatic acids (Fig. 8). The only exception was the Cu<sub>0,1</sub> + Pb<sub>0,5</sub> variant where an increase in the formation of the analyzed acids was observed.

## Discussion

According to FOASTAT [25], China is the greatest producer of cultivated mushrooms, whose production reached 8,948,099 tonnes in 2019. The determined Cu and Pb contents in this work range from 2.22 to 87.9 mg kg<sup>-1</sup> for Cu and from 0.37 to 9.70 mg kg<sup>-1</sup> for Pb. Royse et al. [1] found that 22% of the world's cultivated mushrooms are of the *Lenzula* genus (over 7 million tons). Therefore, the enrichment of Cu and/or Pb fruit bodies is of significance.

Copper is an essential element for living organisms if present in a lesser amount, although while in excessive amounts, the element exerts detrimental effects [26]. Lead is the second most toxic heavy metal owing to its non-degradable nature, which has no beneficial role in biological systems [27]. The presence of Cu and Pb in the growing substrates can significantly affect mycelia growth and yields of fruit bodies. Generally, the higher the concentration of Cu and other supplemented elements, the lower biomass of *Ganoderma lucidum* fruit bodies reported in one of our recent papers [28], where 0.6 mM selenium and Cu addition caused a decrease of over 20% of biomass as compared to mushrooms growing with the addition of Se only. Furthermore, the investigation by Dulay et al. [29] indicated a marked growth inhibition of *Pleurotus* species (up to almost 50%) exposed to Pb in a concentration-dependent manner (from 1 to 100 mg kg<sup>-1</sup> of Pb). The results of our present study showed that the lowest yield from the variant with the maximal concentrations (Cu<sub>0,5</sub> + Pb<sub>0,5</sub>) was less than 30% of the control. The findings add further evidence that Cu and Pb in the substrate can result in growth disturbances in fruit bodies.

Mushrooms are known for their ability to (hyper)accumulate various elements, both essential and detrimental from the nutritional point of view, taken from the growth environment [30–32]. Therefore, it is not surprising that the specimens subjected to the described experiment showed a significantly higher accumulation of Cu and Pb compared to the non-supplemented control variant if these elements were added to the substrate. The addition of 0.1 mM Cu to the substrate resulted in an almost 7-times higher accumulation of this element in *L. edodes* fruit bodies, and a fivefold higher concentration ( $\text{Cu}_{0.5}$ ), nearly 38-fold higher content compared to the control mushrooms. A lower but still relevant increase of Cu content (more than threefold, compared to the control) in *Ganoderma lucidum* after supplementation of the substrate with  $100 \text{ mg kg}^{-1}$  of  $\text{CuSO}_4$  was reported by Postemsky et al. [33]. Increasing accumulation of Cu in *G. lucidum* with an increase of the metal concentration in the growth liquid medium was also described by Matute et al. [34]. The authors obtained more than a 1.5-fold higher Cu content in fruit bodies when  $25 \text{ mg kg}^{-1}$  Cu was added, and further almost 2-fold and more than 2-fold higher at 50 and  $100 \text{ mg kg}^{-1}$ , respectively, of the metal in the substrate.

Similar observations were obtained for Pb in the present study. The addition of  $\text{Pb}_{0.1}$  to the substrate increased the metal content in *L. edodes* over 8-fold, and  $\text{Pb}_{0.5}$  by about 15-fold in comparison with the control variant. Valuable results comparing the range of Pb and other elements in six mushroom species (*Agrocybe cylindracea*, *Clitocybe maxima*, *Flammulina velutipes*, *G. lucidum*, *L. edodes* and *P. eryngii*) growing in two substrates differing chemically were described in one of our previous studies [35]. A higher Pb content in fruit bodies growing on the substrate with a higher concentration of this metal was found for all the studied species.

Mutual interactions among individual elements can significantly affect the efficiency of their uptake by living organisms. Studies addressing such interactions have become increasingly important because they can dramatically modulate the toxicity of chemical individuals [36]. In animal and plant research, many interactions between chemical elements have been observed to date [37–39]. Such a situation also applies to research dealing with the uptake of elements by mushrooms. Unfortunately, only limited attention has been paid to it so far. The series of papers by Rzymiski et al. [10, 28] and Poniedziałek et al. [11] addressed the topic of the interactions between Cu, Se and Zn in *A. bisporus*, *G. lucidum*, *P. ostreatus* and *P. eryngii* following substrate supplementation. The authors claimed that the simultaneous presence of Cu and Zn in the substrate decreased the accumulation of Se in the fruit bodies. Furthermore, even though some competitive sequestration between Cu and Zn may have occurred, the content of the elements generally increased with their concentration in the

substrate. In the present study, the interactions of Cu and Pb present in the substrate were confirmed by significant relationships between the content of elements in individual experimental variants. At a low concentration ( $\text{Pb}_{0.1}$ ), Pb did not affect the accumulation of Cu in *L. edodes*, but in high concentration ( $\text{Pb}_{0.5}$ ) it increased the Cu content in fruit bodies. Similarly, the addition of Cu led to an increase of Pb uptake when it was present in a higher concentration ( $\text{Cu}_{0.5}$ ).

Some factors, e.g. metals or UV light, affect phenolic compound synthesis in mushrooms [20, 40, 41]. Phenolic compounds are metabolites involved in defense mechanisms against stressors, including heavy metals [42, 43]. Their action in response to the presence of metal(s) in the growing substrate is related to their structure and the chemical properties of elements. This enables the detoxification of elements in different pathways, e.g. based on the scavenging and chelating ability of the phenolic compounds [44–47]. Caffeic and *p*-coumaric acids were recognized as strong antioxidants [48]. However, the suppression of the synthesis was observed, but other antioxidants belonging to C6-C1 and C6-C3, and flavonoids were activated. Moreover, the action based on chelation was also activated because of the intensive biosynthesis of protocatechuic acid, which is known for its chelating ability [49]. The content of the acid increased in all contaminated mushrooms. It indicates the important role of protocatechuic acid in the detoxification of Cu and Pb in *L. edodes*, probably via the chelation of Pb and Cu ions. The quantitative changes in the profile of phenolic compounds suggested that different paths of detoxification were activated. The increase of TP was probably connected with the activity of enzymes participating in the biosynthesis of the phenolic compounds detected in mushrooms [41, 50, 51]. Additionally, the correlation between DPPH and TP was confirmed ( $r=0.735$ ) in the present study, which indicated that radical scavenging was one of the detoxification pathways. The obtained results also suggest that the effect of both the elements may be different from that of only a single element on the synthesis of individual phenolic compounds and their sum, TP and the ability to scavenge radicals (Fig. 7).

Similarly, aliphatic acids are known to play an important role in metal detoxification [52]. They react with metals, forming complex compounds with reduced toxicity, and consequently, they can be accumulated in mushroom fruit bodies [53]. It should also be noted that mushroom biomass shows excellent metal-binding properties due to the high percentage of cell wall material [54]. Therefore, fruit bodies have a high tolerance to metal contamination of substrate [55, 56]. The above relationships are confirmed by the data of Table 2 and Fig. 8. A significant increase of almost all the produced acids was observed in most of the experimental variants with Cu and Pb addition to the substrate. However, the  $\text{Cu}_{0.1}$  and  $\text{Pb}_{0.1}$  variants did not affect the total level of

the acids when compared to the control variant due to the lack of any significant differences among these three variants (Fig. 8).

According to the literature data, oxalic acid dominates among aliphatic acids in mushroom fruit bodies growing under metal contamination [57, 58]. In this study, its content significantly increased only in the variants combining Cu and Pb addition (Table 2). This may confirm that oxalic acid can be an efficient metal chelating ligand in mushrooms, important in the detoxification process. Sayer and Gadd [59] also reported the production of oxalic acid by white-rot fungi, which provides a means of immobilizing soluble metal ions or complexes as insoluble oxalates, thus decreasing bioavailability and increasing metal tolerance.

However, in our research, oxalic acid was not the acid with the highest content. *L. edodes* was characterized by a much higher content of succinic, citric, malic and fumaric acids (Table 2). Chen et al. reported that these acids are characteristic and prevailing in this species. Moreover, it was also shown that succinic acid has the greatest content changes depending on the cultivation conditions [60]. This is confirmed by the data in Table 2. The content of succinic acid increased significantly, along with the increase in the content of metals in the substrate (except for the variants with Cu and Pb at the level of 0.1 mM). Therefore, succinic acid may be the acid that can reduce the effects of metals. Further, the contents of malic, quinic and acetic acids also increased significantly with changes in the content of the metals in substrates. Their contents were each time higher compared to the control variant, which was apparent mainly in the variants containing a mixture of the metals. This may indicate that the acids play an important biological function in lessening the toxicity of metals and reducing stress because of the presence of carboxylic functional groups responsible for the biosorption of heavy metals [57]. Due to the fact that chemical forms are one of the most important factors in metal detoxification [61], it is, therefore, indicated that significant changes in the content of aliphatic acids can determine the ability of a given mushroom species to accumulate excess metal ions without adverse effects on their yield [62].

## Conclusions

Appropriate conditions and actions must be respected during all stages of food production and trade to ensure the safety of the lives and health of consumers. Selection of a safe substrate and its components is, therefore, essential in mushroom production. This study shows that *L. edodes* easily accumulates Cu and Pb ions from substrate in fruit bodies; yield reduction is a further consequence. It should

be remembered that the accumulation of elements in fruit bodies depends not only on their availability but also on the mutual quantitative and qualitative relations in the substrate. Thus, the key issue is the selection of substrates containing as low as possible levels of heavy metals. The synthesis of tested bioactive compounds was related to Cu and Pb content hence pointing to the necessity for a determination of the content of some bioactive compounds together with metal/metalloid concentration. The mutual quantitative ratios of the elements in growing media are an important factor determining the quality of fungi.

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## Declarations

**Conflict of interest** The authors declare that there is no conflict of interest.

**Compliance with ethics requirements** This article does not contain any studies with human participants or animals performed by any of the authors.

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## References

1. Roysse DJ, Baars J, Tan Q (2017) Current overview of mushroom production in the world. In: Cunha ZD, Pardo-Giménez A (eds) Edible and medicinal mushrooms: technology and applications. Wiley, pp 5–13
2. Ozçelik E, Pekşen A (2007) Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). Biores Technol 98:2652–2658
3. Siwulski M, Jasińska A, Sobieralski K, Sas-Golak I (2011) Comparison of chemical composition of fruiting bodies of some edible mushrooms cultivated on sawdust. Ecol Chem Eng A 18:89–96

4. Kovacs H, Báthory C, Mentés D (2019) Distribution of Zn, Cu, Cd, Pb, Cr, Ni, Co in *Quercus robur* and *Populus tremula* has grown on heavy metal contaminated brownfield land. *Internat J Energy Water Food Nexus* 1:42–47
5. Stojnić S, Kebert M, Drekić M, Galić Z, Kesić L, Tepavac A, Orlović S (2019) Heavy metals content in foliar litter and branches of *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. observed at two ICP forests monitoring plots. *South-East Europ Forest* 10:151–157
6. Breckle S-W, Kahle H (1992) Effect of toxic heavy metals (Cd, Pb) on growth and mineral nutrition of beech (*Fagus sylvatica* L.). *Vegetatio* 101:43–53.
7. Nicewicz D, Szczepkowski A (2008) The content of heavy metals in the wood of healthy and dying beech trees (*Fagus Sylvatica* L.). *Acta Sci Pol Silvarum Colendarum Ratio Ind Lignaria* 7:35–44
8. Mleczeek M, Siwulski M, Mikołajczak P, Gąsecka M, Sobieralski K, Szymańczyk M, Goliński P (2015) Content of selected elements in *Boletus badius* fruiting bodies growing in extremely polluted wastes. *J Environ Sci Health Part A* 50:767–775
9. Mleczeek M, Niedzielski P, Siwulski M, Rzymiski P, Gąsecka M, Goliński P, Kozak L, Kozubik T (2016) Importance of low substrate arsenic content in mushroom cultivation and safety of final food product. *Europ Food Res Technol* 242:355–362
10. Rzymiski P, Mleczeek M, Siwulski M, Gąsecka M, Niedzielski P (2016) The risk of high mercury accumulation in edible mushrooms cultivated on contaminated substrates. *J Food Compos Anal* 51:55–60
11. Poniedziałek B, Mleczeek M, Niedzielski P, Siwulski M, Gąsecka M, Kozak L, Komosa A, Rzymiski P (2017) Bio-enrichment *Pleurotus* mushrooms for deficiency control and improved antioxidative protection of human platelets? *Eur Food Res Technol* 243:2187–2198
12. Siwulski M, Budzyńska S, Rzymiski P, Gąsecka M, Niedzielski P, Kalač P, Mleczeek M (2019) The effects of germanium and selenium on growth, metalloids accumulation and ergosterol content in mushrooms: experimental study in *Pleurotus ostreatus* and *Ganoderma lucidum*. *Eur Food Res Technol* 245:1799–1810
13. Kabata-Pendias A, Szeke B (2015) Trace elements in abiotic and biotic environment. CRC Press Taylor & Francis Group, LLC, Boca Raton, pp 58–59
14. Koutrotsios G, Danezis G, Georgiou C, Zervakis GI (2020) Elemental content in *Pleurotus ostreatus* and *Cyclocybe cylindracea* mushrooms: correlations with concentrations in cultivation substrates and effects on the production process. *Molecules* 25:2179
15. Chiu S, Chan Y, Law S, Cheung K, Moore D (1998) Cadmium and manganese in contrast to calcium reduce yield and nutritional values of the edible mushroom *Pleurotus pulmonarius*. *Mycol Res* 102:449–457
16. Kalač P (2019) Mineral composition and radioactivity of edible mushrooms. Academic Press/Elsevier
17. Mleczeek M, Rzymiski P, Budka A, Siwulski M, Jasińska A, Kalač P, Poniedziałek B, Gąsecka M, Niedzielski P (2018) Elemental characteristics of mushroom species cultivated in China and Poland. *J Food Comp Anal* 66:168–178
18. Mleczeek M, Siwulski M, Rzymiski P, Budka A, Kalač P, Jasińska A, Gąsecka M, Budzyńska S, Niedzielski P (2018) Comparison of elemental composition of mushroom *Hypsizygus marmoreus* originating from commercial production and experimental cultivation. *Sci Horticultur* 236:30–35
19. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 299:152–178
20. Gąsecka M, Mleczeek M, Siwulski M, Niedzielski P, Kozak L (2015) The effect of selenium on phenolics and flavonoids in selected edible white rot fungi. *LWT Food Sci Technol* 63:726–731
21. Gąsecka M, Magdziak Z, Siwulski M, Jasinska A, Budzyńska S, Rzymiski P, Kalač P, Niedzielski P, Pankiewicz J, Mleczeek M (2020) Effect of *Thymus vulgaris* post-extraction waste and spent coffee grounds on the quality of cultivated *Pleurotus eryngii*. *J Food Process Preserv* 44:e14648. <https://doi.org/10.1111/jfpp.14648>
22. Dong J, Zhang M, Lu L, Sun L, Xu M (2012) Nitric oxide fumigation stimulates flavonoid and phenolic accumulation and enhances antioxidant activity of mushroom. *Food Chem* 135:1220–1225
23. Abdi H, Williams LJ (2010) Principal component analysis. Wiley Interdiscip Rev Comput Stat 2:433–459
24. Morrison DF (1990) Multivariate statistical methods, 3rd edn. McGraw-Hill Co, New York
25. FAOSTAT (2019) Food and Agriculture Organization of the United Nations. Database. Rome, Italy: FAO. Retrieved March 30, 2021 from <http://www.fao.org/faostat/en/#data>.
26. Kumar V, Pandita S, Sidhu GPS, Sharma A, Khanna K, Kaur P, Bali AS, Setia R (2021) Copper bioavailability, uptake, toxicity and tolerance in plants: a comprehensive review. *Chemosphere* 262:127810
27. Zulfiqar U, Farooq M, Hussain S, Maqsood M, Hussain M, Ishaq M, Ahmad M, Anjum MZ (2019) Lead toxicity in plants: impacts and remediation. *J Environ Manage* 250:109557
28. Rzymiski P, Mleczeek M, Niedzielski P, Siwulski M, Gąsecka M (2016) Potential of cultivated *Ganoderma lucidum* mushrooms for the production of supplements enriched with essential elements. *J Food Compos Anal* 81:C587–C592
29. Dulay RMR, Castro MEGD, Coloma NB, Bernardo AP, Cruz AGD, Tiniola RC, Kalaw SP, Reyes RG (2015) Effects and myco-accumulation of lead (Pb) in five *Pleurotus* mushrooms. *Int J Biol Pharm Appl Sci* 4:1664–1677
30. Dhanker R, Tyagi P, Kamble SS, Gupta D, Hussain T (2021) Advances in fungi: rejuvenation of polluted sites. In: Sharma VK, Shah MP, Parmar S, Kumar A (eds) *Fungi bio-prospects in sustainable agriculture, environment and nano-technology*. Academic Press, pp 251–275
31. Dowlati M, Sobhi HR, Esrafil A, Farzadkia M, Yeganeh M (2021) Heavy metals content in edible mushrooms: a systematic review, meta-analysis and health risk assessment. *Trend Food Sci Technol* 109:527–535
32. Mleczeek M, Siwulski M, Budka A, Mleczeek P, Budzyńska S, Szostek M, Kuczyńska-Kippen N, Kalač P, Niedzielski P, Gąsecka M, Goliński P, Magdziak Z, Rzymiski P (2021) Toxicological risks and nutritional value of wild edible mushroom species—a half-century monitoring study. *Chemosphere* 263:128095
33. Postemsky PD, Delmastro SE, Curvetto NR (2014) Effect of edible oils and Cu (II) on the biodegradation of rice by-products by *Ganoderma lucidum* mushroom. *Int Biodeterior Biodegrad* 93:25–32
34. Matute RG, Serra A, Figlas D, Curvetto N (2011) Copper and zinc bioaccumulation and bioavailability of *Ganoderma lucidum*. *J Med Food* 14:1273–1279
35. Siwulski M, Rzymiski P, Budka A, Kalač P, Budzyńska S, Dawidowicz L, Hajduk E, Kozak L, Budzulak J, Sobieralski K, Niedzielski P (2019) The effect of different substrates on the growth of six cultivated mushroom species and composition of macro and trace elements in their fruiting bodies. *Europ Food Res Technol* 245:419–431
36. Alonso ML, Montaña FP, Miranda M, Castillo C, Hernández J, Benedito JL (2004) Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo,

- Ni, Se, Zn) elements in the tissues of cattle from NW Spain. *Biomaterials* 17:389–397
37. Adamczyk-Szabela D, Lisowska K, Romanowska-Duda Z, Wolf WM (2020) Combined cadmium-zinc interactions alter manganese, lead, copper uptake by *Melissa officinalis*. *Sci Rep* 10:1675
  38. Gambling L, Andersen HS, McArdle HJ (2008) Iron and copper, and their interactions during development. *Biochem Soc Trans* 36:1258–1261
  39. Tao S, Liang T, Cao J, Dawson RW, Liu C (1999) Synergistic effect of copper and lead uptake by fish. *Ecotoxicol Environ Saf* 44:190–195
  40. Vieira FPA, Gontijo DC, Vieira BC, Fontes EAF, de Assunção LS, Leite JPV, Goretí M, de Oliveira A, Kasuya MCM (2013) Antioxidant activities, total phenolics and metal contents in *Pleurotus ostreatus* mushrooms enriched with iron, zinc or lithium. *LWT Food Sci Technol* 54:421–425
  41. Xiaokang W, Brunton NP, Lyng JG, Harrison SM, Carpes ST, Papoutsis K (2020) Volatile and non-volatile compounds of shiitake mushrooms treated with pulsed light after twenty-four hour storage at different conditions. *Food Biosci* 36:100619
  42. Drzewiecka K, Gąsecka M, Rutkowski P, Magdziak Z, Goliński P, Mleczek M (2018) Arsenic forms and their combinations induce differences in phenolic accumulation in *Ulmus laevis* Pall. *J Plant Physiol* 220:34–42
  43. Gąsecka M, Drzewiecka K, Magdziak Z, Piechalak A, Budka A, Waliszewska B, Szentner K, Goliński P, Niedzielski P, Budzyńska S, Mleczek M (2021) Arsenic uptake, speciation and physiological response of tree species (*Acer pseudoplatanus*, *Betula pendula* and *Quercus robur*) treated with dimethylarsinic acid. *Chemosphere* 263:127859
  44. Kováčik J, Klejdus B, Hedbavny J, Bačkor M (2009) Comparison of cadmium and copper effect on phenolic metabolism, mineral nutrients and stress-related parameters in *Matricaria chamomilla* plants. *Plant Soil* 320:231
  45. Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *P J Environ Stud* 15:523–530
  46. Mohammadi S, Pourakbar L, Moghaddam SS, Popovic-Djordjevi J (2021) The effect of EDTA and citric acid on biochemical processes and changes in phenolic compounds profile of okra (*Abelmoschus esculentus* L.) under mercury stress. *Ecotoxicol Environ Saf* 208:111607
  47. Sharma A, Shahzad B, Rehman A, Bhardwaj A, Landi M, Zheng B (2019) Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules* 24:1–22
  48. Benbettaieb N, Nyagaya J, Seuvre AM, Debeau F (2018) Antioxidant activity and release kinetics of caffeic and *p*-coumaric acids from hydrocolloid-based active films for healthy packaged food. *J Agric Food Chem* 66:6906e6916
  49. Kováčik J, Klejdus B, Hedbavny J, Bačkor M (2010) Effect of copper and salicylic acid on phenolic metabolites and free amino acids in *Scenedesmus quadricauda* (Chlorophyceae). *Plant Sci* 178:307–311
  50. Jiang TJ, Jahangir MM, Jiang ZH, Lu XY, Ying T-J (2010) Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity and texture of postharvest shiitake (*Lentinula edodes*) mushrooms during storage. *Postharvest Biol Technol* 56:209–215
  51. Kuijpers TF, van Herk T, Vincken JP, Janssen RH, Narh DL, van Berkel WJ, Gruppen H (2014) Potato and mushroom polyphenol oxidase activities are differently modulated by natural plant extracts. *J Agric Food Chem* 62:214–221
  52. Chen Q, Yang L, Liu L, Qian L, Tian K, Zhang Q, Cao D (2021) Combined forms of Pb and its detoxification and absorption in *Cladophora rupestris* subcells. *Spectrochim Acta A* 248:119190
  53. Magdziak Z, Siwulski M, Mleczek M (2017) Characteristics of organic acid profiles in 16 species of wild growing edible mushrooms. *J Environ Sci Health Part B* 52:784–789
  54. Zafar S, Aqil F, Ahmad I (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. *Biores Technol* 98:2557–2561
  55. Mleczek M, Magdziak Z, Gąsecka M, Niedzielski P, Kalač P, Siwulski M, Rzymiski P, Zalicka S, Sobieralski K (2016) Content of selected elements and low-molecular-weight organic acids in fruiting bodies of edible mushroom *Boletus badius* (Fr.) Fr. from unpolluted and polluted areas. *Environ Sci Pollut Res* 23:20609–20618
  56. Muszyńska B, Kała K, Lazur J, Włodarczyk A (2020) *Imleria badia* culinary-medicinal mushroom with interesting biological properties. *Food Biosci* 37:100663
  57. Gupta Ch, Balakrishnan RM, Priyanka U, Pugazhendhi A (2019) Mycosensing of soil contaminants by *Ganoderma lucidum* and *Omphalotus subilludens* including the insights on growth media requirements. *Biocatal Agric Biotechnol* 20:101239
  58. Sembratowicz I, Rusinek-Prystupa E (2012) Content of cadmium, lead, and oxalic acid in wild edible mushrooms harvested in places with different pollution levels. *Pol J Environ Stud* 21:1825–1830
  59. Sayer J, Gadd GM (1997) Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspergillus niger*. *Mycol Res* 106:653–661
  60. Gao S, Huang Z, Feng X, Bian Y, Huang W, Liu Y (2020) Bio-conversion of rice straw agroresidues by *Lentinula edodes* and evaluation of non-volatile taste compounds in mushrooms. *Sci Rep* 10:1814
  61. Wang X, Liu YG, Zeng GM, Chai LY, Song XC, Min ZY, Xiao X (2008) Subcellular distribution and chemical forms of cadmium in *Bechmeria nivea* (L.) Gaud. *Environ Exp Bot* 62:389–395
  62. Chen W, Li W, Yang Y, Yu H, Zhou S, Feng J, Li X, Liu Y (2015) Analysis and evaluation of tasty components in the pileus and stipe of *Lentinula edodes* at different growth stages. *J Agric Food Chem* 63:795–801

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