

A screening study of elemental composition in 12 marketable mushroom species accessible in Poland

Przemysław Niedzielski¹ · Mirosław Mleczek² · Anna Budka³ · Piotr Rzymiski⁴ · Marek Siwulski⁵ · Agnieszka Jasińska⁵ · Monika Gąsecka² · Sylwia Budzyńska²

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Abstract Cultivated mushroom species are becoming an increasingly consumed commodity owing to their nutritional value and potential biological activities. There is a strict necessity to conduct a thorough screening of their chemical composition to ensure the quality and safety of final food products. The present study analyzed the content of 67 macro- and microelements and detected 36 of them (macroelements: Ca, K, Mg, Na, P; micronutrients: Cu, Fe, Mn, Mo, Zn; toxic metals: Ag, Al, Cd, Ni and Pb; metalloids: As, B, Ge, Te; platinum group elements (PGEs): Os, Pt, Rh; rare earth elements (REES): Gd, Ho, La, Nd, Pr; other elements: Ba, Bi, Ga, In, Sr, Ti, U, V, Zr) in 12 mushroom species (*Agrocybe cylindracea*, *Auricularia polytricha*, *Clitocybe maxima*, *Coprinus comatus*, *Flammulina velutipes*, *Grifola frondosa*, *Hericium erinaceus*, *Laetiporus sulphureus*, *Pholiota nameko*, *Stropharia rugosoannulata*, *Trametes versicolor*, *Tremella fuciformis*) obtained between 2008 and 2016 from the Polish market but originating from both Poland (small scale local production) and China (available in selected oriental or internet shops only).

Elemental composition was highly species-specific and did not follow a taxonomical pattern on the family level. As revealed, *G. frondosa* contained high concentrations of minerals (Ca, Cu, Fe, Mg, Mn, P and Zn). Most of the studied mushrooms were characterized by a relatively high level of PGEs. Serious food contaminants such as Al, As, Cd and Pb were within safety limits set by the FAO/WHO. Additionally, the study presented Andrews curves as a convenient tool to analyze trends on multidimensional data on chemical composition.

Keywords Accumulation · Contamination · Cultivated mushrooms · Elements · Food

Introduction

Edible mushrooms have been valued for centuries for their sensory characteristics and culinary suitability and are well recognized for their nutritional and health benefits. Both fresh and dried mushrooms are a good source of polysaccharides (37–48%), proteins (20–25%), fiber (13–24%), vitamins (e.g., B₁, B₂, B₃, B₇, C) and minerals (e.g., K, P, Na, Ca, Mg) while being low in fat content (4–5%) and caloric value [1, 2]. They also contain a number of secondary metabolites, such as phenolic compounds, polyketides, terpenes and steroids which possess various properties beneficial for health [3–5]. Specialty mushrooms have been recognized for their anti-bacterial (e.g., *Lentinus edodes*), and anti-viral activities (e.g., *Agrocybe aegerita* and *Hypsizygus mamoreus*), immune-modulating and anti-tumor properties (e.g., *Agaricus blazei*, *Cordyceps sinensis*, *Grifola frondosa*, *Ganoderma lucidum*, and *Trametes versicolor*), and have also been documented as functional foods [6–10].

✉ Mirosław Mleczek
mirekmm@up.poznan.pl

¹ Faculty of Chemistry, Adam Mickiewicz University in Poznań, Poznań, Poland

² Department of Chemistry, Poznan University of Life Sciences, Poznań, Poland

³ Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, Poznań, Poland

⁴ Department of Environmental Medicine, Poznan University of Medical Sciences, Poznań, Poland

⁵ Department of Vegetable Crops, Poznan University of Life Sciences, Poznań, Poland

Globally, several hundred wild mushroom species are recognized as edible although only around twenty are commonly cultivated and consumed [11] and only about 10 species are produced on a commercial scale [12]. The most popular species used for dietary purposes include *Agaricus bisporus* (white button mushroom), *Pleurotus ostreatus* (oyster mushroom) and *Lentinula edodes* (Shiitake mushroom).

Mushrooms are well known to accumulate different elements, including potentially toxic metals and metalloids, and have been proposed as potential bioindicators of environmental pollution [13]. Numerous studies have addressed the chemical composition of wild species, including recently reported values of PGEs and REEs [14–22]. Cultivated specimens have usually been analyzed with respect to the content of some elements in selected mushroom species only [23–26], in general, those important to trade. As experimentally proven, mushrooms cultivated on artificially contaminated substrates can uptake and accumulate health-threatening concentrations of toxic elements such as cadmium, lead [27, 28], mercury [28], silver [29] or arsenic [30]. This highlights the need to perform multi-elemental investigations of cultivated mushrooms available commercially as foodstuffs (Table 1).

Nevertheless, few such “market surveys” have been reported, and those that have been performed to date were limited to the analyses of only a few elements in mushrooms collected from 1 year [23]. As far as the European market is concerned, the only multi-elemental and long-term investigations were conducted for mushroom species of the genus *Pleurotus* cultivated in Poland [46]. The study described inter-specific differences in element accumulation and concluded that levels of most toxic elements were low, apart from Pb and Nd contents, which in particular cases were of some concern [46]. *Pleurotus* mushrooms are, after *Agaricus* sp., the most widely cultivated mushroom species in Poland, with a significant share in global production. In 2015 the production of *Pleurotus* was over 20 thousand tons and *Agaricus* about 300 thousand tons. More than 80% of these mushrooms were exported to European Union countries. However, there are some less popular mushroom species which are occasionally available in trade in Poland which have never been characterized in respect to their chemical composition (Table 1).

The present study was undertaken to explore the multi-elemental composition of twelve less popular mushroom species available on the Polish market over an 8-year period (2008–2016) using an inductively coupled plasma optical emission spectrometer (ICP-OES). To the best of our knowledge, the results of this study offer, to date, the most comprehensive insight into the observed elemental contents of the studied species.

Materials and methods

Experimental material

Collected mushroom species were purchased between 2008 and 2016 from the Polish market. Some species originated from China and were distributed in some oriental or internet shops only. The fruiting bodies of three species were obtained from small scale local production in Poland. Their characteristics and the substrates used in cultivation are presented in Table 2. With the exception of *C. maxima* (from 2010 and 2014) and *L. sulphureus* (from 2011, 2013 and 2014), mushrooms were purchased in varying quantities, from relatively few to several fruiting bodies of each species growing in each of the 9 years (about 100 g of fresh or 20 g of dry matter per year). Based on these materials, 3 initial samples were prepared (each of 5 g). For a comparison of mushroom species during the 9 years, although not between particular years, initial samples from all 9 years of the experiment were summed ($45\text{ g} = 5\text{ g} \times 9\text{ years}$). In the case of mushrooms where fruit bodies were not available in all the years of the research, the missing initial samples were prepared based on the rest of the collected bodies. In this way we obtained 36 analytical samples ($12\text{ species} \times 3\text{ initial samples}$).

Preparation of fruit bodies for element analysis

All collected fruit bodies were dried at $50 \pm 2\text{ }^\circ\text{C}$ for 30 h and at $80 \pm 1\text{ }^\circ\text{C}$ for 12 h in an electric oven (SLW 53 STD, Pol-Eko, Wodzisław Śląski, Poland) to dry matter. The 3 initial samples were composed of 5 g of particular mushroom species collected in each year with the above mentioned exceptions. All materials were ground with a laboratory Cutting Mill SM 200 (Retsch GmbH, Haan, Germany) to a powder fraction. Each of the three initial samples collected in all the years was summed to one sample (45 g) and homogenized using a B-400 homogenizator (Buchi Labortechnik AG, Switzerland). This allowed 3 analytical samples per one mushroom species to be obtained (36 samples total).

Accurately weighed $0.3000 \pm 0.0001\text{ g}$ of a dry sample of each mushroom species was ultrasonically extracted using phosphoric acid of 1 mol L^{-1} (Honeywell, USA). After extraction all samples were filtered and diluted with ultrapure water to a final volume of 15.0 mL. Each of the samples was analyzed in triplicate using the whole sample preparation procedure.

Analytical methods

Element determination was conducted using the inductively coupled plasma with optical emission detection model

Table 1 The summary of literature data on elements content (mg kg⁻¹ dm) reported for some mushroom species

Element	<i>Agrocybe cylindracea</i>	<i>Clitocybe maxima</i>	<i>Coprinus comatus</i>	<i>Auricularia polytricha</i>	<i>Flammulina velutipes</i>	<i>Hericium erinaceus</i>	<i>Laetiporus sulphureus</i>	<i>Pholiota nameko</i>	<i>Stropharia rugoso-annulata</i>	<i>Trametes versicolor</i>
Ca	45.7	962	1150	590–6070	1175.5	174	1500–2170	3.1–226	1371	
K	84.8	26,430		5884–12,432	28,976	28,803		37,352	16,320	
Mg	18.8	520	1610	593–1360	1430.4	948	2200–3800	1572–1672	1135	
Na	5.9	1692		449–8584	754.5		800		411	
P		5390		944–1118	9403.3	7619	2100–3300	10,888	7290	0.28
Ag	0.074–0.41		3.85				0.26–4.17			
Al			31.2				5.8–8.3			
As	0.44–1.4	0.05	1.0		0.035–0.194			0.195–1.18		
Be							0.082–0.249			
Ba	27					27				
Cd	0.011–27.8	0.29	0.08–15.3	6.1–8.1	0.005–0.838	0.29	0.68	0.013–0.938	1.17	0.21
Ce	9	11				6				
Co	3	3	0.08–3.61			3	2.7–53.9			
Cr	6–75.6	7	2.0–15.17			7–22.6	58.3			82.7
Cs	5					5				
Cu	24–54	52–93	7.88–137	3.0–6.2		4.0–8.75	22.7–300	5.6	29	326
F	84.5									
Fe	19.6	179	308	110–163	94–160	89–225		70.21–182	80–130	37.6–476
Hg	0.34		2.6–55.2		0.087–96.3			0.031–0.125		
Ga	1	1				1				
Li					<0.2					
Mn	20–39	33	11.1–68.4	6.2–13.0	9.6	15.2	30.7–160	10.1	59	90.7
Mo							0.33–0.67			
Nb	5	5				4				
Ni	2.08–39	3	0.92–11.6			2.0–2.62	5.82–21.7			31
Nd	4	3				1				
Pb	0.018–8.2	1.44–4.0	2.21–30.5	4.9–5.5	0.025–9.17	3.04–39.2	24.5		0.07	0.91
Rb	35	21				18				
Sc							1.67			
Se	14.4		20.5–36.4		<0.5					
Si							80–230			
Sn							0.415–0.5			
Sr	15	15				15				

Table 1 continued

Element	<i>Agrocybe cylindracea</i>	<i>Clitocybe maxima</i>	<i>Coprinus comatus</i>	<i>Auricularia polytricha</i>	<i>Flammulina velutipes</i>	<i>Hericium erinaceus</i>	<i>Laetiporus sulphureus</i>	<i>Pholiota nameko</i>	<i>Stropharia rugoso-annulata</i>	<i>Trametes versicolor</i>
Th	2	2				2				
V	2	15	<0.5			3				
U	1	4				1				
Zn	0.82–89	102–127	4.85–102	10.0–16.7	42.9–91.3	15–121	8.3–16.3	50–75.3	102	
Zr	2	2				1	0.91–4.18			
Literature data	[17, 28, 29]	[31, 34]	[17, 32–37]	[44, 45]	[38–40]	[33, 34, 41]	[17, 42]	[23, 41, 43]	[31]	[17]

[17]—Doğan et al. (2006); [23]—Huang et al. (2015); [31]—Liu et al. (2012); [32]—Cocchi et al. (2006); [33]—Zhu et al. (2011); [34]—Campos et al. (2012); [35]—Michelot et al. (1998); [36]—[36]; [37]—Severoglu et al. (2013); [38]—Kim et al. (2012); [39]—Smiderle et al. (2008a); [40]—Smiderle et al. (2008b); [41]—Rodrigues et al. (2015); [42]—Agafonova et al. (2007); [43]—Florczak et al. (2009); [44]—Manjunathan et al. (2011); [45]—Anno et al. (2016)

5100 ICP-OES (Agilent, USA). Evaluation of the content of 67 elements was performed according to the following: for multi-elemental determination common conditions were used: Radio Frequency (RF) power 1.2 kW, nebulizer gas flow 0.7 L min⁻¹, auxiliary gas flow 1.0 L min⁻¹, plasma gas flow 12.0 L min⁻¹, viewing height for radial plasma observation 8 mm, detector Charge Coupled Device (CCD) temperature -40 °C, signal accusation time 5 s for 3 replicates. The detection limits were found at the level of 0.01 mg kg⁻¹ dry weight (DW) for all elements determined (as 3-sigma criteria). The uncertainty for the total analytical procedure (including sample preparation) was at the level of 20%. Traceability was checked using reference materials CRM S-1—loess soil; CRM NCSDC (73349)—bush branches and leaves; CRM 2709—soil; CRM 405—estuarine sediments; CRM 667—estuarine sediments and a recovery (80–120%) was acceptable for most of the elements determined. For uncertified elements recovery was defined using in the standard addition method.

Statistical analysis

Estimation of the content of selected elements (dependent variable) in fruiting bodies of mushroom species (independent variable) was carried out. The mean of element content in particular mushrooms was compared. One-way analysis ANOVA with the F-Fisher test ($\alpha = 0.05$) was used to verify the general hypothesis with respect to the equality of mean content of particular elements in the analyzed mushroom species. In case the null hypothesis was rejected, the Tukey test for multiple comparisons was applied to divide the studied mushroom species into homogenous groups ($\alpha = 0.05$) [47].

Andrew curves were applied to present differences in elemental composition among mushrooms. This data visualization allowed the selection of those species in which the content of all elements jointly was relatively lower or higher than in others [48, 49].

Principal Component Analysis (PCA) was used to illustrate the relationships between independent variables (content of elements) for the tested mushroom species. The initial population of variables (mean content of particular elements) X_1, X_2, \dots, X_p , where $p = 1, 2, \dots, 36$ was transformed into the population of principal components (Z_1, Z_2, \dots, Z_p). Correlation coefficients between particular variables and components was calculated and based on these results the relationship between the element content of the analyzed mushroom species was interpreted. Moreover, the similarities and differences in the element content of the analyzed mushroom species were determined [50].

To group the tested mushroom species according to similarities in the content of all elements jointly,

Table 2 Characteristics of compared cultivated mushroom species

Mushroom species name	Family/order	Ecology characteristics	Substrates used in cultivation	Form/origin
<i>Agrocybe cylindracea</i> (DC. ex Fr.) Maire (syn. <i>A. chaxiangui</i>)	Strophariaceae/Agaricales	Saprotrophic/parasitic ^b	Sawdust	Dried, China
<i>Auricularia polytricha</i> (Mont.) Sacc.	Auriculariaceae/Auriculariales	Saprotrophic ^a	Composted sawdust	Dried, China
<i>Clitocybe maxima</i> (Berk.) Torrend	Tricholomataceae/Agaricales	Saprotrophic ^a	Sawdust	Dried, China
<i>Coprinus comatus</i> (Müll.) Pers.	Agaricaceae/Agaricales	Saprotrophic ^c	Compost	Dried, China
<i>Flammulina velutipes</i> (Curtis) Singer	Physalacriaceae/Agaricales	Saprotrophic ^a	Sawdust	Fresh, Holland and Germany
<i>Grifola frondosa</i> (Dicks.) Gray	Meripilaceae/Polyporales	Parasitic ^a	Sawdust	Dried China
<i>Hericium erinaceus</i> (Bull.) Pers.	Hericiaceae/Russulales	Parasitic ^a	Sawdust	Dried China
<i>Laetiporus sulphureus</i> (Bull.) Murrill	Fomitopsidaceae/Polyporales	Parasitic ^a	Sawdust	Fresh, small scale Experimental production, Poland
<i>Pholiota nameko</i> (T. Itô) S. Ito & S. Imai	Strophariaceae/Agaricales	Saprotrophic ^b	Sawdust	Fresh, small scale production, Poland
<i>Stropharia rugosoannulata</i> Farl. ex Murrill	Strophariaceae/Agaricales	Saprotrophic ^c	Sawdust and wood chips	Fresh, small scale production, Poland
<i>Trametes versicolor</i> (L.) Lloyd	Polyporaceae/Polyporales	Saprotrophic ^a	Sawdust	Dried, China
<i>Tremella fuciformis</i> Berk.	Tremellaceae/Tremellales	Parasitic (of <i>Hypoxylon</i> sp.) ^a	Sawdust	Dried, China

^a Lignicolous species; ^b lignicolous but sometimes aboveground, ^c terricolous species

Hierarchical Cluster Dendrograms were applied. Homogenous groups were created by the ward.D2 agglomeration method (hclust {stats}) with Euclidean Distance.

Results

A total of 36 of 67 of analyzed elements (Ag, Al, As, B, Ba, Bi, Ca, Cd, Cu, Fe, Ga, Gd, Ge, Ho, In, K, La, Mg, Mn, Mo, Na, Nd, Ni, Os, P, Pb, Pr, Pt, Rh, Sr, Te, Ti, U, V, Zn and Zr) were identified above the limit of detection for each investigated mushroom species. Therefore, the comparisons and relationships presented in the following subsections were elaborated on this group of elements.

Differences between mushroom species respecting particular element content

Characteristics of the similarities and differences between 12 cultivated mushroom species respecting the accumulation of the thirty-six elements are presented in Table 3.

Fruiting bodies of *G. frondosa* exhibited the highest content (mg kg⁻¹ dm) of Ba (2.5), Ca (2481), Cd (1.9), Fe

(214), Ge (1.3), Mg (1453), Mn (32), Mo (0.57), Ni (0.65), P (26,910), Pt (6.0), Rh (0.30), Sr (8.8) and Zn (246) but the lowest concentration of Pb (0.38). *L. sulphureus*, in turn, was characterized by the lowest levels of Cu (5.0), K (16,023), Mg (481), Mn (6.1), P (10,554), Te (0.37) and Zn (51) but the highest content of Al (23), Pb (1.8), Ti (0.39) and V (0.04). The highest content of Ag (0.40) and Os (0.20) but the lowest content of Ca (155), La (<0.01), Na (41) and Sr (317) was observed in *A. cylindracea*. The highest concentrations of B (57) and Na (463) were observed for *A. polytricha*. As (1.2) and K (40,583) in *T. fuciformis*, *A. polytricha* and *T. fuciformis* were the next species to reveal a higher content of two elements only (B (56), Na (463) and As (1.2), K (40,583), respectively) and the lowest content of four other elements (Cd (0.04), Gd (<0.01), Os (<0.01), Zr (0.01) and Mo (0.01), Na (39), Ti (0.04), V (<0.01), respectively). *C. comatus* contained the highest content of Cu (44) and Pr (1.7) and the lowest, trace amounts of As (<0.01), Bi (<0.01), Ga (0.08), Gd (<0.01) and Ho (<0.01). Similar characteristics were found for *F. velutipes* and *H. erinaceus*, where the lowest content of Ga (0.33), Ho (0.30), Te (5.8) and Bi (1.6), In (7.5) and Nd (0.45), were observed in these two, respectively. It is worth underlining that these species were simultaneously

Table 3 continued

	Pt	Rh	Sr	Te	Ti	U	V	Zn	Zr
F	4.170	6.551	13.282	7.793	3.382	3.946	2.437	5.830	13.201
<i>p</i> value	<i>p</i> ≤ 0.05	<i>p</i> ≤ 0.05	<i>p</i> ≤ 0.05	<i>p</i> ≤ 0.05	<i>p</i> ≤ 0.05	<i>p</i> ≤ 0.05	0.033	<i>p</i> ≤ 0.05	<i>p</i> ≤ 0.05
<i>A. cylindracea</i>	3.6 ^{ab} ± 0.5	0.29 ^a ± 0.10	0.32 ^c ± 0.27	1.1 ^{bcd} ± 0.3	0.11 ^{abc} ± 0.03	0.43 ^{ab} ± 0.10	0.03 ^a ± 0.01	110 ^{ab} ± 25	0.19 ^{ab} ± 0.01
<i>A. polytricha</i>	5.2 ^a ± 0.8	0.08 ^{abc} ± 0.01	1.6 ^{cd} ± 0.1	1.0 ^{bcd} ± 0.1	0.08 ^{abc} ± 0.01	0.34 ^{ab} ± 0.05	0.01 ^{ab} ± 0.01	95 ^{ab} ± 13	0.01 ^f ± 0.01
<i>C. maxima</i>	1.6 ^b ± 0.9	0.22 ^{ab} ± 0.08	1.6 ^{cd} ± 1.3	2.6 ^{abc} ± 1.5	0.11 ^{abc} ± 0.05	0.73 ^a ± 0.22	0.03 ^a ± 0.02	89 ^b ± 24	0.21 ^a ± 0.03
<i>C. comatus</i>	5.1 ^a ± 0.3	0.16 ^{abc} ± 0.01	1.9 ^{bcd} ± 0.1	0.58 ^d ± 0.04	0.21 ^{ab} ± 0.01	0.27 ^{ab} ± 0.02	0.02 ^a ± 0.01	93 ^{ab} ± 6	0.06 ^{de} ± 0.01
<i>F. velutipes</i>	5.2 ^a ± 0.4	<0.01 ^c	1.0 ^{cd} ± 0.1	5.8 ^a ± 0.5	0.11 ^{abc} ± 0.01	0.66 ^a ± 0.05	0.02 ^a ± 0.01	77 ^b ± 6	0.15 ^{abc} ± 0.01
<i>G. frondosa</i>	6.0 ^a ± 0.3	0.30 ^a ± 0.03	8.8 ^a ± 1.6	4.5 ^a ± 0.2	0.24 ^{ab} ± 0.12	0.67 ^a ± 0.05	0.03 ^a ± 0.01	246 ^a ± 62	0.08 ^{cde} ± 0.02
<i>H. erinaceus</i>	4.0 ^{ab} ± 1.0	0.07 ^{bc} ± 0.05	0.75 ^{cde} ± 0.08	0.51 ^d ± 0.32	0.11 ^{abc} ± 0.01	0.20 ^b ± 0.15	<0.01 ^b	78 ^b ± 37	0.05 ^{de} ± 0.01
<i>L. sulphureus</i>	3.2 ^{ab} ± 0.7	0.11 ^{ab} ± 0.07	1.6 ^{cd} ± 0.3	0.37 ^d ± 0.05	0.39 ^a ± 0.20	0.28 ^{ab} ± 0.04	0.04 ^a ± 0.02	51 ^b ± 36	0.07 ^{cde} ± 0.01
<i>P. nameko</i>	4.8 ^{ab} ± 1.5	0.17 ^{ab} ± 0.06	1.0 ^{cd} ± 0.1	3.8 ^{ab} ± 2.7	0.10 ^{abc} ± 0.04	0.50 ^{ab} ± 0.22	0.03 ^a ± 0.02	100 ^{ab} ± 21	0.12 ^{abcd} ± 0.06
<i>S. rugosoannulata</i>	5.1 ^a ± 0.6	<0.01 ^c	0.49 ^{de} ± 0.05	0.76 ^{cd} ± 0.08	0.05 ^{bc} ± 0.01	0.35 ^{ab} ± 0.04	<0.01 ^b	67 ^b ± 7	0.03 ^{ef} ± 0.01
<i>T. versicolor</i>	3.9 ^{ab} ± 1.4	0.28 ^a ± 0.13	7.4 ^{ab} ± 2.4	3.5 ^{ab} ± 1.1	0.12 ^{abc} ± 0.07	0.53 ^{ab} ± 0.15	0.02 ^a ± 0.01	123 ^{ab} ± 30	0.11 ^{bcd} ± 0.04
<i>T. fuciformis</i>	3.6 ^{ab} ± 0.6	0.28 ^a ± 0.03	3.3 ^{abc} ± 1.7	0.43 ^d ± 0.10	0.04 ^c ± 0.01	0.29 ^{ab} ± 0.19	<0.01 ^b	111 ^{ab} ± 24	0.06 ^{de} ± 0.01

Mean value (*n* = 36) ± SD; F-statistic, different letters (a, b, c....) points at significant differences between content of element in column at *p* ≤ 0.05 (Tukey’s HSD test)

characterized by a lower content of Ag (0.04), Ge (0.06), Mo (<0.01), Nd (0.11), Ni (<0.01), Rh (<0.01) and Al (2.9), Bi (1.6), P (10,277), Pr (0.02), U (0.204) and V (<0.01), respectively. The lowest diversity in the analyzed element contents was found in *P. nameko* and *T. versicolor*. The highest content of Gd (0.05) and La (0.13) with the lowest content of Mo (<0.01) and As (<0.01), was recorded in both these mushroom species. *S. rugosoannulata* revealed the lowest contents of B (<0.01), Ba (0.48), Gd (<0.01), In (1.5), Os (<0.01), Rh (<0.01) and V (<0.01). Additionally, it was characterized by a medium content of the remaining of the elements.

Principal Component Analysis explained 27.07% + 15.42% = 42.49% of total variability meaning that 36 elements for 12 mushroom species were well transformed into a 2 dimensional system for graphical presentation of the obtained results.

Some of the presented relationships indicate a generally higher content of the analyzed elements in *G. frondosa*, *F. velutipes* and *C. comatus* (Fig. 1a). Most of the variability (nearly 100%) for *G. frondosa* was explained with the first principal component. In the case of *F. velutipes* and *C. comatus*, almost the whole variability was explained by the second variable component.

T. fuciformis was localized in the center of the coordinate system thereby indicating that elemental content of this species was more similar to the multidimensional mean value than any other mushroom species investigated in the present study (Fig. 1a). It is difficult to show that such a localization of *T. fuciformis* among the tested mushroom species will be the same each time. To confirm these relationships, it will be necessary to conduct further studies because when considering the effective transport of 36

elements it is essential not to ignore the important role of the interactions between elements and, in particular, their influence on the nutritional elements in mushroom bodies.

Other mushroom species were placed above the ring, which suggests a generally higher or lower content of all elements jointly than in other groups of similar species present inside the ellipsoid (Fig. 1b). Differences between the three groups of elements marked with green rings were indicated. According to data presented in Fig. 1b, the content of Pb was adverse to B, Pt, Pr, Mo, Sr, Ca, Cd, Zn, Mg, Mn, Fe and P content.

Simultaneously, content of Zr, Os, Bi, As, Ga, Te, U and Gd in mushrooms was adverse to Ag, Nd, Al, Ti, Cu and Ni content. It is worth noting that content of Zr, Os, Bi, As, Ga and Te was independent of B, Pt, Pr, Mo, Sr, Ca, Cd, Zn, Mg, Mn, Fe, P and U content. The relationships between element contents described in our study are probably an effect of their differences in accumulation and toxicity. Pb is a heavy metal usually characterized by limited transport, whereas especially numerous elements contained within the group placed on the right side are more mobile and in some cases, such as nutritional elements, essential for the proper functioning of mushrooms. Unfortunately, we have no data on the detailed characteristics of the substrate used in production, thus another possible cause of such relationships could also be the concentration of Pb in substrates and their pH and conductivity.

Differences between mushroom species respecting all elements jointly

To compare the analyzed mushroom species with respect to the content of all 36 elements jointly, a cluster analysis was

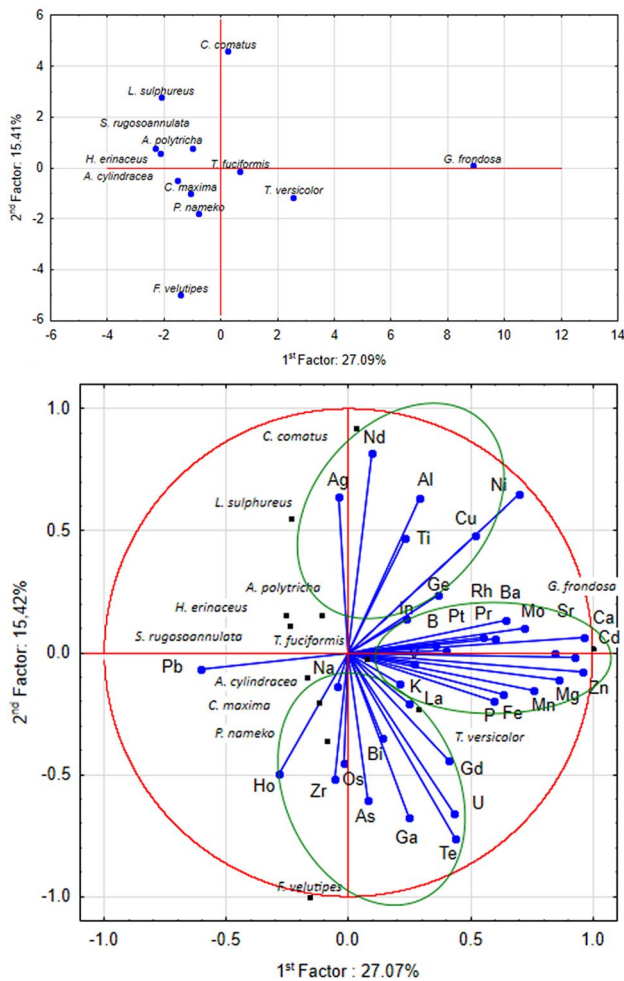
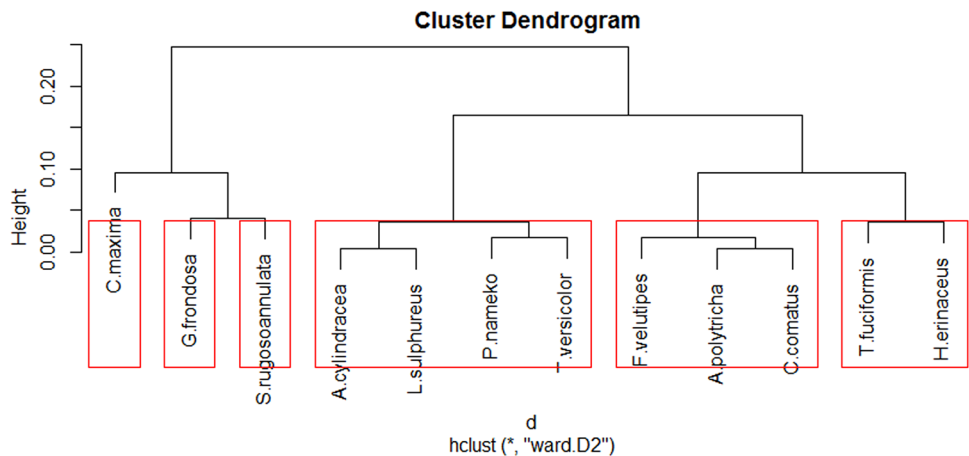


Fig. 1 Principal component analysis for studied elements with regard to the coordinate factor for the cases (mushroom species only—a) and coordinate factor variables (element contents and mushroom species—b)

Fig. 2 Hierarchical cluster analysis on a set of the Euclidean distance measure and method ward.D2 clustering criterion for studied mushroom species



applied which allowed 6 groups of mushrooms to be distinguished (Fig. 2). In the first three groups, only *C. maxima*, *G. frondosa* and *S. rugosoannulata*, were present.

The two latter mushroom species were more similar to each other than *C. maxima*. In the fourth group, the following species were included: *A. cylindracea*, *L. sulphureus*, *P. nameko* and *T. versicolor*. Within this group differences were found in element contents between the first two and the other mushroom species. In the fifth group, *A. polytricha* and *C. comatus* were found to be similar while some differences were noted respecting *F. velutipes*. *T. fuciformis* and *H. erinaceus* are included in the sixth and last group.

The differences between the investigated species were also demonstrated using Andrews curves calculated for all the analyzed fruiting bodies collected from each species (Fig. 3).

As illustrated, the curve drawn for *T. fuciformis* (light blue) had the highest amplitude, indicating that the elemental composition of this species differed from other mushrooms. This was also confirmed by plotting Andrews curves for 3 randomly selected observations (elements) for each species—a similar amplitude of curves was observed (Fig. 3b).

Figure 3a presents the mean values calculated for all analyzed fruiting bodies for particular mushroom species. *T. fuciformis*, the mushroom species located in the center of PCA ring (Fig. 1a), in this case is presented as a blue curve with the highest amplitude. This suggests that this species differs from the others in terms of elemental content. It is interesting to note that for 3 randomly selected observations for particular mushroom species (Fig. 3b), similar tendencies (amplitude of curves) were observed thereby confirming the observations presented in Table 3.

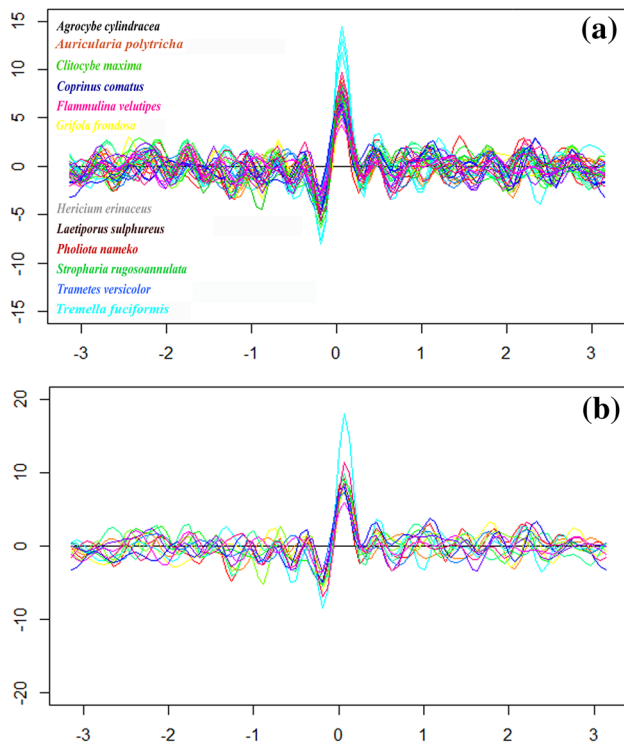


Fig. 3 Andrews curves showing the transformation of multidimensional data into curves calculated for mean content of elements in all mushroom fruit bodies (a) and for 3 randomly selected fruit bodies of particular mushroom species (b)

Discussion

The present study demonstrates the large set of data of multi-elemental compositions of mushroom species that are available on the Polish market. The studied species are less popular than *Pleurotus ostreatus*, *Agaricus bisporus* or *Lentinula edodes* but their popularity, distribution and sale may potentially rise in the near future. This can be expected due to the growing interest in the use of mushrooms in medicinal nutrition therapies and diet [51]. This considered, it is of great importance to screen and compare the chemical composition of mushrooms. However, in the literature, there is a great deal of data from investigations that tend to focus on wild-growing species, while the majority of cultivated specimens are usually studied for the content of some elements in selected mushroom species [23–26]. Contrary to the majority of studies, the present research demonstrates the results of 36 elements in 12 cultivated species whose fruiting bodies were collected over 8 years, and provides some informational values on content for their nutritional value and contamination levels.

As shown using different statistical approaches, the investigated species differ in the content of certain elements including minerals, and potentially toxic metals and metalloids. Particularly useful visualization of multidimensional

data was acquired by plotting Andrews curves in which outliers appear as a single curve that differs from the rest in its amplitude. Although such an approach to the illustration of data has been successfully applied in biomedical and pharmacological sciences [52, 53], we are unaware of any similar application in food chemistry. If one considers that chemical screenings often require the collection of large data sets, Andrews curves may be helpful in their further interpretation, and selection of those items which clearly differ from others.

The cluster analysis revealed that there is no particular pattern as regards elemental composition related to taxonomic position (family) of the studied mushrooms or their ecology (saprotrophic or parasitic). Similarly, the PCA analysis did not demonstrate any existence of such a pattern. Therefore, the observed differences can be attributed to: (1) different composition of substrate used for their cultivation, and/or (2) inter-specific differences in mycelia uptake [54].

Compared to other species, the fruiting bodies *G. frondosa* were revealed to be the greater source of Ca, Cu, Fe, Mg, Mn, P or Zn [13, 55, 56] and lower in the case of, e.g., sum of REEs [22]. All of these elements play a pivotal role in various physiological processes while their deficiencies have been implicated in the etiology of number of disorders [57]. Fe and Zn deficiency in particular appears to be a global health issue, affecting the populations of both developed and undeveloped countries [58]. Our finding highlights the nutritional value of *G. frondosa* which was previously shown to contain high concentrations of starch and total dietary fiber [59]. Although the bioavailability of Ca, Cu, Fe, Mg, Mn, P and Zn in *G. frondosa* remains to be studied more fully, this species would appear to be a promising candidate for the dietary source of different minerals in the human diet.

There is an increasing interest in the content of REEs in different environmental, biological and food samples [60]. Over the last decade, there has been a significant increase in their use, mostly in the industrial sector, and their subsequent release to the environment is being observed. Despite this, toxicological investigations on REE-related adverse effects to human health are still scarce, and not much is known as to their presence in mushrooms. Recently, some studies have reported the presence of REEs in wild-edible species [22], and our previous studies have described their content in cultivated mushrooms from the *Pleurotus* genus [46]. In the present study, each sample contained detectable levels of five REEs whose mean content generally decreased in the following order: Pr > Nd > Ho > La > Gd. Particularly high Pr content, exceeding 1 mg kg⁻¹ was found in *C. comatus*, *T. fuciformis*, *F. velutipes*, *G. frondosa*, *L. sulphureus* and *T. versicolor*. So far the only existing

regulation, implemented in China, sets a maximum content of total REEs in food at 0.7 mg kg^{-1} fresh weight [61], which accounts for 7 mg kg^{-1} dw (if one considers the usual level of 10% water content). This maximum was not exceeded by any studied species in the present study. Nevertheless, the present study draws attention to the fact that in any further legislation concerning this elemental group mushrooms should be considered as a potentially important dietary source of REEs, particularly Pr and Nd.

The present study also identified some PGEs in cultivated mushrooms; namely Pt, Rh and Os. It has been shown that environmental concentrations of PGEs are steadily increasing due to their use in automobile catalytic converters. Chronic, sub-clinical exposure to these elements may pose a health risk, particularly in vulnerable groups such as children. It has been suggested that nearly half of human PGE exposure occurs through diet [62, 63]. In spite of this, information respecting PGEs in different foodstuffs, including mushrooms, is scarce. Our previous screening of 20 wild-edible species (including *G. frondosa* and *F. velutipes*, whose specimen from cultivation were tested in the present study) revealed generally low content of PGEs which for Pt, Rh and Os was in range of $0.01\text{--}0.03 \text{ mg kg}^{-1}$ dw [22]. The present study observed levels higher by orders of magnitude but in line with our previous findings in different cultivated mushrooms from the *Pleurotus* genus [46]. The general mean content of PGEs in the present study decreased in the following order: Pt > Rh > Os. Particularly high Pt concentrations (exceeding 5 mg kg^{-1} dw) were found for *G. frondosa*, *C. comatus*, *F. velutipes* and *S. rugosoannulata*. All in all, this highlights that cultivated mushrooms may be an important dietary source of PGEs; the reasons behind it are yet to be elucidated. As previously shown, the increased concentrations of various pollutants in fruiting bodies of cultivated mushrooms can be associated by their increased content in overgrown substrate [23]. Therefore, it would be highly advisable to screen PGE content in cultivation substrates as their origin may be distinctively different. Typical substrates which are used include wheat straw, gypsum, oak and beech sawdust, or chicken manure [64, 65]. All of the above can potentially be contaminated, particularly if collected from polluted areas.

As previously demonstrated experimentally, As presence in substrate can result in high levels of accumulation of this toxic metalloid in cultivated mushrooms. This strongly advocates the necessity to control As levels in different species which are available in trade to ensure their safety for human health. The present study found distinctively different mean As levels for the investigated mushrooms species ranging from 0.001 mg kg^{-1} dm (*C. comatus*, *H. erinaceus* and *T. versicolor*) to over 1.0 mg kg^{-1} dm (*F. velutipes* and *T. fuciformis*). Similar

or higher levels of As have been found in commercial mushrooms from cultivation [23, 46], as well as other food products and dietary supplements [66]. As toxicity largely depends on its form (with inorganic compounds being more toxic than organic) and the provisional tolerable weekly intake (PTWI) of total As was set at $15 \text{ } \mu\text{g kg}^{-1}$ body weight (later withdrawn with no new PTWI set) [67, 68].

Maximum allowance levels of Cd and Pb in mushrooms (set only for *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*) set by the European Commission are 0.2 and 0.3 mg kg^{-1} dm, respectively which accounts for 2.0 and 3.0 mg kg^{-1} dw, respectively, if one considers the usual level of 10% water content. Mean concentrations obtained for the mushrooms investigated in the present study did not exceed this. If one will further consider that the bioaccessibility of toxic elements from the investigated mushrooms in the human gastrointestinal tract can be reduced by cooking treatments such as boiling or microwaving with water [69], consumption of the investigated species would not contribute significantly to Cd or Pb exposure.

Finally, some mushrooms examined in the present study contained relatively high Al content, exceeding 20 mg kg^{-1} dm for *L. sulphureus*. It should, however, be stressed that Al, which is the most ubiquitous metal in the earth's crust and has no known biological function, is poorly absorbed in the gastrointestinal tract (in the range of a 0.1–1.0% oral dose), and in healthy subjects almost all the absorbed Al is excreted readily from the body [70]. The provisional tolerable weekly intake (PTWI) for Al is 2 mg kg^{-1} bodyweight [71]. Considering an average single serving is 300 g of fresh mushrooms, i.e., about 30 g of dry matter (as a 10% level is used for calculations with an unknown factual dm level), the content determined in the investigated mushrooms would contribute insignificantly to PTWI for an adult weighing 60 kg.

Conclusions

The present study screened the elemental content of fruiting bodies of 12 mushroom species. The studied mushrooms revealed differences in chemical content as shown using different statistical tools, including Andrews curves which appear to be a convenient method for illustrating multidimensional data on food composition. As revealed, *G. frondosa* appeared to be a highly nutritional mushroom with an exceptional Ca content. Relatively high levels of PGEs were found in the studied species. Further studies are yet to be conducted to elucidate the strikingly high content of PGEs in some of the mushrooms.

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Compliance with ethical standards

Conflict of interest Przemysław Niedzielski declares that he has no conflict of interest. Mirosław Mleczek declares that he has no conflict of interest. Anna Budka declares that he has no conflict of interest. Piotr Rzymiski declares that he has no conflict of interest. Marek Siwulski declares that he has no conflict of interest. Agnieszka Jasińska declares that he has no conflict of interest. Monika Gąsecka declares that she has no conflict of interest. Sylwia Budzyńska declares that she has 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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