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ORIGINAL ARTICLE

In vitro cultures and fruiting bodies of culinary-medicinal *Agaricus bisporus* (white button mushroom) as a source of selected biologically-active elements

Bożena Muszyńska[1] · **Agata Krakowska**[2] · **Katarzyna Sułkowska-Ziaja**[1] · **Włodzimierz Opoka**[3] · **Witold Reczyński**[2] · **Bogusław Baś**[2]

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Abstract Concentrations of the chosen elements (Cu, Zn, Mg, Fe, Cd and Ni) in fruiting bodies of *Agaricus bisporus*, in mycelium from in vitro cultures of this species, and in mycelium from in vitro cultures with the addition of L-tryptophan and Zn hydroaspartate to the medium were measured by the Atomic Absorption Spectroscopy Method (AAS). Fruiting bodies of *A. bisporus* contains a high level of Mg, its concentration ranging from 1545.00 to 2275.00 μg g^{-1} DW. The concentration of Mg in in vitro cultures was at a similar level. With the addition of Zn to the culture medium, a substantial increase of Mg accumulation was observed. Zn was present in the fruiting bodies of *A. bisporus* in the concentration range 70.39–112.75 μg g^{-1} DW. Enrichment of the culture medium with L-tryptophan increased the bioavailability of Zn by two-fold in in vitro culture. The concentration of Fe in the fruiting bodies of *A. bisporus* was in a wide range: 13.90–161.75 μg g^{-1} DW. For the substrate enrichment with L-tryptophan, Fe concentrations ranged between 118.65 and 311.65 μg g^{-1} DW. Fruiting bodies of *A. bisporus* were characterized by different levels of Cd (0.06–4.76 μg g^{-1} DW). In vitro cultures levels of Cd were recorded at a lower level than in the fruiting bodies (0.04–1.08 μg g^{-1} DW). Mycelia of *A. bisporus* cultured in vitro constitute a good source of the selected biologically-active elements. The method of in vitro cultures proposed here proved that the essential micronutrients are effectively taken up and accumulated in the mycelia.

Keywords *Agaricus bisporus* · Zn · L-Tryptophan · Atomic absorption spectroscopy

✉ Bożena Muszyńska
muchon@poczta.fm

[1] Department of Pharmaceutical Botany, Jagiellonian University Collegium Medicum, Medyczna Street 9, Kraków 30-688, Poland

[2] Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Kraków, Poland

[3] Department of Inorganic and Analytical Chemistry, Jagiellonian University Collegium Medicum, Medyczna Street 9, Kraków 30-688, Poland

Introduction

The medicinal use of mushrooms has a very long tradition (Barros et al. 2007, 2008; Dembitsky et al. 2010; Ey et al. 2007; Sułkowska-Ziaja et al. 2005). *Agaricus bisporus* (J. E. Lange) Imbach., commonly named the white button mushroom, is one of the most popular edible mushrooms collected from natural sites and from commercial cultivations. *A. bisporus* contains high levels of assimilable proteins, free amino acids, polyphenols, polysaccharides, ergothioneine, vitamins and elements (Koyalamudi et al. 2008; Markowa 2009; Roberts 2008; Zaidman et al. 2005). This species is also characterized by high levels of linoleic acid and aromatase, the enzyme catalyzing sex hormone metabolism in humans (Grube 2001). Thus, *Agaricus bisporus* (*A. bisporus*) exhibits many biological activities, such as antioxidant, antibacterial, anti-inflammatory, antitumor and immunomodulatory ones (Markowa 2009; Roberts 2008). The medicinal and dietary properties of this species are a result of an excellent combination of these activities.

In recent years there has been a significant increase in the total sales volume of non-prescription pharmaceuticals. This development has been influenced by the speed of modern urban lifestyles and, most importantly, by the lack of time for the consumption of a balanced diet. All these facts explain the increased interest in the pharmacological balancing of elements, administered in an easy form and sold via a network

of pharmacies, chemists and hypermarkets. Currently, basic research in the field of the specialized cultivation of the mycelium of *A. bisporus* supplemented with elements (macro- and microelements) is especially important for the explanation of the mechanisms of their accumulation and distribution in in vitro cultures. It is commonly known that high absorption rates of elements from natural sources are caused by the presence of fiber (chitin and chitosan). The choice of *A. bisporus* has been influenced by practical aspects: the possibility for mass production and also the habits of consumers (due to taste and smell properties). Currently, the fruiting bodies of *A. bisporus* are irradiated by UV light to increase vitamin D levels during production (Koyalamudi et al. 2008).

In closed laboratory conditions, it is easier to control the accumulation and release of chosen elements and substance metabolites. Determination of any connection between biomass growth and the composition of culture media, as well as achieving improvements in biomass growth in in vitro cultures of the examined *A. bisporus*, constitute an innovatory approach allowing optimization of the absorption process which might be used in future for natural microelement supplementation for consumers or patients. The concentrations of Cu, Zn, Mg, and Fe were determined to compare accumulation efficiency of mycelium from in vitro culture with the fruiting bodies of naturally grown mushroom (control). What should be underlined, nutritive sources in the form of diet supplements of those metals are still scarce but are needed because of their antidepressant, antiphlogistic and regenerative activity (Guerrera et al. 2009; Opoka et al. 2010).

The aims of the study were to establish an in vitro culture of *A. bisporus* and to investigate the accumulation of the following elements – Cu, Zn, Mg, Fe, Cd and Ni using the Atomic Absorption Spectroscopy (AAS) method in: fruiting bodies of *A. bisporus*, in mycelium from in vitro cultures of this species, in mycelium from in vitro cultures with the addition of L-tryptophan to medium and mycelium from in vitro cultures with the addition of Zn hydroaspartate. These two components were added to culture medium to determine their absorption and mutual influence on the degree of accumulation in the mycelium. Due to the fact that this species is so widely used as a food and that it has a natural ability to accumulate elements, it is advisable to obtain fruiting bodies of *A. bisporus* rich in selected physiologically active compounds and elements (for example, L-tryptophan, Zn and other micronutrients). The aim of the presented research was also to analyze the accumulation of toxic microelements: Cd and Ni (Cempel and Nikel 2006; Wan and Zhang 2012) in naturally grown fruiting bodies and in mycelium from in vitro culture of *A. bisporus*.

This research was made to evaluate the safety of mycelium from in vitro culture as a potential dietary supplement in future. We think that this information, resulting from our basic research in specialized culturing of mycelia of edible mushrooms, might be used in the future to develop cultivations of mushrooms rich with physiologically active elements.

The history of "industrial" white mushroom production begins in the second half of the 20th century. That is why the possibility of implementation of the obtained results to the technological aspects of the production of white mushrooms supplemented with elements seems to be real. All this can be effective after performing additional technological experiments not being in the scope of our project. As far as we know this is the first analysis of endogenous accumulation of elements in mycelium from in vitro culture enriched with them.

Materials and methods

Material for analysis The studies were conducted on young fruiting bodies of *A. bisporus* harvested from natural sites in southern Poland in 2011 (near Kraków) and of commercial origin, purchased at a supermarket. After taxonomic identification according to Knudsen and Vesterholt (2008) (representative samples of mushrooms were deposited at the Department of Pharmaceutical Botany, Jagiellonian University Collegium Medicum, Kraków, Poland), some of the young sporocarps from natural states were used to derive culture in vitro from which the obtained mycelium formed the material for further analysis.

The pieces of fruiting bodies were defatted with 70 % ethyl alcohol for 15 s and then sterilized in 15 % HClO for 5 min. After being rinsed several times with sterile redistilled water, mycelium fragments were transferred to Petri dishes containing agar-solidified medium with a composition according to Oddoux (1957). After growing on solid medium, the pieces of mycelium were placed in an Erlenmeyer flask (500 mL) containing 250 mL of liquid medium with modified 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 a temperature of 25±2 °C under 16 h light (900 lx/8 dark). The agitated liquid cultures of *A. bisporus* were maintained for 2 weeks and after this time were subcultured.

Experimental culture in vitro Both agitated liquid cultures of *A. bisporus* on Oddoux medium with the addition of 0.5 g L^{-1} of L-tryptophan and cultures on the same medium but with the addition of 0.1 g L^{-1} of Zn hydroaspartate were maintained for 2 weeks. After 2 weeks, the biomass was separated from the liquid medium using filter paper on a Büchner funnel and rinsed with redistilled water. The obtained fresh biomass consisted of: mycelium from in vitro cultures of *A. bisporus*, mycelium of this species from in vitro cultures with addition of L-tryptophan to the medium, mycelium from the same medium but with the addition of Zn hydroaspartate,

and fruiting bodies of *A. bisporus*. Fifty grams samples of each of the materials were frozen and immediately dried by lyophilization (lyophilizer Freezone 4.5. Labconco, temperature: − 40 °C). Trace elements quantitative determination was performed by means of the Atomic Absorption Spectroscopy (AAS) method.

Chemicals L-Tryptophan was from Sigma-Aldrich, (St Louis, Mo, USA) and Zn hydroaspartate came from Farmapol (Poland), conc. HNO_3 and H_2O_2 for AAS analysis were from Merck, Suprapure® (Germany), HClO solution was manufactured by Unilever (Hungary). Water for liquid medium preparation was purified by redistillation and filtered through Milipore (Millex, Milipore Corporation, USA) under reduced pressure.

Determination of elements with the AAS method The usefulness of the Atomic Absorption Spectroscopy method in biological sample trace elemental analysis is well elaborated in the literature (David 1978; Fricke et al. 1979). Substantial improvement has been made regarding the sample preparation methods, in particular, the use of microwave digestion has been widely discussed (Muller 1998).

Prior to the elements quantitative analysis, the lyophilized mushrooms samples were wet digested (conc. HNO_3 and H_2O_2, Merck, Suprapure®, Germany) in a microwave system (Multiwave 3000, Anton Paar, Switzerland). The concentrations of the elements were determined by the Atomic Absorption Spectroscopy (AAS) using flame and electrothermal techniques. Each sample was analyzed in triplicate and the results presented below are the mean values.

Concentrations of Fe, Mg and Zn were determined using the flame technique (Perkin Elmer AAS Model 3110 spectrometer, USA) under standard conditions. Ni and Cd were determined using the electrothermal technique (Perkin Elmer HGA 600 graphite furnace, USA). ET AAS measurement conditions were optimized before analysis with the use of the Method Development program. Graphite furnace operating conditions are presented in Table 1. Measurements were made using the pyrocoated graphite tubes and for Cd an electrodeless discharge lamp was used.

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

Table 1 Optimized analytical conditions for graphite furnace operation

Stage/Element	Ni	Cd
Pretreatment Temperature (°C)	1400	600
Atomization Temperature (°C)	2500	1600

Statistical analyses For each of the mycelium from in vitro cultures and fruiting bodies of *A. bisporus*, five samples were used for the determination of every compound and all the analyses were conducted in three repetitions. Statistical analyses were conducted using Microsoft Excel 2010 and commercially available package GraphPad Prism v.3.02 (GraphPad Software, San Diego, USA). The data are presented as mean±standard deviation. One way ANOVA was applied to check for any differences between different groups of fruiting bodies and mycelium of from in vitro cultures of *A. bisporus*. Tukey post-hoc test was used to reveal the differences between paired groups of mushroom materials. *P* values below 0.05 were considered to be statistically significant.

Results and discussion

We have established that good mycelial mass growth for *A. bisporus* could be obtained in solid cultures and agitating liquid cultures on modified Oddoux (1957) medium at 25± 2 °C under a 16 h photoperiod (900 lx/8 h dark). 20-fold fresh biomass growth in cultures in liquid medium was obtained within a 14-day growth cycle. The biomass growth in the initiated cultures averaged 7.9 g DW (dry weight) per L of medium. The obtained biomass increments and dynamics of *A. bisporus* mycelium growth did not differ from the results that we obtained in our earlier studies for *Tricholoma equestre* (L.: Fr.) Kumm., *Xerocomus badius* (Pers.: Fr.) Fr. (Muszyńska et al. 2009), *Sarcodon imbricatus* L. (Sułkowska-Ziaja et al. 2012) and *Cantharellus cibarius* Fr. cultures (Muszyńska et al. 2013). The addition of L-tryptophan and Zn hydroaspartate to the medium stimulated the mycelium growth and resulted in a significant increase in biomass in in vitro cultures (the biomass growth averaged 11.2 and 10.8 g DW per L of medium, respectively). Liquid medium allowed easy and accurate dosing of solutions of metals' ions, separation of mycelium for analysis and ensured homogenous composition of the whole volume of the medium. The presented research included determination of the levels of individual trace elements (Cu, Mg, Zn, Fe, Cd, and Ni) in fruiting bodies of *Agaricus bisporus* and in mycelium from in vitro cultures. The concentrations of the elements in mycelium from in vitro cultures allowed evaluation of the relationship between the degree of accumulation and the driving conditions of the culture. In addition, identification of similarities between objects was possible based on the results of cluster analysis. Cu (Table 2) concentration in the fruiting bodies of *A. bisporus* oscillated in the range from 21.95 to 51.49 μg g^{-1} DW (Table 2).

The obtained mycelial cultures were characterized by lower levels of Cu, ranging from 5.56 to 20.96 μg g^{-1} DW, in relation to the fruiting bodies. It was observed that addition of L-tryptophan to the culture medium caused an increase in

Table 2 Concentration of micronutrients in fruiting bodies of *Agaricus bisporus* and in their mycelium from in vitro cultures (1.1–5.1 fruiting bodies of *A. bisporus* (control); 1.2–5.2 mycelium of *A. bisporus* from in vitro cultures; 1.3–5.3 mycelium of *A. bisporus* from in vitro cultures with addition of L-tryptophan; 1.4–5.4 mycelium of *A. bisporus* from in vitro cultures with addition of Zn) (mean±SD; n=3)

Sample	Cu	Mg	Zn [μg g^{-1} DW]	Fe	Cd	Ni
1.1	21.95±0.93	1545.00±7.07	70.39±20.68	161.75±0.26	2.75±0.00	3.99±4.04
2.1	51.49±1.14	1785.00±21.21	112.75±3.75	88.09±2.95	2.02±0.04	1.00±0.44
3.1	41.20±0.38	2275.00±7.07	76.69±2.68	71.42±3.75	4.76±0.36	2.74±0.61
4.1	34.57±1.10	1970.00±28.28	99.94±9.98	76.80±2.82	1.08±0.78	1.22±0.10
5.1	23.27±3.21	1100.50±65.76	54.81±4.45	13.90±0.53	0.06±0.00	0.35±0.014
Mean of 1.1–5.1	34.50±11.78 [a,b,c]	1735.10±419.22 [d,e,f]	82.91±23.33 [i,j,k]	82.39±51.84 [o]	2.13±1.70 [p,r,s]	1.86±1.95
1.2	5.56±0.24	403.00±1.41	169.70±1.98	255.55±0.91	0.12±0.00	1.45±0.47
2.2	20.96±5.21	438.50±20.51	172.75±15.63	147.05±3.04	0.35±0.26	0.93±0.12
3.2	11.97±0.01	372.00±5.66	127.45±6.43	125.15±6.43	0.16±0.01	2.63±0.20
4.2	7.24±2.75	633.50±14.85	160.55±3.32	118.11±2.94	1.08±0.07	1.22±0.91
5.2	6.81±0.01	445.70±0.57	100.07±4.20	60.85±5.23	0.05±0.01	0.79±0.02
Mean of 1.3–5.3	10.51±6.28 [a]	458.54±96.71 [d,g]	146.10±30.19 [i,l,m]	141.39±57.32	1.61±0.84 [p]	1.61±0.14
1.3	7.41±1.18	343.00±13.44	238.90±0.42	311.65±1.13	0.21±0.04	0.96±0.15
2.3	26.91±5.80	302.50±31.82	293.30±17.11	187.00±0.07	0.18±0.02	1.82±0.07
3.3	17.06±2.18	396.00±11.31	272.45±26.09	170.45±15.90	0.41±0.28	2.62±0.64
4.3	21.01±3.85	375.00±9.90	262.20±4.95	145.75±8.69	0.20±0.00	1.92±0.08
5.3	13.92±0.57	618.60±3.39	131.04±2.32	118.65±6.35	0.04±0.00	0.64±0.01
Mean of 1.2–5.2	17.26±7.35 [b]	417.92±112.59 [e,h]	239.58±61.03 [j,l,n]	186.70±74.95 [o]	1.59±0.78 [r]	1.59±0.15
1.4	1.14±0.22	315.00±32.53	541.85±16.90	135.70±1.32	0.05±0.01	0.82±0.01
2.4	16.75±0.42	1300.00±14.14	561.00±3.96	179.60±1.55	0.19±0.03	0.80±0.09
3.4	12.98±2.72	868.00±12.73	664.85±36.70	219.85±10.67	0.19±0.02	1.91±0.77
4.4	13.51±0.73	1145.00±7.07	577.30±16.83	114.05±14.49	0.16±0.00	1.24±0.16
5.4	21.31±0.68	789.40±13.15	463.55±1.06	111.61±0.16	0.10±0.01	0.73±0.04
Mean of 1.4–5.4	13.14±7.12 [c]	877.48±352.76 [f,g,h]	561.71±69.73 [k,m,n]	152.16±44.39	1.098±0.53 [s]	1.098±0.06

Significant differences were presented as the same letter in each row (for g, h, i, l, o *p<0.05 and for a, b, c, d, e, f, j, k, m, n, p, r, s **p<0.001)

accumulation of Cu in the mycelium. Fruiting bodies of *A. bisporus* had a high content of Mg (Table 2), its concentration was in the range from 1545.00 to 2275.00 μg g^{-1} DW. The concentration of Mg in in vitro cultures was at a similar level, in the range from 403.00 to 633.50 μg g^{-1} DW. With the addition of Zn to the culture medium, a substantial increase of Mg accumulation (range from 315.00 to 1300.00 μg g^{-1} DW) was observed. Zn was present in the fruiting bodies of

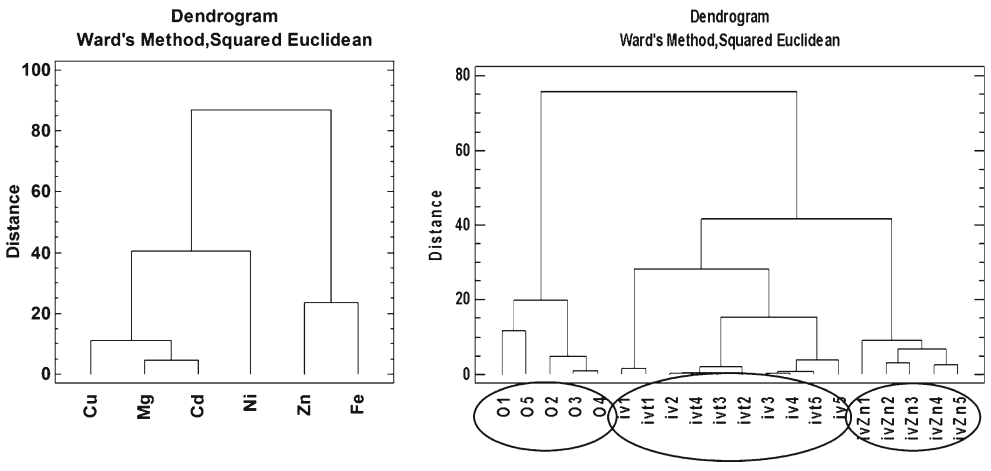

Fig. 1 Cluster analysis of the mycelium from fruiting bodies of *Agaricus bisporus* and in mycelium from in vitro cultures (the Euclidean distance square, Ward's algorithm); o – fruiting bodies of *Agaricus bisporus*; iv - mycelium from in vitro cultures of *A. bisporus*; ivt - mycelium from in vitro cultures of *A. bisporus* with L-tryptophan; ivZn - mycelium from in vitro of *A. bisporus* cultures with zinc hydroaspartate

Table 3 Factor loads for three first main principal components (PC1, PC2 and PC3 are linear combinations of the original variables multiplied by the charges shown in the table)

	Principal component 1	Principal component 2	Principal component 3
Cu	0.49	−0.15	0.21
Mg	0.50	−0.09	0.44
Zn	−0.34	0.03	0.85
Fe	−0.31	0.60	0.18
Cd	0.51	0.27	0.09
Ni	0.23	0.73	−0.12

Variables PC1, PC2 and PC3 are linear combinations of the original variables multiplied by the charges shown in the table

A. bisporus in the concentration range from 70.39 to 112.75 μg g^{-1} DW. Enrichment of the culture medium with L-tryptophan increased the bioaccumulation of Zn two-fold in mycelium from in vitro culture. A similar relationship was observed in the case of enrichment of the medium with Zn. The concentration of Fe in the fruiting bodies of *A. bisporus* was in a wide range: 13.90–161.75 μg g^{-1} DW. In this case, a similar relationship was observed as for Zn, i.e., addition of L-tryptophan or Zn into the culture medium increases the preferential accumulation of Fe in the mycelium of *A. bisporus*. For the substrate enrichment with L-tryptophan, Fe concentrations ranged between 118.65 and 311.65 μg g^{-1} DW, while Zn was in the range from 131.04 to 293.30 μg g^{-1} DW. For mycelium from in vitro cultures where the concentration of Fe was in the range of 60.85–255.55 μg g^{-1} DW. Our earliest study on plant material in opposite to present results, showed that the addition of L-tryptophan, caused only a very small increase in the accumulation of elements in *Bacopa monnieri* and for example addition of L-tryptophan to the cultures resulted in decrease of Mg accumulation in biomass (Łojewski et al. 2014). Fruiting bodies of *A. bisporus* were characterized by different levels of Cd (0.06–4.76 μg g^{-1} DW). The reason may be that the fruiting bodies were collected from the vicinity of Kraków (pollution). In vitro cultures levels of Cd were recorded at a lower level than in the fruiting bodies from natural sites, and this was due to the maintenance of the sterile conditions and controlled medium composition, and the average content of Cd was 0.22 μg g^{-1} DW. Ni was found in lower concentrations (0.64–2.63 μg g^{-1} DW) in mycelium from in vitro culture of *A. bisporus* than in fruiting bodies of this species (0.35 to 3.99 μg g^{-1} DW). The material from commercial origin was very poor in examined elements and the amounts of all of them were lower than in fruiting bodies from natural states and than in mycelium from in vitro culture.

Cluster analysis (CA) can identify groups of similar objects, when these objects are described by more than one feature. The basis of CA is the concept of the so-called distance between the studied objects. The distance between objects is defined by a selected distance measure. Objects that are considered to be similar have a near position in the multidimensional space. It is possible to group objects with high similarity. The result of the analysis of similarity is often presented in graphical form of a dendrogram (Fig. 1). Labels of the x-axis and y-axis, these do not correspond numerically. The x-axis shows the name of the analyzed object and the y-axis the distance between the objects. In this study, the distance between the objects is defined by Euclidean distance squared (Fig. 1).

The analyzed set of objects consisted of fruiting bodies of *A. bisporus*, mycelium from in vitro cultures of this species, and mycelium from in vitro cultures enriched with L-tryptophan or Zn form 3 clear clusters. The first cluster on the dendrogram (Fig. 1) grouped the fruiting bodies of mushrooms.

The second cluster consists of a large group in which there are both mycelium from in vitro cultures and in vitro cultures enriched with L-tryptophan. The mycelium from in vitro mycelium cultures of *A. bisporus* form a separate third cluster

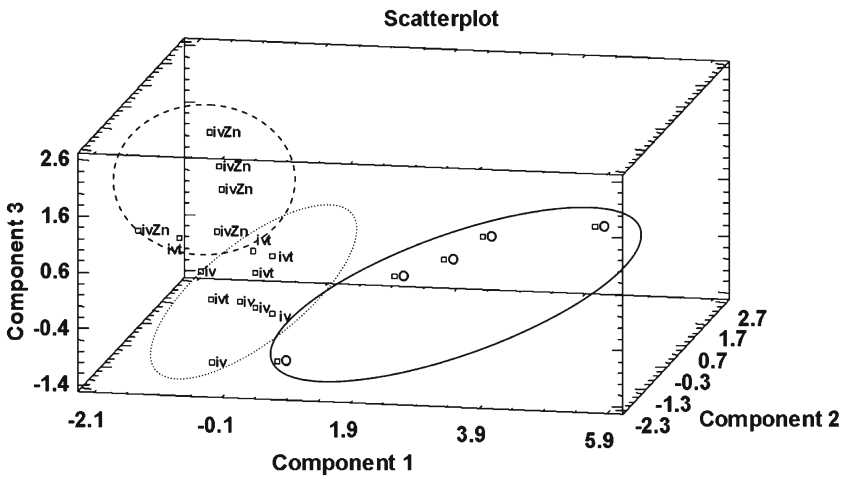

Fig. 2 Diagram of 3 principal components PC1, PC2, and PC3 in three dimensional spaces, representing the data clusters

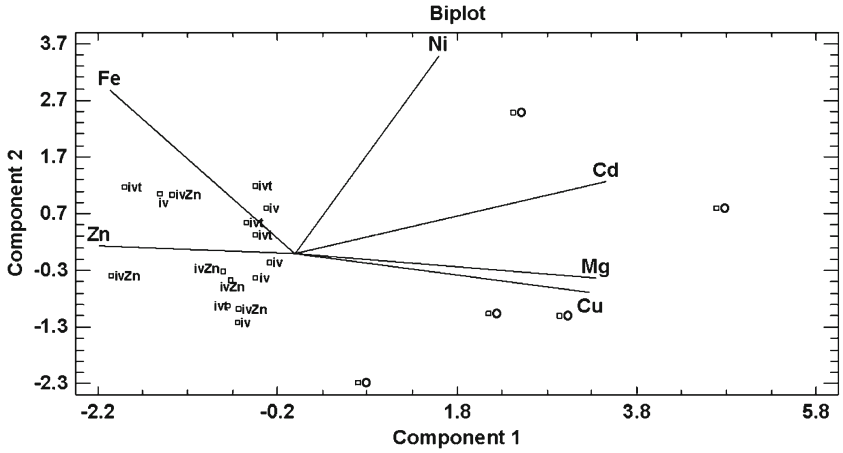

Fig. 3 Biplot graph based on principal components PC1, PC2

which proves that there is a similarity with mycelium from in vitro cultures enriched with Zn. However, there is no similarity in composition between the samples derived from mycelium from in vitro cultures and fruiting bodies of mushrooms. Based on the analysis of similarity (Fig. 1), it was observed that subgroups of the parameters describing the concentrations of the individual elements are characterized by similar variability. It means that the course of concentration changes is similar. On this basis, the 2 clusters are distinguished, which is reflected in the high correlation between the concentrations of the analyzed elements. The first cluster is formed of Fe and Zn, while to the second cluster comprises Cu, Mg, Ni and Cd. It may be concluded that the medium composition (addition of L-tryptophan and Zn aspartate) influences the accumulation of Zn and Fe in the mycelium, and has only a limited effect on the accumulation of other elements.

Principal Component Analysis (PCA) is considered to be a computational method used to reduce the number of variables required to describe the observed phenomena. Based on PCA analysis, it was concluded that 85.82 % of the variation off elements' concentrations in the analyzed objects is described only by the first three principal components (PC1 – 50.18 %, PC2 – 71.49 %, PC3 – 85.82 %). In further analyses, other components were not taken into consideration. The resulting variables (PC1, PC2, and PC3) were a linear combination of input variables (elements' concentrations) multiplied by their assigned loads. Load corresponds to the saturation of the variable and is fixed to the factor equal to the correlation coefficient for the primary variables. As a result, the greatest impact on a change of the main component has a high-value ratio (Mazerski 2000; Massart et al. 1998). Table 3 shows the values of loads for each of the three major components. It was found that the input variables have a high impact on components PC1, PC2, PC3. Variables – concentrations of Cu, Mg and Cd had the most significant influence on the size of component PC1. Similarly, components PC2 and PC3 can be assigned to the original variables – Fe and Zn concentrations, respectively. This procedure allowed analysis of the results in three-dimensional space that has been created, using as a base the main components (Fig. 2).

Based on Fig. 2, it can be seen that three groups of objects are formed. The first group gathers mycelium from in vitro cultures of mushroom with mycelium from in vitro culture carried on a substrate of L-tryptophan (dotted line). The

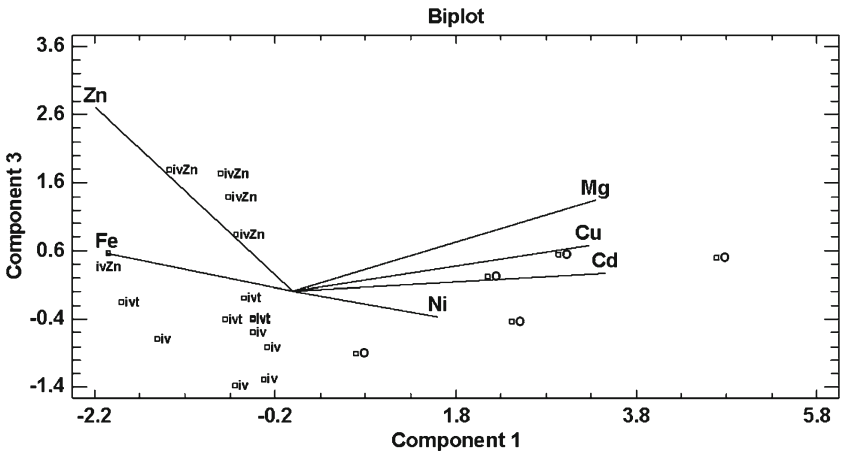

Fig. 4 Biplot graph based on principal components PC1, PC3

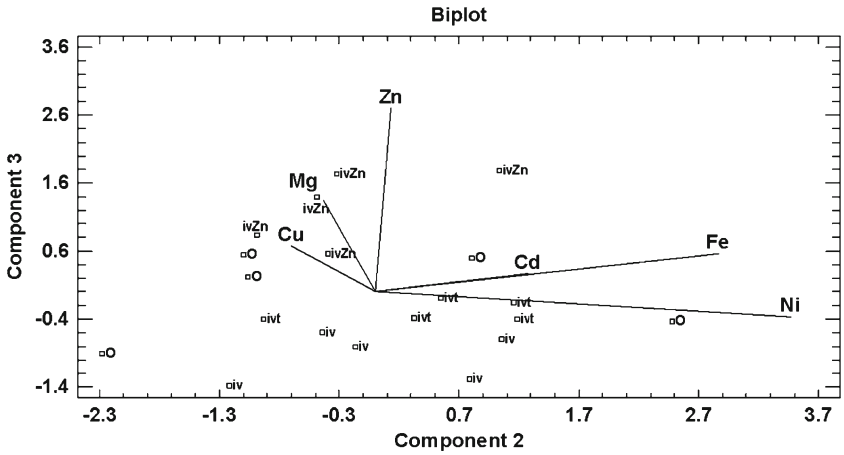

Fig. 5 Biplot graph based on principal components PC2, PC3

second group contains mycelium cultures enriched with Zn (dashed line), while the third group is formed by fruiting bodies of *A. bisporus* (solid line). The points located close to each other show significant similarity between the analyzed characteristics (concentrations) within the examined groups. Biplot graphs (Figs. 3, 4, and 5) for the main components allow simple tracking of changes in levels of the specific elements within and between cultures.

From graphs in Figs. 3, 4, and 5, it was found that the fruiting bodies of mushrooms have relatively high Mg, Cd and Cu levels. In addition, mycelium from in vitro cultures enriched with L-tryptophan and Zn are characterized by a high capacity for accumulation of Fe and Zn, in contrast to the mycelium from in vitro cultures and fruiting bodies of *A. bisporus*. Fruiting bodies harvested from the vicinity of Kraków, in contrast to the mycelium cultured in vitro, show substantially lower levels of Zn, Fe.

Interestingly, *A. bisporus* and its mycelium from in vitro cultures is characterized by higher concentrations of the analyzed elements than the previously investigated fruiting bodies and mycelia from in vitro cultures of two popular wild growing species: *Cantharellus cibarius* (the Chantarelle) and *Boletus badius* (Bay bolete). For example, the mean values of Mg were respectively (in $\mu g\ g^{-1}$ DW): mycelium of *C. cibarius* cultured in vitro – 541.8, fruiting bodies – 1004.1, mycelium of *B. badius* cultured in vitro – 928.9 and fruiting bodies – 906.4.

The mean concentrations of Zn were: in mycelium from in vitro cultures of *B. badius* 442.7 $\mu g\ g^{-1}$ DW and in fruiting bodies 172.1 $\mu g\ g^{-1}$ DW, in case of *C. cibarius* in mycelium from in vitro cultures 131.9 $\mu g\ g^{-1}$ DW and 95.5 $\mu g\ g^{-1}$ DW in fruiting bodies (Reczyński et al. 2013). Determined concentrations of the chosen elements are below humans' daily demand, thus consumption of mushrooms can only partially meet required physiological dose (mean consumption of mushrooms in Czech Republic and in Poland is over 10 kg per year) (Kalač 2010). In the authors' opinion, all conclusions related to natural, complex processes should be based on objective observations and interpreted using available tools. The statistical analysis presented in this paper confirmed, that by manipulation of culture medium composition, we are able to obtain mycelia and fruiting bodies with the desired levels of physiologically active elements in in vitro culture. Moreover, this approach can be aimed at obtaining material enriched with a given element at a required concentration. We think that actually research will be important for the development of basic sciences (biochemistry, physiology, chemistry, analytical chemistry and agricultural sciences) as well as the production of easily absorbed special dietary components enriched with physiologically important mineral and organic substances.

Conclusions

The proposed here method of in vitro cultures proved that the essential micronutrients are effectively taken up and accumulated in the mycelia. For the first time, these studies on the accumulation of the elements of cultured in vitro mycelia may allow an understanding of the biochemical mechanisms of these processes. It was particularly valuable to demonstrate that the cultures enriched with L-tryptophan significantly enhanced ability to accumulate Zn, Mg, Fe and Cu. The ability to accumulate the essential amino acid and simultaneously physiologically active elements, which are important for the proper functioning of the human body, could lead to development of conditions for the commercial crop production of enriched *A. bisporus* fruiting bodies in the future. *A. bisporus* cultured in vitro exhibited very low levels of Cd. This may be achieved only in such controlled cultures, as mushrooms grown naturally in contaminated environments also accumulate certain amounts of toxic metals. Hence, the possibility to transfer the obtained results on the basis of technologically fortified varieties of the selected elements and micronutrients is considered to be very real. 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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