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Comparative Study of Metals Accumulation in Cultured In Vitro Mycelium and Naturally Grown Fruiting Bodies of *Boletus badius* and *Cantharellus cibarius*

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Abstract *Cantharellus cibarius* Fr. (chanterelle) and *Boletus badius* Pers. (bay bolete) harvested from natural sites in Poland were used to derive in vitro cultures. The optimal medium composition for cultures was developed. Concentrations of the chosen elements (Zn, Cu, Fe, Mg, Ni, and Cd) in mycelium samples were measured by means of atomic absorption spectrometry. Fe concentration in the analyzed mushroom materials was in the range 215.4–680.3 μg/g dry weight. Mean values of Mg were respectively (in micrograms per gram dry weight) 541.8 for mycelium of *C. cibarius* cultured in vitro and 1,004.1 for *C. cibarius* fruiting bodies and 928.9 for the mycelium of *B. badius* cultured in vitro and 906.4 for *B. badius* fruiting bodies. The mean concentrations of Zn were 442.7 μg/g dry weight in mycelium from in vitro cultures of *B. badius* and 172.1 in *B. badius* fruiting bodies and 131.9 in the case of *C. cibarius* in mycelium from in vitro cultures and 95.5 for the *C. cibarius* fruiting bodies. Cu exhibited a reversal tendency, i.e., the element concentrations in naturally grown mushrooms were significantly higher (43.57 μg/g dry weight for *C. cibarius* and 43.54 μg/g for *B. badius*) than in cultured in vitro mycelium (12.47 μg/g for *C. cibarius* and 4.17 μg/g for *B. badius*). Ni was found in lowest concentrations ranging from 0.33 to 1.88 μg/g dry weight. Toxic metal Cd was found in relatively high concentrations in naturally grown species (0.79 μg/g dry weight—1.02). The lowest was the concentration of Cd in *C. cibarius* mycelium from in vitro culture—0.06 μg/g dry weight—a bit higher than it was in the *B. badius* mycelium (0.21 μg/g).

Keywords AAS · Elements · Edible mushrooms · In vitro cultures · Dietary supplements

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Introduction

According to the Food and Agriculture Organization of the United Nations, 1,350 species are considered as edible mushrooms. In numerous countries, picking wild grown mushrooms is a habitual activity [1]. Biologically and therapeutically active mushroom metabolites are used in therapy of serious diseases, like for example cancer, arteriosclerosis, diabetes, and blood circulation disorders [2, 3]. In conventional medicine, mushroom polysaccharides in oncological treatment are used for a long time; therefore, this group of metabolites is best known [4, 5]. Other well-recognized groups of metabolites are: phenolic and indolic compounds, terpenes, vitamins, and elements, among the others selenium. This element was found in over 200 mushroom species, including Boletaceae family (*Boletus edulis*—20 μg/g dry weight (DW) [6]. It is a characteristic feature that mycelium uptakes from the environmental mineral compounds and accumulates them in various mushroom parts. Usually, element concentration in caps is higher than in other parts of fruiting bodies. Data concerning Hg accumulation in fruiting bodies of Basidiomycota come from 1973 [7]. Other

Table 1 Optimized analytical conditions of graphite furnace operation

Stage/element	Ni	Cd
Pretreatment temperature (°C)	1,400	600
Atomization temperature (°C)	2,500	1,600

element (K, Na, Mg, Ca, Fe, Zn, Mn, Cu, Pb, Cd, Hg, and Ag) accumulations in edible mushrooms from North Poland were also reported [8–12].

It should be remembered that edible mushrooms constitute an increasing fraction of our diet; for example, in the Czech Republic, statistically each person consumes over 10 kg of fresh mushrooms per year [1].

The chosen mushroom species for the experiments were *Boletus badius* (bay bolete) and *Cantharellus cibarius* (chantarelle) which contain antioxidant substances, i.e., phenols, flavonoids, terpenes (sesquiterpenes and triterpenes), sterols, ascorbic acid, ergothioneine, carotenoids, and bioelements [13, 14]; they also contain metabolites having curative (polysaccharides, phenolic compounds, statins, and antibiotics) and dietetic (balanced proteins, unsaturated fatty acids, vitamins soluble in water and in fats) actions, characteristic for Basidiomycota [13]. Moreover, each of the analyzed species contains certain characteristic of metabolites having beneficial salubrious properties. *C. cibarius* contains significant amount of β-carotene (13.56 μg/g DW) and ergocalciferol (D_2) [15, 16]. It was shown by Turkish scientists that *B. Badius* exhibits excellent antioxidant properties. During the linolic acid oxidation test, they have proven that the inhibition rate of methanol extracts (with a concentration of 100 μg/mL) reached 99.2 % [17].

Due to large capacity of mycelium to absorb metals and the possibility of screening in laboratory, in vitro cultures of the selected edible mushrooms *C. cibarius*, *B. badius* constitute a useful model for studying metabolism and accumulation of microelements. The aim of the study was the derivation of in vitro cultures from the fruiting bodies of these species and then, for the first time, analysis of the

Table 2 Concentration of micronutrients in in vitro cultures and in fruiting bodies of *C. cibarius* and *B. badius*

Sample	Zn (μg/g dry weight)	Cu (μg/g dry weight)	Fe (μg/g dry weight)	Mg (μg/g dry weight)	Ni (μg/g dry weight)
ccm 1	134.30±7.21*	24.56±0.40	694.5±11.30*	541.5±5.34	0.31±0.05
ccm 2	139.40±1.13	20.02±0.66	650.7±6.10	608.5±1.08	0.15±0.03
ccm 3	112.65±0.07**	8.00±0.08**	414.0±0.61**	453.0±4.17	0.10±0.01
ccm 4	126.25 ±1.06	3.72±0.28**	395.0±16.22*	440.5±1.67	0.67±0.02
ccm 5	169.05±0.49*	5.16±0.16**	296.6±0.41**	474.5±3.91	0.64±0.02**
ccm 6	109.95±0.64*	13.38±0.17*	296.6±0.50**	733.0±2.56*	0.15±0.04
cco 7	106.05±2.76**	46.58±0.61	72.9±58.30	1,055.0±5.34	2.28±0.06
cco 8	103.75±3.32*	45.54±0.86	587.3±33.13	1,050.0±3.92	2.09±0.01*
cco 9	92.16±1.86*	40.67±1.53	404.3±8.30	971.0±2.19	1.72±0.08
cco 10	82.34±0.61*	40.70±0.49	303.5±7.61	993.5±5.33	1.98±0.06*
cco 11	102.32±10.73	48.78±0.13	307.1±46.00	1,060.0±2.32	1.61±0.06
cco 12	86.11±0.53	39.17±3.39*	438.9±57.82	895.0±2.24	1.61±0.05
bbm 13	711.85±37.69*	3.53±0.02*	423.4±9.11	947.0±3.95**	0.19±0.02*
bbm 14	254.25±12.01*	5.53±0.00*	672.0±44.30	840.0±5.34**	1.26±0.09
bbm 15	468.95±12.94*	4.17±0.32*	1,486.0±12.35*	1,013.0±2.31*	1.06±0.07
bbm 16	555.40±22.34*	2.47±0.00	386.4±6.21	1,085.0±6.82**	0.09±0.02*
bbm 17	277.60±5.66	5.08±0.13*	657.6±7.41	842.0±11.90	0.95±0.05
bbm 18	388.15±53.09	4.24±1.65	535.5±35.90	846.5±6.64	0.19±0.03
bbo 19	219.60±8.63	59.91±1.58**	457.1±3.22**	994.0±9.02	0.39±0.03
bbo 20	193.05±2.47*	35.15±0.69	136.0±0.50	925.5±8.86*	0.22±0.03
bbo 21	137.60±3.61**	40.80±0.08**	215.4±6.60**	795.5±3.04*	0.33±0.03
bbo 22	166.05±5.57	51.94±1.63**	245.9±15.60**	1,005.0±8.24*	0.50±0.04
bbo 23	150.50±9.76*	38.56±0.19*	253.6±11.11**	906.0±6.41	0.10±0.01
bbo 24	165.70±7.92*	34.91±0.03	232.5±1.11**	812.5±7.80*	0.57±0.02*

Data were presented as the mean±SD; n=4 repetitions

ccm C. cibarius mycelium from in vitro cultures, *cco C. cibarius* fruiting bodies, *bbm B. badius* mycelium from in vitro cultures, *bbo B. badius* fruiting bodies

*p<0.05, **p<0.01 by Statistica 10 (StatSoft, Poland)

content of physiologically active microelements, cupper, iron, magnesium, zinc, and nickel, in mycelia from in vitro cultures using atomic absorption spectrometry (AAS) and evaluation of the accumulation potential in respect to the elements of interest in order to enrich the mycelium in elements. It should be stressed that nutritive sources in the form of diet supplements of those metals are still scarce and in request because of their antidepressant, antiphlogistic, and regenerative activity [18–20].

The same element concentrations were also determined in fruiting bodies of *B. badius* and *C. cibarius* to compare accumulation efficiency of mycelium from in vitro culture with the fruiting bodies of naturally grown mushrooms. The aim of the presented research was also the analysis of accumulation of a toxic microelement, cadmium, in naturally grown fruiting bodies and in mycelium from in vitro culture of *B. badius* and *C. cibarius*. This research was made for the evaluation of safety of mycelium from in vitro culture as potential dietary supplement in the future.

Materials and Methods

Material for Analysis

The studies were conducted on young fruiting bodies of *C. cibarius* Fr. (chanterelle) and *B. badius* Pers. (bay bolete) harvested from a natural site in mixed and coniferous forest in southern Poland (Brodła, near Cracow) in autumn 2009–2011. After taxonomic identification according to Knudsen and Vesterholt [21] and online keys (http://www.mycokey.com/) (representative samples of mushrooms were deposited in the Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Cracow, Poland), fresh mushrooms (50 g of each species) and medium for culture in vitro (in liter) were frozen and immediately dried by lyophilization (lyophilizer Freezone 4.5, Labconco, Germany; temperature, −40 °C) for quantitative metal analysis. The soil from the place where the mushrooms are growing (coniferous forest in Brodła) also was used for analysis. Some of the young sporocarps of mushrooms were used to derive culture in vitro from which mycelium formed material for further analysis was obtained.

Initial Cultures

The pieces of mushroom fruiting bodies were defatted with 70 % ethyl alcohol for 15 s then sterilized in 15 % Domestos solution for 5 min (HClO content 15 %, manufactured by Unilever, Hungary). After being rinsed several times with sterile redistilled water, mycelium fragments were transferred to Petri dishes containing agar-solidified medium with composition according to Oddoux [22]. After growing on solid medium, the pieces of mycelium were placed in an Erlenmeyer flask (500 mL) containing 250 mL of liquid Oddoux medium and the initial biomass amounted to 0.1 g. The cultures were shaken at a rate of 140 rpm (shaker Altel, Poland). Cultures were incubated at the temperature 25±2 °C. The agitated liquid cultures of *B. badius* and *C. cibarius* were maintained for 2 weeks and after this time subcultured.

Optimization of Culture Conditions

To find suitable conditions for the growth of mycelium, various types of solid and liquid medium were tested (Oddoux, Pachlewski, MNM).

To investigate the effect of light on mycelial growth, cells were cultivated under 600–1,200 lx in different combination of time in light or dark. The pH value of the media was adjusted to values from 3.0 up to 7.5, at 0.5 increments. Changes in pH were checked after 1 week using a digital pH meter. The effect of temperature on liquid stationary cultures

Table 3 Concentration of cadmium in in vitro cultures and in fruiting bodies of *C. cibarius* and *B. badius*

Sample	Cd (μg/g dry weight)
ccm 1	0.04±0.005*
ccm 2	0.04±0.002
ccm 3	0.04±0.003
ccm 4	0.12±0.021
ccm 5	0.08±0.002*
ccm 6	0.04±0.002
cco 7	0.73±0.032
cco 8	0.95±0.073
cco 9	0.70±0.017
cco 10	0.822±0.043
cco 11	0.77±0.069
cco 12	0.77±0.029
bbm 13	0.14±0.004**
bbm 14	0.19±0.002**
bbm 15	0.52±0.005**
bbm 16	0.11±0.004**
bbm 17	0.18±0.005
bbm 18	0.15±0.011
bbo 19	1.53±0.041**
bbo 20	0.41±0.007
bbo 21	1.49±0.034**
bbo 22	1.31±0.101*
bbo 23	0.69±0.045
bbo 24	0.67±0.023**

Data were presented as the mean±SD; $n=4$ repetitions

ccm C. cibarius mycelium from in vitro cultures, *cco C. cibarius* fruiting bodies, *bbm B. badius* mycelium from in vitro cultures, *bbo B. badius* fruiting bodies

*$p<0.05$, **$p<0.01$ by Statistica 10 (StatSoft, Poland)

Table 4 Elements concentration rates

	Medium	Mycelium from in vitro cultures of *C. Cibarius*	Mycelium from in vitro cultures of *B. badius*
Mg:Fe	4.5	1.2	1.7
Zn:Cu	11.0	10.6	10.6
Mg:Zn	40.2	4.1	2.1
Fe:Zn	8.9	3.5	1.3

was determined in an incubator at various temperatures (15, 20, 25, 30, and 35 °C).

Experimental In Vitro Cultures

The agitated liquid cultures of *B. badius* and *C. cibarius* were maintained for 2 weeks. Cultures were incubated at the temperature 25±2 °C under 16-h light (900 lx/8 h dark). After 2 weeks, the biomass was separated from the liquid medium using a filter paper on Büchner funnel and rinsed with redistilled water. The obtained fresh biomass and fruiting bodies of *B. badius* and *C. cibarius* were frozen and immediately dried by lyophilization.

Metals Quantitative Analysis

The usefulness of atomic absorption spectrometry method in biological samples trace elemental analysis is well elaborated in literature [23, 24]. Substantial improvement refers to the sample preparation methods; especially, the use of microwave digestion is widely discussed [25].

Prior to metals quantitative analysis, the lyophilized mushrooms samples were wet digested (conc. HNO_3 and H_2O_2, Suprapur, Merck, Germany) in a microwave system (Multiwave 3000, Anton Paar, Switzerland). Concentration of the elements was determined by means of AAS using flame and electrothermal techniques. Each sample was analyzed in quadruplicate and the results presented below are the mean values.

Concentrations of Fe, Mg, and Zn were determined using flame technique (PerkinElmer AAS Model 3110 spectrometer, USA) in standard conditions. Ni and Cd were determined using electrothermal technique (PerkinElmer HGA-600 graphite furnace, USA). Determination conditions were optimized before analysis with the use of the method development program. Experimental conditions are presented in Table 1. Measurements were made using pyrocoated graphite tubes and for cadmium an electrodeless discharge lamp.

Quality of the performed analyses was tested using certified reference plant material INCT-MPH-2 (Mixed Polish Herbs). All the results were in agreement with certified values of the elements concentrations (for Fe only informational value was available).

Statistical Analysis

The statistical analyses were performed using Student's test. For each of mycelium from in vitro cultures and mushroom fruiting bodies, six samples were used for the determination of every compound and all the analyses were carried out in four repetitions. The results were expressed as mean values and standard deviation (SD). All analyses were conducted using Statistica 10 (StatSoft, Poland) and Statgraphics Centurion XVI (Poland). Statistical significance was defined at $p<0.05$.

Results

In the presented study, it was established that a good mycelial mass growth of *C. cibarius* and *B. badius* could be obtained in agitating liquid cultures and solid cultures on modified Oddoux [22] medium at 25±2 °C under 16 h photoperiod (900 lx/8 h dark). A 20-fold fresh biomass growth in cultures on solid medium and a 15-fold growth in liquid cultures were obtained within a 14-day growth cycle. Maximum growth of biomass was observed at the initial pH=6. The biomass growth in the initiated cultures averaged 8.3 g DW/1 L of medium. The obtained biomass increments and dynamics of mycelium growth did not differ from the results that were obtained for *Calocera viscosa* (Pers.: Fr.) Fr., *Sarcodon imbricatus* L., and *Tricholoma equestre* (L.: Fr.) Kumm. [16, 26] cultures studied earlier.

The mycelia from in vitro cultures and fruiting bodies of these species were used for evaluation of quantitative composition of trace elements: essential micronutrients such as cupper, iron, magnesium, zinc, and nickel and potentially harmful element cadmium.

Table 5 Elements concentrations in in vitro culture medium and soil from the place in which the fruiting bodies were collected

Element	Mg (μg/g dry weight)	Fe (μg/g dry weight)	Zn (μg/g dry weight)	Cu (μg/g dry weight)	Ni (μg/g dry weight)	Cd (μg/g dry weight)
Medium	4.42±0.05	0.98±0.03	0.11±0.01	0.02±0.002	0.01±0.002	0.001±0.0001
Soil	2,083.30±0.50	6,720.3±0.70	53.23±5.21	17.3±0.71	5.13±5.80	0.44±0.49

Data were presented as the mean±SD; $n=4$ repetitions. Significant differences was tested at $p\leq0.05$ by Statistica 10 (StatSoft, Poland)

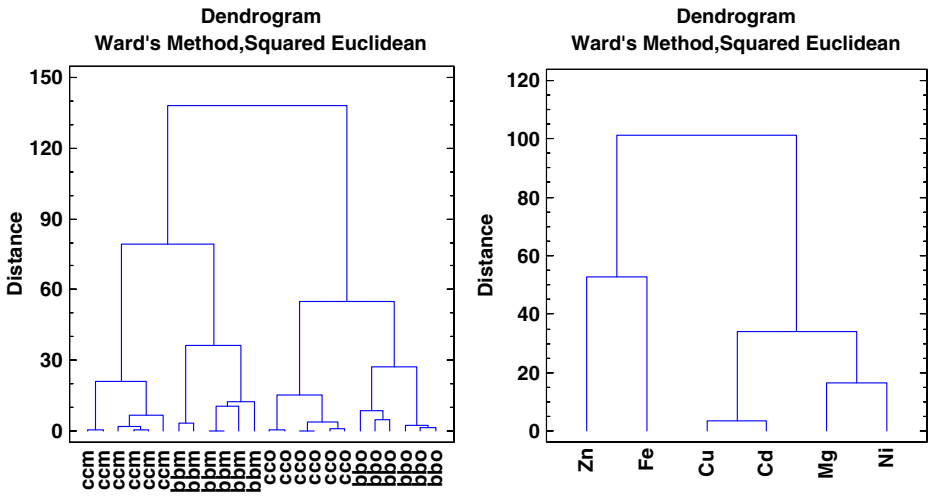

Fig. 1 Cluster analysis of the mycelium from in vitro cultures and fruiting bodies of mushrooms (the Euclidean distance square, Ward's algorithm; *bbm—B. badius* mycelium from in vitro cultures, *bbo—B. badius* fruiting bodies, *ccm—C. cibarius* mycelium from in vitro cultures, *cco—C. cibarius* fruiting bodies)

Micronutrients

Iron (Table 2) concentration in the analyzed mushroom materials was in the range 215–680.3 μg/g DW. Mean values of Fe concentration differed little between mycelium cultured in vitro (457.9 μg/g DW) and fruiting bodies (435.7 μg/g DW) of *C. cibarius*, the highest mean concentration was found in in vitro cultures (559.2 μg/g DW), and the lowest in the fruiting bodies (256.7 μg/g DW) of *B. badius*.

Magnesium (Table 2) was the element found in highest concentrations in all analyzed materials. Mean values were respectively (in micrograms per gram DW) 541.8 for the mycelium of *C. cibarius* cultured in vitro and 1,004.1 for fruiting bodies and 928.9 for the mycelium of *B. badius* cultured in vitro and 906.4 for fruiting bodies.

The distribution of zinc in mushroom tissues was quite different. Noteworthy is the fact that mycelia from in vitro cultures accumulated substantial amounts of Zn in respect to naturally grown mushrooms (fruiting bodies). The mean value of Zn concentration in mycelium from in vitro cultures of *B. badius* (442.7 μg/g DW) was 2.5 times higher than its concentration in fruiting bodies (172.1 μg/g DW). This difference was less pronounced in case of *C. cibarius* (131.9 for mycelium from in vitro cultures and 95.5 μg/g DW for fruiting bodies; Table 2).

Copper (Table 2) exhibited a reversal tendency, i.e., the element concentrations in naturally grown mushrooms (*C. cibarius*, 43.57 μg/g DW; *B. badius*, 43.54 μg/g DW) than in cultured in vitro mycelium (*C. cibarius*, 12.47 μg/g DW; *B. badius*, 4.17 μg/g DW) were significantly higher. Nickel was found in lowest amounts: 0.33 μg/g DW in the fruiting bodies of *B. badius* and 0.62 in mycelium from in vitro culture of this species and 1.88 in fruiting bodies of *C. cibarius* and 0.33 in mycelium from its in vitro culture.

Toxic Microelement

Toxic metal Cd due to obvious reasons (medium for in vitro cultures contained only trace amounts less than 5 ng/L) was found in relatively high concentrations in naturally grown species (0.79 μg/g DW of Cd in fruiting bodies of *C. cibarius* and 1.02 in fruiting bodies of *B. badius*). The lowest was concentration of Cd in *C. cibarius* mycelium from in vitro cultures—0.06 μg/g DW—a bit higher in *B. badius* mycelium (0.21 μg/g DW; Table 3).

Accumulation of the analyzed micronutrients must be referred to the concentration of elements in in vitro culture medium. These were as follows: Fe—0.98 mg/L, Mg—4.42 mg/L, Zn—0.11 mg/L, and Cu—0.01 mg/L. Determined concentrations confirmed that the elements are selectively taken up by the mycelia of mushrooms cultured in vitro. Considering concentrations ratios (Table 4), it may be concluded that the values obtained for the medium are in all instances different from those calculated for the mycelia.

The concentration of elements in medium for mushrooms culture in vitro and in soil on which fruiting bodies were collected was determined.

The highest amounts of all examined elements were found in the soil from which fruiting bodies were collected and ranged from 5.12 to 6,720 μg/g DW (Table 5). The dominant elements in this material were Fe (6,720 μg/g DW) and Mg (2,083 μg/g

Table 6 Table of component weights

	Component 1	Component 2	Component 3
Zn	0.305729	0.503537	−0.552023
Cu	−0.568130	−0.120129	−0.009330
Fe	0.205245	0.530955	0.520284
Mg	−0.365023	0.555131	−0.348414
Ni	−0.349652	0.376696	0.511837
Cd	−0.534915	−0.005941	−0.202777

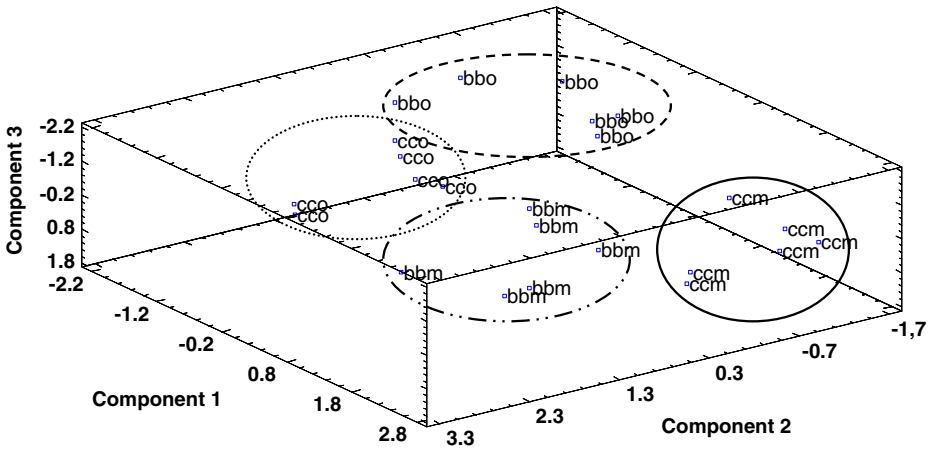

Fig. 2 Analyzed components in three-dimensional data space (*bbm*—*B. badius* mycelium from in vitro cultures, *bbo*—*B. badius* fruiting bodies, *ccm*—*C. cibarius* mycelium from in vitro cultures, *cco*—*C. cibarius* fruiting bodies)

DW). On the other hand, concentrations of elements in the soil compared to their concentrations in the medium were very low and ranged from 0.01 to 4.42 μg/g DW. The same elements found in the soil, Fe and Mg, were determined in highest amounts in the medium, but Mg was dominant (4.42 μg/g DW). The amount of Fe in this material was 0.98 μg/g DW. In case of Cd, the amount was much greater in the soil than in the medium (0.49 and 0.001 μg/g DW, respectively).

Discussion

Chemometric Analysis and Cluster Analysis

Chemometric tools facilitate the process of data analysis and interpretation. These enable extracting out of significant information about relationships between measured variables or objects. In respect to the presented results herein, using cluster analysis variations in element concentrations vs. sample type was discussed.

Cluster analysis is based on the distance between objects. It is assumed that analyzed objects are similar if their position in the multidimensional space of variables is close. As a result, the analyzed objects are classified into groups characterized by high level of similarity. In order to visualize the outcome of cluster analysis graphs, dendrograms need to be constructed (Fig. 1). In the presented dendrograms, *x* axis represents object names and *y* axis denotes the distance between them.

In Fig. 1 it can be seen that the analyzed samples can be divided into four distinct groups which directly represent four kinds of analyzed samples: *B. badius* mycelium from in vitro cultures, *B. badius* fruiting bodies, *C. cibarius* mycelium from in vitro cultures, and *C. cibarius* fruiting bodies. It means that there are meaningful variations in metals accumulation in mushrooms, and despite the fact that in vitro medium was identical for mycelium culture for both fungi genera, element uptake and accumulation profile are in fact immanent features of the species. Additionally, it is clear that there is a significant similarity between mycelium from in vitro cultures of both mushroom species than between the mycelium and fruiting bodies of the same species.

Considering analyzed elements (Fig. 1), two groups (clusters) can be distinguished—Zn and Fe and Cu, Cd, Mg, and Ni. Assuming that the elements are selectively taken up, the vital elements belonging to the first cluster are accumulated to high extent. Copper, although it is also micronutrient, is considered as Zn antagonist so uptake of this element is much less efficient. Its similarity to Cd may

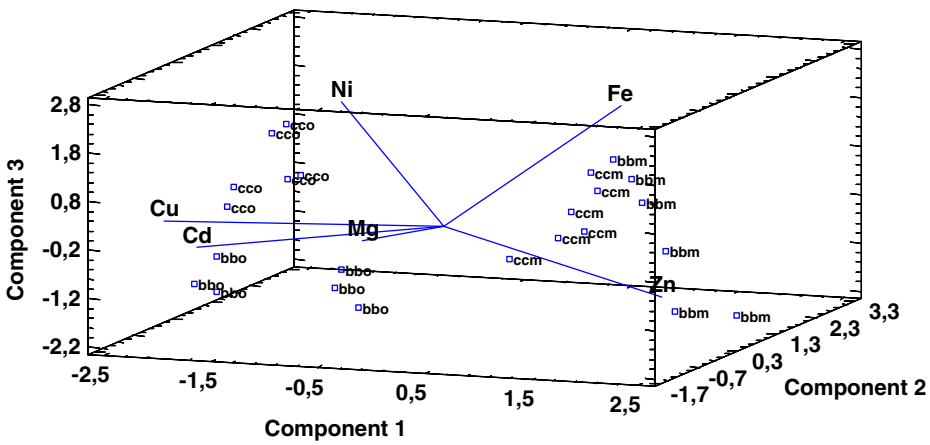

Fig. 3 Biplot graph creates a three-dimensional space (created on the basis of three main components; *bbm*—*B. badius* mycelium from in vitro cultures, *bbo*—*B. badius* fruiting bodies, *ccm*—*C. cibarius* mycelium from in vitro cultures, *cco*—*C. cibarius* fruiting bodies)

be also the result of its low concentration in the culture medium. High concentration of Mg in the analyzed samples is a result of its high concentration in the medium and important physiological function. Cd and Ni being contaminants are taken up passively; thus, higher concentrations of these elements were found in naturally grown fungi fructifications.

Planning the in vitro cultures of mushrooms mycelium, the authors expected high efficiency of Zn and Fe accumulation. In this respect, the goal was achieved what was confirmed by the results of chemometric analysis (Fig. 1).

Principal Component Analysis

Principal component analysis is a calculation method that reduces data space. This reduction is carried out by transformation of interdependent data into entirely new so-called main components. According to the principal component analysis, it was concluded that 88 % of variation of the analyzed data is described by three main components. Therefore, to carry out further research, the variability of the other components was not taken into consideration. The resulting variables C1, C2, and C3 correspond to linear combinations of the original variables. They were multiplied by the corresponding factor loads. The factor load is adequate to the level of saturation of the variable; also, it is the coefficient of correlation with the original variables. In practice, the higher is the value of the correlation coefficient, the greater is the change of the main components.

Table 6 shows the number of factor loads for three main components. It was identified which variables significantly affect the components C1, C2, and C3. The concentration of Zn, Cu, and Cd has the most significant impact on the value of the component C1. In the same way, one can connect other components C2 and C3 with the original variables, as it was done for the component C1. As a result, this procedure allows the analysis of the three-dimensional space that was created based on the main components (Fig. 2).

In vitro cultivation of *C. cibarius* and *B. badius* mycelium as well as fruiting bodies created clusters in three-dimensional space of the main components. The close location of points shows a significant similarity in features (concentration) within the analyzed groups.

Biplot diagram (Fig. 3) was made for the main component and it provided an easy and transparent way to analyze how content of each element in the cultivation is changing.

On the basis of Fig. 3, it was found that in vitro cultivation of *C. cibarius* and *B. badius* is characterized by high accumulation of such elements as Zn and Fe. This suggests that the prepared cultivation media for the fungi are rich in these elements. Fruiting bodies gained from the forest, in contrast to in vitro grown mycelia, exhibit much lower content of elements (Zn and Fe).

Generally, both fruiting bodies and mycelia cultured in vitro constitute a very good source of elements for humans. The proposed method, herein, of in vitro cultures proved that the essential micronutrients are effectively up taken and accumulated in the mycelia. The cultures of *B. badius* due to high concentrations of Fe, Mg, and Zn seem to be most advantageous. Lower accumulation of Cu was observed, probably due to competitive effect of Zn uptake. Mushrooms cultured in vitro exhibited very low level of toxic compounds. This may be achieved only in such cultures as naturally grown in contaminated environment mushrooms accumulate also certain amounts of toxic metals. The obtained results indicate that *B. badius* and *C. cibarius* in vitro cultures can be a good model for the studies on accumulation and metabolism of elements in mushrooms. Although much less concentration of the elements was found in the tested medium for mushrooms in vitro cultures than in the soil, the mycelium from cultures of both species showed greater accumulation. In summary, in vitro cultures of mushrooms create better conditions for the accumulation of the examined elements. These results demonstrate that actually examined mushroom mycelia from in vitro cultures accumulated physiologically active elements. This suggests that the next step should be to estimate the release and bioavailability of elements from in vitro cultured mycelia in in vitro and in vivo conditions.

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