Changes in mineral composition of six strains of *Pleurotus* after substrate modifications with different share of nitrogen forms

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Abstract

The chemical characteristics of substrate are one the most significant factors influencing the growth and development of cultivated mushroom species. The aim of this study was to determine the mineral composition of six *Pleurotus* species (*P. cystidiosus*, *P. djamor*, *P. ostreatus*, *P. ostreatus* var. *florida*, *P. pulmonarius* and *P. sajor-caju*) growing on three wheat straw substrates with the addition of agricultural fertilizer rich in ammonium and with addition of salt solution rich in nitrates. Significant differences in the concentration of Al, Ca, Cu, Ir, Ni, Ru, Sn and Te were observed in all substrates used in this experiment. Cultivation on chemically-enriched substrates did not result in changes in yield with the exception of *P. sajor-caju*, which had a lower yield when grown on ammonium-rich substrate. No macroscopic alterations in fruit bodies were observed for any species regardless of the applied substrate. A higher concentration of selected elements was not correlated with their higher content in particular mushroom species, or such a relationship was present only in selected mushroom species. The efficiency of element accumulation depends on their concentration in the substrate (positive values of r_s), although the mushroom species and the nitrogen form concentration may also have a significant impact (negative r_s values). The obtained results show that cultivation of different *Pleurotus* strains on substrates enriched with a different share of ammonium and nitrate may cause changes in their mineral composition in spite of the similarity in the concentration of the majority elements in substrates.

Keywords *Pleurotus* mushrooms · Substrate composition · Yield · Mineral content

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Introduction

Species belonging to the *Pleurotus* genus are globally one of the most popular marketable mushrooms, not only because of their taste and nutritional value but also because of their relatively easy cultivation and ability to grow on many types of lignocellulosic biomass [1]. *Pleurotus* species belong to white-rot fungi that are known to produce a wide variety of polysaccharide and lignin-degrading enzymes (affected by pH, temperature, and other typical fermentation factors) which are capable of degrading different lignocellulose materials [2, 3]. Carbohydrates are necessary for the growth and development of mycelia. Therefore, most types of organic matter containing lignin, cellulose, and hemicellulose with a different C/N ratio, such as sawdust, corn stalks, and stalk, cotton waste, rice straw, pomace, wheat stalks or banana leaves can be successfully employed as mushroom substrate [4–7]. The type of substrate used in cultivation depends on

many factors affecting the quality of production, including the ability to secrete enzymes (ligninase, laccase, manganese peroxidase, cellulase, xylanase, and tannase) which are involved in the use of lignocellulosic substrates, and its costs [8, 9]. In Europe, cereal straw, mainly of wheat and rye, is the most commonly used substrate for growing mushrooms from the *Pleurotus* species. For development, fungi require macronutrients such as C, N, P, K and Mg, as well as trace elements such as Fe, Se, Zn, Mn, Cu, and Mo [10]. As saprophytes, *Pleurotus* species uptake molecules pivotal for their growth, such as C, N, mineral compounds, and vitamins through mycelium [11]. Therefore, the type of substrate used in cultivation has an effect on the chemical and nutritional composition of fruit bodies [12]. Generally, lignocellulosic materials are a weak source of mineral content and to increase the efficiency of mushroom yield, organic and mineral additives are used [13]. These organic additives usually include waste materials originating from agriculture or the food industry. They mostly serve as an additional source of N that is supplied with the protein content. Mineral additions, in the form of mineral salts and/or fertilizers, enrich the substrate with substances essential for the proper development of mycelium and fruit bodies. Some of additions can also be a source of N in the form of ammonium compounds, which are better absorbed by fungi than nitrate or nitrite forms.

Nitrogen is an essential element in the synthesis of many organic compounds (proteins, nucleic acid) and cell wall components in fungi [14–18]. There are various potential sources of N that include ammonium nitrate, ammonium acetate, ammonium chloride, ammonium phosphate dibasic, ammonium sulfate, ammonium tartrate, potassium nitrate, sodium nitrate, amino acids, hydrolyzed proteins urea, and yeast extract [15, 16, 19]. Nitrogen supplementation can increase the crop efficiency of *Pleurotus* to a certain level. However, higher nitrogen content can inhibit the fruitification of mushrooms [20]. A high level of N can repress the ligninolytic enzyme production, while at low availability of nitrogen, this metabolic pathway can be up-regulated [20].

Due to the different bioavailability of various forms of N, which is often the main factor determining the growth of mycelium and which may stimulate fructification, attention was focused on changes in the mineral composition of fruit bodies. The aim of the present study was to determine the content of 29 elements in six cultivated *Pleurotus* species growing in control conditions on substrates enriched with additives containing a different share of ammonium and nitrate nitrogen. It was hypothesized that *Pleurotus* species growing on a substrate enriched with nitrogen, present mainly as ammonium form, will be characterized by a higher elemental content in their fruit bodies.

Materials and methods

Mushroom strains and spawn

In the present experiment, six strains of *Pleurotus* were used: B122 (*P. cistidiosus*), NR8 (*P. djamor*), B204 (*P. ostreatus*), PFL1 (*P. ostreatus* var. *florida*), B76 (*P. pulmonarius*) and B51 (*P. sajor-caju*). All of these strains were obtained from the Mushroom Collection of Poznań University of Life Science (Department of Vegetable Crops). The spawn for inoculation of substrates was prepared according to the method described by Stamets (2000) [21].

Substrates and environmental conditions of cultivation

Wheat straw was the main component of the substrates used in the experiment. The straw was minced using a mechanical crusher to a particle size of 1–2 cm. Three types of substrates, marked as A, B and C, were prepared. All of the substrates consisted of 97% straw and 3% wheat bran. To the mixture wheat straw and bran 2% of CaSO_4 (in relation to substrate d.m.) was added for substrate A. For substrate B 1% CaCO_3 and 1% fertilizer Azofoska (INCO, Poland) were added, while for substrate C, 2 ml of salt solution (in the relation of 1 kg of substrate d.m.). Azofoska contained: 13.6% N (5.53% nitrate, 8.09% ammonium), 6.41% P_2O_5 , 19.1% K_2O , 4.53% MgO , 23.1% SO_3 , 0.045% B, 0.184% Cu, 0.175% Fe, 0.270% Mn, 0.041% Mo and 0.045% Zn. The salt solution contained: 15.73% N (11.64% of nitrate and 4.09% of ammonium), 5.14% of P_2O_5 , 17.2% of K_2O , 5.72% of MgO , 15.5% of Si, 0.011% of B, 1.043% of Cu, 0.226% of Fe, 0.351% of Mn, 0.01% of Mo and 0.101% of Zn. The substrates for the experiment were moistened to a moisture content of 70% using distilled water and were then pasteurized at 60 °C for 24 h. Next the substrates were mixed with *Pleurotus* species mycelium, which constituted 3% in relation to the wet weight of the substrate and placed in bags of perforated foil. Each bag contained 1 kg of the substrate. Incubation was conducted at a temperature of 25 °C and air relative humidity 85–90%. Once the substrate was totally covered with the mycelium it was transferred to the cultivation room in which the temperature was maintained at 16 ± 1 °C for *P. ostreatus*, *P. o.* var. *florida*, *P. columbinus*, *P. pulmonarius* and 21 ± 1 °C for *P. cistidiosus*, *P. djamor* and *P. sajor-caju*. Air relative humidity in the cultivation room was maintained at 85–90%. The cultivation was additionally lighted with fluorescent light of 500 lx intensity with the formula 12/12 h (day/night). The cultivation room was aerated in such a way as to maintain

CO₂ concentration below 1000 ppm. Carpophores were consecutively harvested as they matured. Yield included whole carpophores.

Analytical procedure

Samples of mushrooms and substrates were dried and ground to a particle size below 0.02 mm in an agate mill. The portion 0.300 to 0.500 g (with an accuracy of 0.001 g) of a sample was digested in the microwave sample preparation system Mars 6 Xpress (CEM, USA) in closed Teflon containers by 10 mL of concentrated nitric acid (65%; Sigma-Aldrich, USA). After cooling, the samples were filtered (Qualitative Filter Papers Whatman) and diluted to a final volume of 15.0 mL using demineralized water (Direct-Q system, Millipore, USA).

An inductively coupled plasma spectrometer with optical emission detection (Agilent 5110 ICP-OES, Agilent USA) was used for sample analysis. Standard conditions were applied for elemental analysis (determination of Ca, K, Mg, Na, P, Al, Ba, Be, Cd, Cr, Cs, Cu, Fe, Hg, Ir, La, Mn, Nd, Ni, Pb, Pr, Rb, Ru, Sb, Si, Sn, Sr, Te, Tm, Zn): plasma argon flow 12.0 L min⁻¹, nebulizer argon flow 0.7 L min⁻¹, auxiliary argon flow 1.0 L min⁻¹, Radio Frequency (RF) power 1.2 kW. The most sensitive analytical wavelengths were used for all element determinations. Commercial ICP analytical standards (Romil, England) and demineralized water were used for calibration. The limits of detection were determined in the range of 0.01–0.09 mg kg⁻¹ dry weight (based on criteria of 3-sigma) for the total procedure, including sample preparation. Uncertainty was estimated at the level of 20%. The certified reference materials (sediments: CRM 667 and CRM 405; soils: CRM and CRM S-1; CRM NCSDC (73,349)—bush branches and leaves), and the procedure of standard additions were applied in quality control. The recovery level (80–120%) was found acceptable.

Statistical analysis

All analyses in this study were performed using the Agri-colle Package (R) and STATISTICA 12.0 software (StatSoft, USA). The two-way ANOVA and also the test post hoc: Tukey's HSD (statistically significant difference) test were used to compare the content of particular major and trace elements in mushroom species growing in specific substrates and the concentration of these elements in cultivation substrates. The Spearman correlation coefficients (r_s) was used to show a relationship between the level of particular elements in substrates, and their content in fruit bodies growing on these substrates.

For a graphical presentation of the obtained results, a Principal Component Analysis (PCA) was performed [22, 23] to show the general distribution of *Pleurotus* species and

their tendency for higher/lower accumulation of determined elements. An additional visualization of multidimensional data was a Heatmap with a cluster analysis performed to show similarities/differences between *Pleurotus* species growing in particular substrates as regards the content of macro- and trace elements, and all 29 elements jointly. The rank-sum was also performed to show which of the *Pleurotus* species growing in which substrate was the most enriched with macro-, trace and all elements jointly.

Results

Macroscopic characteristics and biomass yield of fruit bodies

There were no differences in the macroscopic appearance of fruit bodies for the investigated species of mushrooms regardless of the type of substrate (Fig. 1). The highest biomass yield was found for *P. o.* var. *florida* growing on substrates C, A and B (143, 130 and 124 g, respectively), while the lowest for *P. djamor* growing on substrates B, A and C (70.8; 62.0 and 54.6 g, respectively). With the exception of *P. sajor-caju*, the biomass of fruit bodies of particular *Pleurotus* species was similar independent of substrate composition. The biomass yield of *P. sajor-caju* was the highest for fruit bodies growing on substrate A (99.4 g). The biomass yield of fruit bodies from substrate C was similar (90.6 g), while that obtained from substrate B was significantly lower (64.1 g) than mushrooms collected from the above mentioned substrates (Fig. 2).

Characteristics of macro- and trace elements content in substrates and fruit bodies

There were no significant differences in the concentration of Ba, Be, Cr, K, Mn, Na, P and Si in the used substrates, while in case of Al, Ca, Cu, Ir, Ni, Ru, Sn and Te significant differences between all used substrates were recorded (Tables 1, 2, 3). The concentrations of Cd, Fe, K, Mg and Sb in substrates A and B were similar and significantly higher than in substrate C, while the concentration of Pb, Sr and Tm in substrates B and C was significantly lower than in substrate A.

The content of both macro- and trace elements in fruit bodies was diverse and they were growth dependent on mushroom species and substrate (Tables 1, 2, 3). The highest content of Ca (1910 mg kg⁻¹) was determined in *P. djamor* growing on substrate A, while that of K and Na was found in *P. ostreatus* growing on substrate B (29,000 and 293 mg kg⁻¹, respectively). The content of Ca in the mushroom fruit bodies collected from substrate A was significantly higher than in the fruit bodies from substrate C

Fig. 1 Macroscopic picture of studied *Pleurotus* species

with the exception of *P. pulmonarius*. Fruit bodies of *P. pulmonarius* growing on substrate B were the most enriched with Mg (861 mg kg^{-1}), while the mean content of P was similar in all *Pleurotus* species growing on all substrates (Table 1). The same observations were also recorded for Be. *Pleurotus djamor* revealed the highest content of Hg, Pr, Si and Sr (0.946 ; 0.595 ; 10.7 and 6.38 mg kg^{-1} , respectively) in fruit bodies from substrate A, the highest content of Hg, Ni, Sb and Zn (0.912 ; 1.05 ; 74.8 and 123 mg kg^{-1} , respectively) from substrate B as well as the highest content of Cu and

Ru (14.5 and 1.66 mg kg^{-1} , respectively). The second effectively accumulating *Pleurotus* species was *P. sajor-caju* with the highest content of Ba and Sn (76.1 and 10.2 mg kg^{-1} , respectively) in fruit bodies from substrate A; Cr, Fe and Mn (21.6 ; 87.5 and 16.0 mg kg^{-1} , respectively) from substrate B and Pr and Rb (0.585 and 40.9 mg kg^{-1} , respectively) from substrate C. In case of the remaining *Pleurotus* species, *P. cystidiosus* from substrates B and C contained the highest amount of Cd and Ir (3.55 and 6.77 mg kg^{-1} , respectively), while *P. o. var. florida* growing on the same substrates was

Fig. 2 Biomass [g] of *Pleurostus* species with respect to the kind of substrate **Table 1** Content of major and trace elements (mg kg⁻¹) in *Pleurotus* species and substrates used in experiment

Species/substrates	System	Ca	K	Mg	Na	P	Al	Ba	Be	Cd	Cr
<i>Pleurotus cystidiosus</i>	A	1220 ^{bcd}	22200 ^{c-h}	749 ^{a-d}	204 ^{bcd}	3114 ^a	1.53 ^{hij}	45.0 ^{fgh}	0.103 ^a	3.06 ^b	16.7 ^{bc}
	B	872 ^{c-f}	24300 ^{b-e}	801 ^{ab}	167 ^d	3127 ^a	2.31 ^{bcd}	45.2 ^{fgh}	0.092 ^a	3.55 ^a	17.8 ^{abc}
	C	697 ^{ef}	14000 ⁱ	647 ^{def}	215 ^{bcd}	3014 ^a	1.10 ^{ik}	55.4 ^{def}	0.132 ^a	1.12 ^{ij}	18.3 ^{abc}
<i>Pleurotus djamor</i>	A	1910 ^a	20100 ^{d-h}	679 ^{b-e}	194 ^{bcd}	2967 ^a	1.95 ^{d-h}	45.4 ^{fgh}	0.220 ^a	2.76 ^b	20.9 ^{ab}
	B	1310 ^{bc}	23900 ^{c-f}	706 ^{b-e}	196 ^{bcd}	2983 ^a	2.08 ^{c-g}	51.4 ^{efg}	0.077 ^a	3.19 ^{ab}	19.4 ^{abc}
	C	1080 ^{cde}	20000 ^{e-h}	523 ^f	171 ^d	2838 ^a	1.88 ^{d-i}	66.2 ^{a-d}	0.103 ^a	2.04 ^{cde}	19.2 ^{abc}
<i>Pleurotus ostreatus</i> var. <i>florida</i>	A	958 ^{cde}	18900 ^{gh}	582 ^{ef}	210 ^{bcd}	3146 ^a	1.78 ^{e-i}	41.6 ^{gh}	0.042 ^a	2.00 ^{c-f}	17.8 ^{abc}
	B	758 ^{def}	20600 ^{c-h}	662 ^{cde}	231 ^{bc}	3000 ^a	2.21 ^{cde}	43.0 ^{gh}	0.091 ^a	1.60 ^{e-h}	19.1 ^{abc}
	C	444 ^f	17800 ^{hi}	614 ^{def}	183 ^{cd}	3038 ^a	0.764 ^k	30.0 ⁱ	0.098 ^a	0.859 ^j	16.1 ^c
<i>Pleurotus ostreatus</i>	A	1630 ^{ab}	28700 ^{ab}	725 ^{a-d}	200 ^{bcd}	2721 ^a	1.64 ^{ghi}	56.7 ^{de}	0.117 ^a	2.25 ^c	15.9 ^c
	B	1580 ^{ab}	29400 ^a	683 ^{b-e}	293 ^a	2790 ^a	2.02 ^{c-h}	39.8 ^{hi}	0.110 ^a	1.40 ^{ghi}	17.1 ^{abc}
	C	1040 ^{cde}	24800 ^{a-d}	798 ^{abc}	242 ^{ab}	2898 ^a	1.69 ^{f-i}	61.0 ^{cde}	0.089 ^a	1.65 ^{e-h}	18.8 ^{abc}
<i>Pleurotus pulmonarius</i>	A	980 ^{cde}	19500 ^{fgh}	681 ^{b-e}	186 ^{cd}	3267 ^a	1.40 ^{ij}	64.1 ^{bcd}	0.077 ^a	1.33 ^{hi}	17.6 ^{abc}
	B	815 ^{c-f}	19960 ^{ef}	861 ^a	188 ^{cd}	3069 ^a	3.82 ^a	70.0 ^{abc}	0.071 ^a	1.58 ^{fgh}	19.6 ^{abc}
	C	702 ^{ef}	22991 ^{c-g}	727 ^{a-d}	200 ^{bcd}	3003 ^a	2.47 ^{bc}	50.1 ^{e-h}	0.063 ^a	0.749 ^j	17.2 ^{abc}
<i>Pleurotus sajor-caju</i>	A	1310 ^{bc}	24900 ^{abc}	716 ^{b-e}	206 ^{bcd}	3153 ^a	2.17 ^{c-f}	76.1 ^a	0.072 ^a	2.15 ^{cd}	20.6 ^{abc}
	B	839 ^{c-f}	24600 ^{b-e}	814 ^{ab}	197 ^{bcd}	2792 ^a	2.79 ^b	73.1 ^{ab}	0.184 ^a	1.92 ^{c-f}	21.6 ^a
	C	748 ^{def}	21200 ^{c-h}	716 ^{b-e}	190 ^{bcd}	2931 ^a	1.84 ^{d-i}	74.6 ^{ab}	0.136 ^a	1.79 ^{d-g}	19.4 ^{abc}
Substrate A	A	10900 ^a	7760 ^a	783 ^a	234 ^a	3019 ^a	10.5 ^b	296 ^a	0.110 ^a	0.743 ^a	18.2 ^a
Substrate B	B	8500 ^b	7920 ^a	830 ^a	250 ^a	2765 ^a	13.0 ^a	311 ^a	0.117 ^a	0.712 ^a	19.2 ^a
Substrate C	C	5510 ^c	6700 ^b	671 ^b	246 ^a	2973 ^a	7.29 ^c	357 ^a	0.107 ^a	0.322 ^b	19.1 ^a

$n=3$; identical superscripts (a, b, c...) denote non-significant differences between means in columns for mushroom species and particular substrates separately according to the post-hoc Tukey's HSD test

characterized by the highest content of Tm (0.233 mg kg⁻¹) and also Rb and Te (41.0 and 11.7 mg kg⁻¹, respectively). *Pleurotus ostreatus* contained the highest amount of Nd in fruit bodies from substrate A and Pb from substrate B (0.863 and 17.3 mg kg⁻¹, respectively). The highest contents of Al were determined in *P. pulmonarius* from substrate B and La from substrate C (3.82 and 0.059 mg kg⁻¹, respectively).

A significantly higher content of Al, Cd, Fe, Ru, Sb and Sn was noted in *P. cystidiosus* from substrate B than from substrate C, whereas the opposite situation was observed

for Ir, Nd, Pb, Pr, Si, Te and Tm. *P. djamor* contained a significantly higher content of Cd, Fe, Ni, Pr, Sb, Tm and Zn from substrate B and also Ba, Cu, Ir, Pb, Ru and Te from substrate C. *Pleurotus o. var. florida* growing on substrate B was characterized by the highest content of Al, Ba, Cd, Fe, La, Nd, Ni, Pr, Ru, Si, Sr and Tm, while from substrate C, a significantly higher content of Cu, Ir, Rb and Te. These results suggest that in the case of this *Pleurotus* species, its growth on substrate containing a higher concentration of nitrogen as an ammonium form was related to the most

Table 2 Content of trace elements (mg kg⁻¹) in *Pleurotus* species and substrates used in experiment

Species/substrates	System	Cu	Fe	Hg	Ir	La	Mn	Nd	Ni	Pb	Pr
<i>Pleurotus cystidiosus</i>	A	6.14 ^{ghi}	34.3 ^f	0.461 ^{fg}	1.25 ^{hij}	0.049 ^{abc}	5.89 ^h	0.409 ^{d-g}	0.075 ^h	4.93 ^{def}	0.128 ⁱ
	B	9.59 ^{cde}	31.8 ^{f-i}	0.884 ^{ab}	0.440 ^{ik}	0.040 ^{cde}	6.04 ^h	0.203 ^{ij}	0.614 ^{cd}	3.60 ^{fgh}	0.262 ^{ef}
	C	10.7 ^{bc}	20.8 ^j	0.832 ^{abc}	6.77 ^a	0.040 ^{cde}	5.90 ^h	0.383 ^{e-h}	0.595 ^{cde}	6.02 ^{cde}	0.327 ^d
<i>Pleurotus djamor</i>	A	9.43 ^{c-f}	60.0 ^{bc}	0.946 ^a	2.05 ^{e-h}	0.031 ^e	11.8 ^{cde}	0.298 ^{ghi}	0.013 ^h	1.42 ^{hi}	0.595 ^a
	B	10.2 ^{cd}	46.6 ^c	0.912 ^a	2.77 ^{de}	0.050 ^{abc}	13.4 ^{bcd}	0.307 ^{f-i}	1.05 ^a	0.66 ⁱ	0.525 ^b
	C	14.5 ^a	26.2 ^{g-j}	0.816 ^{abc}	4.13 ^b	0.048 ^{a-d}	11.7 ^{de}	0.289 ^{hi}	0.609 ^{cd}	3.34 ^{fgh}	0.347 ^d
<i>Pleurotus ostreatus</i> var. <i>florida</i>	A	4.41 ^{ij}	33.5 ^{fgh}	0.597 ^{d-g}	0.081 ^k	0.034 ^{de}	8.70 ^{fg}	0.601 ^{bc}	0.146 ^{gh}	4.55 ^{d-g}	0.129 ⁱ
	B	7.05 ^{fgh}	33.7 ^{fg}	0.592 ^{d-g}	1.61 ^{ghi}	0.055 ^{ab}	7.35 ^{gh}	0.616 ^{bc}	0.915 ^{ab}	2.71 ^{f-i}	0.346 ^d
	C	13.0 ^{ab}	25.5 ^{hij}	0.692 ^{cde}	3.31 ^{bcd}	0.010 ^f	7.81 ^{gh}	0.261 ^{ij}	0.507 ^{def}	2.23 ^{ghi}	0.061 ^j
<i>Pleurotus ostreatus</i>	A	6.10 ^{ghi}	34.7 ^f	0.722 ^{bcd}	2.25 ^{e-h}	0.040 ^{cde}	6.87 ^{gh}	0.863 ^a	0.250 ^g	6.10 ^{cde}	0.419 ^c
	B	9.08 ^{c-f}	52.0 ^{cde}	0.422 ^g	2.68 ^{def}	0.041 ^{b-e}	7.34 ^{gh}	0.572 ^{bc}	0.014 ^h	17.3 ^a	0.102 ^{ji}
	C	14.61 ^a	25.3 ^{ij}	0.506 ^{efg}	4.23 ^b	0.049 ^{abc}	7.10 ^{gh}	0.517 ^{cd}	0.015 ^h	3.41 ^{fgh}	0.310 ^{de}
<i>Pleurotus pulmonarius</i>	A	2.65 ^j	63.1 ^b	0.672 ^{cde}	1.66 ^{f-i}	0.043 ^{b-e}	10.5 ^{ef}	0.439 ^{de}	0.016 ^h	3.49 ^{fgh}	0.205 ^{gh}
	B	4.87 ^{hij}	58.1 ^{bcd}	0.615 ^{def}	3.00 ^{cde}	0.040 ^{cde}	12.3 ^{b-e}	0.631 ^{bc}	0.150 ^{gh}	8.01 ^{bc}	0.192 ^h
	C	7.98 ^{d-g}	34.0 ^{fg}	0.759 ^{a-d}	3.56 ^{bcd}	0.059 ^a	14.1 ^{ab}	0.164 ^j	0.457 ^{ef}	3.96 ^{efg}	0.260 ^{efg}
<i>Pleurotus sajor-caju</i>	A	5.04 ^{hij}	50.8 ^{de}	0.580 ^{d-g}	2.55 ^{d-g}	0.054 ^{abc}	11.3 ^{de}	0.654 ^b	0.898 ^b	10.1 ^b	0.226 ^{fgh}
	B	7.59 ^{efg}	87.5 ^a	0.530 ^{efg}	0.917 ^{kji}	0.010 ^f	16.0 ^a	0.421 ^{def}	0.448 ^f	4.22 ^{efg}	0.245 ^{fgh}
	C	11.0 ^{bc}	32.8 ^{f-i}	0.930 ^a	4.00 ^{bc}	0.041 ^{b-f}	14.0 ^{abc}	0.633 ^{bc}	0.682 ^c	6.91 ^{cd}	0.585 ^a
Substrate A	A	6.67 ^c	152 ^a	0.444 ^b	3.24 ^b	0.082 ^a	50.6 ^a	1.10 ^a	0.500 ^b	6.73 ^a	1.03 ^a
Substrate B	B	30.0 ^b	165 ^a	0.730 ^a	2.17 ^c	0.078 ^{ab}	53.8 ^a	0.604 ^b	0.647 ^a	4.61 ^b	0.790 ^b
Substrate C	C	92.3 ^a	115 ^b	0.701 ^a	6.00 ^a	0.067 ^b	46.2 ^a	1.18 ^a	0.020 ^c	4.47 ^b	0.961 ^{ab}

$n=3$; identical superscripts (a, b, c...) denote non-significant differences between means in columns for mushroom species and particular substrates separately according to the post-hoc Tukey's HSD test

effective mineral enrichment of fruit bodies. The reverse situation was observed for *P. sajor-caju*, where a significantly higher content of Cu, Hg, Ir, Nd, ni, Pb, Pr, Ru and Te was present in fruit bodies from substrate C than those from substrate B with a higher content of Al, Fe and Tm only. It is especially interesting that the higher concentration of selected elements in substrates was not correlated with their higher content in particular mushroom species, or such a relationship was present only in selected mushroom species.

Correlations between the concentration of particular elements in substrates and their content in the fruit bodies of the studied *Pleurotus* species were calculated to show whether the increase in the concentration of elements in the substrate forces an increase in their accumulation by fruit bodies. That such a calculation was necessary is due to problem of data interpretation (similarities and differences in the content of particular elements with higher, similar or lower content in fruit bodies growing on particular substrates). Significantly positive correlations ($\alpha=0.05$) for Fe ($r_s=0.960$), Rb ($r_s=0.908$), Sn ($r_s=0.986$), Te ($r_s=0.966$) in *P. cytidious*; Ca ($r_s=0.999$), Ir ($r_s=0.950$), Ni ($r_s=0.968$) and Ru ($r_s=0.936$) in *P. djamor*; Na ($r_s=0.941$), Al ($r_s=0.987$), Fe ($r_s=0.999$), Sn ($r_s=0.921$) and Tm ($r_s=0.975$) in *P. o. var. florida*; Cd ($r_s=0.990$) and Ni ($r_s=0.986$) in *P. ostratus*; K ($r_s=0.981$), Mg ($r_s=0.994$), Nd ($r_s=0.999$), Ni ($r_s=0.961$)

and Ru ($r_s=0.959$) in *P. pulmonarius* and also Be ($r_s=0.997$), Fe ($r_s=0.967$) and Ru ($r_s=0.936$) in *P. sajor-caju* were determined. On the other hand, significantly negative correlations for Ni ($r_s=-0.968$) and P ($r_s=-0.940$) in *P. cystidiosus*; P ($r_s=-0.949$), Pr ($r_s=-0.997$), Rb ($r_s=-0.971$) and Te ($r_s=-0.916$) in *P. djamor*; Rb ($r_s=-0.999$) and Te ($r_s=-0.986$) in *P. o. var. florida*; Mg ($r_s=-0.978$), Na ($r_s=-0.969$), Al ($r_s=-0.999$), Ir ($r_s=-0.999$) and Sn ($r_s=-0.927$) in *P. ostratus*; Cr ($r_s=-0.999$), Pr ($r_s=-0.938$) and Rb ($r_s=-0.903$) in *P. pulmonarius* and also Mg ($r_s=-0.973$), Cd ($r_s=-0.936$), Mn ($r_s=-0.983$) and Ni ($r_s=-0.988$) in *P. sajor-caju* were determined. The obtained results clearly indicated that the efficiency of element accumulation depends on their concentration in the substrate (positive values of r_s), but also depends on the mushroom species and other factor(s), where concentration of nitrogen form may also have a significant impact (negative r_s values).

Importance of substrate enrichment for the mineral composition of mushroom species

Pleurotus species growing on particular substrates were characterized by diverse mineral composition but to explain which of them was characterized by the highest content of

Table 3 Content of trace elements (mg kg⁻¹) in *Pleurotus* species and substrates used in experiment

Species/substrates	System	Rb	Ru	Sb	Si	Sn	Sr	Te	Tm	Zn
<i>Pleurotus cystidiosus</i>	A	33.5 ^{abc}	1.09 ^{c-f}	52.8 ^{c-f}	4.89 ^{efg}	1.81 ^{de}	3.10 ^{e-i}	9.74 ^{bc}	0.097 ^{hij}	117 ^{ab}
	B	35.2 ^{abc}	1.31 ^{bc}	68.1 ^{ab}	3.64 ^{gh}	2.35 ^d	2.51 ^{ij}	8.78 ^{cd}	0.084 ^{ijk}	92.8 ^{c-f}
	C	35.5 ^{abc}	0.956 ^{ef}	53.2 ^{c-f}	5.58 ^{def}	0.765 ^{fgh}	3.14 ^{e-i}	11.4 ^a	0.125 ^{fg}	106 ^{a-d}
<i>Pleurotus djamor</i>	A	37.8 ^{ab}	1.50 ^{ab}	48.0 ^{efg}	10.7 ^a	1.12 ^{fg}	6.38 ^a	5.74 ^{fg}	0.196 ^b	113 ^{abc}
	B	33.8 ^{abc}	1.12 ^{cde}	74.8 ^a	8.86 ^{bc}	0.111 ⁱ	4.11 ^{cd}	6.60 ^{ef}	0.138 ^{ef}	123 ^a
	C	39.4 ^{ab}	1.66 ^a	60.1 ^{b-c}	9.11 ^b	0.201 ^{hi}	3.37 ^{d-h}	8.65 ^{cd}	0.072 ^{jk}	98.4 ^{b-e}
<i>Pleurotus ostreatus</i> var. <i>florida</i>	A	32.1 ^{bc}	0.906 ^{efg}	62.8 ^{a-d}	3.37 ^h	0.537 ^{ghi}	2.83 ^{ghi}	3.26 ^{ij}	0.071 ^k	78.2 ^{fgh}
	B	32.7 ^{abc}	0.691 ^g	44.2 ^{fgh}	3.31 ^h	0.123 ⁱ	2.91 ^{f-i}	8.07 ^{de}	0.233 ^a	66.4 ^h
	C	41.0 ^a	0.217 ^h	51.0 ^{c-g}	1.16 ⁱ	0.140 ⁱ	1.68 ^j	11.7 ^a	0.106 ^{ghi}	70.2 ^{gh}
<i>Pleurotus ostreatus</i>	A	28.5 ^c	1.03 ^{ef}	73.6 ^a	3.33 ^h	0.597 ^{ghi}	3.81 ^{cde}	1.33 ^k	0.188 ^{bcd}	79.9 ^{e-h}
	B	39.2 ^{ab}	1.02 ^{ef}	42.3 ^{fgh}	6.17 ^d	1.25 ^{ef}	5.10 ^b	2.88 ^{ij}	0.163 ^{de}	108 ^{a-d}
	C	35.1 ^{abc}	1.07 ^{def}	68.5 ^{ab}	5.78 ^{de}	4.03 ^b	3.74 ^{c-f}	3.94 ^{hi}	0.165 ^{cd}	88.3 ^{d-g}
<i>Pleurotus pulmonarius</i>	A	37.3 ^{ab}	1.08 ^{def}	63.4 ^{abc}	4.48 ^{fgh}	1.11 ^{fg}	3.18 ^{e-i}	2.02 ^{jk}	0.199 ^b	110 ^{abc}
	B	31.6 ^{cb}	0.880 ^{fg}	53.3 ^{c-f}	7.67 ^c	0.983 ^{fg}	2.46 ^{ij}	3.08 ^{ij}	0.038 ^l	89.2 ^{d-g}
	C	37.0 ^{ab}	1.29 ^{bcd}	45.5 ^{fgh}	5.70 ^{def}	3.09 ^c	3.41 ^{d-g}	5.08 ^{gh}	0.117 ^{fgh}	105 ^{a-d}
<i>Pleurotus sajor-caju</i>	A	32.9 ^{abc}	1.09 ^{c-f}	50.4 ^{d-g}	5.04 ^{def}	10.2 ^a	4.41 ^{bc}	1.95 ^{jk}	0.139 ^{ef}	98.2 ^{b-e}
	B	33.8 ^{abc}	1.00 ^{ef}	38.7 ^{gh}	3.73 ^{gh}	2.07 ^d	2.94 ^{f-i}	2.69 ^{ijk}	0.190 ^{bc}	105 ^{a-d}
	C	40.9 ^a	1.39 ^b	34.5 ^h	3.76 ^{gh}	1.99 ^d	2.56 ^{hi}	11.2 ^{ab}	0.101 ^{ghi}	96.5 ^{c-f}
Substrate A	A	32.5 ^b	1.42 ^b	64.6 ^a	19.3 ^a	2.15 ^a	47.5 ^a	6.76 ^c	0.276 ^a	24.0 ^{ab}
Substrate B	B	42.7 ^a	0.765 ^c	68.5 ^a	16.3 ^a	1.38 ^b	13.3 ^b	8.71 ^b	0.158 ^b	21.2 ^b
Substrate C	C	40.8 ^a	1.92 ^a	50.6 ^b	15.8 ^a	0.028 ^c	15.1 ^b	14.9 ^a	0.130 ^b	25.2 ^a

$n=3$; identical superscripts (a, b, c...) denote non-significant differences between means in columns for mushroom species and particular substrates separately according to the post-hoc Tukey's HSD test

Fig. 3 Principal Component Analysis for all studied *Pleurotus* species concerning the content of macro- (a), trace (b) and all elements jointly (c)

determined elements, a PCA was calculated separately for macro- (Fig. 3a), trace (Fig. 3b) and all elements jointly (Fig. 3c).

According to data presented in Fig. 3a, in which 66.8% (44.6+22.2) of total variability was explained, *Pleurotus* species growing on substrate C were distributed on the right side of the diagram with the exception of *P. ostreatus*, which indicates that this *Pleurotus* species is characterized

by a relatively high content of macroelements. On the other hand, distribution of *Pleurotus* species growing on substrate B mainly at the bottom of the diagram suggest that fruit bodies of *P. cystidiosus*, *P. djamor*, *P. pulmonarius*, *P. sajor-caju* in particular but also *P. ostreatus*, placed on the left side of diagram, confirm the generally higher content of macroelements in these species. It is worth emphasizing that the location of *P. cystidiosus* and *P. sajor-caju* growing on substrate

A indicates that these species might contain the highest content of all macroelements jointly. PCA for trace elements explained 38.61% ($20.63 + 17.98$) of total variability with a clear presentation of studied *Pleurotus* species placed mainly at the top of the diagram. This suggests that the majority of them accumulate selected trace elements only in higher amounts (e.g., Al in *P. pulmonarius*, Fe in *P. sajor-caju*, or Pb in *P. ostreatus*). Similar observations for mushrooms growing on substrate B were observed, while all studied *Pleurotus* species growing on substrate C were placed at the bottom of the diagram. Thus it can be assumed that growth of species on substrate C led to the uptake of only selected elements to their fruit bodies. This is especially interesting in the case of selected elements such as Ni, where a high accumulation in relation to a relatively low content of this metal in substrate C (barely 0.020 mg kg^{-1}) was recorded. With respect to the transformation of a 29-dimensional system to a 2-dimensional picture in Fig. 3c, PCA for all determined elements explained only 36.26% ($19.39 + 16.87$) of the total variability. In this case, the distribution of mushroom species growing on particular substrates was similar to that

presented in Fig. 3b, with a slight displacement of points, as regards macroelemental content (Fig. 3a).

For improved visualization of similarities/differences between *Pleurotus* species, a Heatmap with a cluster analysis was performed separately for macro- (Fig. 4a), trace (Fig. 4b) and all elements jointly (Fig. 4c). In the case of macroelements, four groups of *Pleurotus* species were marked, the first included: *P. cystidiosus*, *P. o. var. florida* and *P. djamor* from substrate C; the second: *P. cystidiosus* and *P. pulmonarius* from substrate B; the third: *P. ostreatus* from substrates A, B, C and *P. sajor-caju* from substrate B; the fourth group: the remaining species (Fig. 4a).

All mushroom species were divided into five groups with a similar accumulation of all trace elements jointly: (1) *P. cystidiosus*, *P. djamor* and *P. ostreatus* growing on substrate C; (2) *P. djamor* from substrates A and B with *P. sajor-caju* from substrate C; (3) *P. cystidiosus* and *P. o. var. florida* from substrates A and B with *P. ostreatus* from substrate A; (4) *P. o. var. florida* from substrate C; (5) the rest of the species (Fig. 4b). A Heatmap prepared for all determined elements jointly allowed five individual groups of *Pleurotus* species

Fig. 4 Correlations between studied *Pleurotus* species concerning the content of macro- (a), trace (b) and all elements jointly (c) (heatmap) in mean values with presentation of a hierarchical tree plot

to be distinguished: (1) *P. o. var. florida* from substrate A, *P. pulmonarius* from substrates A, B and C with *P. sajor-caju* from substrate A; (2) *P. cystidiosus* from substrates A and B with *P. o. var. florida* from substrate B; (3) *P. ostreatus* from substrates A, B, C with *P. sajor-caju* from substrate B; (4) *P. o. var. florida* from substrate C; (5) the remaining species (Fig. 4c).

To summarize the results described in the visual presentations and estimate which of the studied *Pleurotus* species contained the highest amount of all elements belonging to particular groups of elements, a rank-sum was calculated.

According to data shown in Fig. 5, fruit bodies of *P. sajor-caju* and *P. cystidiosus* growing on substrate A contained the highest amount of all macroelements jointly, while the lowest content was observed in *P. cystidiosus*, *P. djamor*, *P. o. var. florida* and *P. sajor-caju* from substrate C.

The *Pleurotus* species most enriched with trace elements were *P. sajor-caju* from substrate C and *P. djamor* growing on substrates B and A, while the lowest content of these elements was recorded in *P. o. var. florida* fruit bodies growing on all forms of substrate. In the case of the rank-sum calculated for all elements jointly, the most enriched species

Fig. 5 Graphical presentation of rank sum according to increase of the macro- (a), trace (b) and all elements jointly (c) in the studied *Pleurotus* species growing on particular substrates

were *P. djamor* growing on substrates B and A and *P. sajor-caju* from substrate A, while the least enriched was *P.o. var. florida* from substrates A, B and C. The obtained results show that cultivation of *Pleurotus* species on substrates with a different share of ammonium and nitrate may be the cause of changes in the mineral composition of mushroom species in spite of the similar content of the majority of detectable elements in substrates.

Discussion

The selection of the substrate for mushroom cultivation is a pivotal step to obtain a satisfactory yield of fruit bodies. Such substrate can be further modified chemically by the addition of particular compounds to increase nutritional value, obtained biomass or even biological properties. Various studies have tested the effect of different types of substrate or their supplementation with mineral salts on the outcome of the cultivation of *Pleurotus* mushrooms [24–27]. The present research further investigated whether changes in the source of N in the substrate can modify yield, macroscopic appearance of fruit bodies, and the mineral content of selected cultivable species from the *Pleurotus* genus. Although the effects of N availability on mushroom growth were already subject to some previous studies [28, 29] regarding the *Pleurotus* genus, only limited observations have been reported for *P. ostreatus*, *P. sajor-caju* and *P. eryngii* [15, 30, 31]. Here, a direct comparison of the growth, appearance, and macro- and trace element composition of six species in the presence of an increased substrate level of N is presented for the first time.

Earlier studies have shown that some N compounds can significantly increase the growth of mushrooms belonging to this genus. For example, *P. sajor-caju* sustained the highest growth of mycelium in the presence of N originating from yeast extract, casein and glycine [30]. *P. ostreatus*, in turn, displayed the highest growth of mycelium in the presence of ammonium citrate or yeast extract [32]. Increased biomass of *P. ostreatus* fruit bodies was also observed following the addition of ammonium salts to the substrate, in this case Mn(II) was simultaneously supplemented [33]. The yield of *P. o. var. florida* was also increased following the addition of ammonium sulfate to a substrate based on corn cub [34]. This enhancement may be due to the increased activity of ligninolytic enzymes that play a key role in white-rot fungi in the utilization of lignocellulosic biomass. However, some of these fungi, e.g., *Phanerochaete chrysosporium*, revealed higher ligninolytic properties when N-starved [35], various other species from this group, including *P. ostreatus*, have been shown to increase such properties when additional sources of this element were available [36–38].

The present study, which focused on the biomass of yielded fruit bodies, demonstrated that the addition of N in the form of ammonium or nitrate salts to substrate based on wheat straw and bran did not increase the biomass of any of the tested mushroom species, including *P. o. var. florida*, which produced the highest yield from all studied *Pleurotus* species. These results indicate that ammonium and nitrate salts are not the most suitable sources for N supplementation for increasing the commercial production of various *Pleurotus* mushrooms. We hypothesize that ammonium and nitrate nitrogen may be too readily available, particularly if applied prior to spawning, to produce a beneficial effect on fruitification. It may, therefore, be more suitable to employ the chemical formulas that slowly release N to the substrate or increase N availability when the substrate is already overgrown by the mycelium. This would, however, require further verification. One should also note that in the case of *P. sajor-caju*, growth on ammonium-rich substrate resulted in a lower yield of fruit bodies when compared to the results from cultivation on nitrate-rich and unsupplemented media. All in all, the present study suggests that the addition of inorganic N compounds to the wheat substrate at the end of its preparation will not lead to satisfactory results regarding the production of *Pleurotus* mushrooms. These findings should be taken into account when considering the modification of the cultivation process of these, increasingly economically important species.

The cultivation of mushrooms on the compared substrates, varying in chemical composition has, unsurprisingly, resulted in different contents of macro- and trace elements in the fruit bodies. It is known that uptake of elements by mycelium and further translocation to the fruit bodies depends predominantly on their ambient levels, i.e., the overgrown substrate [39–41]. It may, however, reveal significant interspecies differences in this regard, also between species representing the same genus [27, 42–44]. The present study compared the content of essential macroelements (Ca, K, Mg, Na, and P), essential trace elements (Cr, Cu, Fe, Mn, Si, and Zn), and toxic elements (Al, Cd, Ni, Hg, and Pb). Considering that, with the exception of *P. sajor-caju*, the investigated *Pleurotus* species did not reveal differences in yield regardless of the applied substrate, it is of interest to evaluate whether some beneficial modifications with respect to their nutritional value were observed, but also to assess if the application of some substrates led to increased accumulation of toxic elements.

In the case of macroelements K, Mg, Na, and P, the highest contents were usually found in fruit bodies grown and harvested from cultivation on substrate B, and this closely reflected their levels in the substrate. The exception was the level of Ca, which, out of all the investigated *Pleurotus* species, was the highest when grown on the Ca-richest substrate A. However, the level of trace elements in the fruit bodies

did not always follow their content in the substrate with the exception of Cu. For example, the content of Zn was the highest for *P. djamor*, *P. ostreatus*, and *P. sajor-caju* when cultivated on substrate B, which was characterized by the lowest level of this element. One should also note that differences in the level of minerals such as Cr, Zn, Cu, Fe, and Mn in the fruit bodies were too low to recommend the addition of agricultural fertilizer or salt solution to the substrate to obtain a relevant nutritional enhancement.

The content of toxic elements varied in the applied substrates. The highest levels of Al were found in substrate B, enriched with the addition of commercial fertilizer, and this was reflected by the increased content of this metal in the fruit bodies of all *Pleurotus* species with *P. pulmonarius* revealing the highest level. Similarly, Cd levels were the highest for unsupplemented substrate A and substrate B that was supplemented with a salt solution, and species grown under such conditions exhibited the highest accumulation of this element. A content of Cd exceeding 2.0 mg kg^{-1} , which is the maximum level set for cultivated mushrooms by the European Food Safety Authority, was exceeded in the case of *P. cystidiosus* grown on A and B substrates, *P. ostreatus* and *P. sajor-caju* grown on A substrate, and *P. djamor* grown on all substrates [45]. Importantly, decreased Cd contents were mostly found for fruit bodies resulting from cultivation on C substrate, which was also found to contain the lowest level of this metal. Assuring a low level of Cd in foodstuffs is of high importance, given that it is classified as a human carcinogen [46]. Rather disturbing results were observed regarding the content of Pb in the fruit bodies. For the majority of studied species, including *P. ostreatus*, its levels exceeded the maximum threshold of 3.0 mg kg^{-1} set by the EFSA regardless of the applied substrate [45]. Increased Pb content in commercially available *P. ostreatus* was already reported previously [27]. Moreover, in the present study, the decidedly highest content of this metal was found for this species when grown on substrate B, which was not characterized by the highest Pb levels. This indicates that under specific conditions Pb uptake and translocation can be promoted. This therefore highlights the need to further monitor the content of this metal not only in substrates used for cultivation but also in *P. ostreatus* fruit bodies that are available for consumers. The significantly increased content of Hg in the substrate B and C is a worrisome finding, but interestingly, it was not always reflected by the highest Hg content in the fruit bodies of cultivated mushrooms. The highest level of this toxic metal across all the species and applied substrates was found for *P. djamor* grown on substrate A which was characterized by at least 35% lower Hg content when compared to substrates B and C. This confirms the previous research in which very high translocation of externally applied Hg in *P. djamor* was

observed [47]. In the case of Ni, the lowest accumulation was found for *P. ostreatus*, which is the most commonly cultivated *Pleurotus* species in the world [48, 49]. Interestingly fruit bodies of *P. o. var. florida* revealed significantly higher levels of this metal, indicating some relevant differences in Ni uptake in this variation. With the exception of *P. ostreatus*, all species grown on substrate C, which revealed several-fold lower Ni levels, had a tendency to uptake and accumulate high contents of this element in fruit bodies. Although there are no regulations regarding the levels of Ni in food, its increased content in some foodstuffs is of concern and highlights the need for monitoring this element to decrease dietary exposures [50–52].

Although chemical modifications of the substrate can potentially lead to beneficial results regarding the yield and/or nutritional value of the cultivated mushrooms, they can sometimes result in macroscopic changes to the fruit bodies. These alterations can include changes in shape or color and can eventually affect consumer acceptance and value of the final product [53–56]. In the present study, none of the tested *Pleurotus* species exhibited any visible changes to the fruit bodies regardless of the employed substrate. This also indicates that despite an increased content of toxic elements, the yield and appearance of fruit bodies may not always be affected.

In summary, the present study was the first to compare the growth of six different *Pleurotus* mushroom species cultivated under typical industrial conditions on three wheat substrates: unsupplemented, enriched with agricultural fertilizer, and high in ammonium, and enriched with salt solution and high in nitrate nitrogen. As demonstrated, the chemical modification of substrates did not result in a beneficial increase in yield of fruit bodies, while in the case of *P. sajor-caju*, the cultivation on the ammonium-rich substrate led to a decreased production of biomass. The macroelemental composition of fruit bodies followed that in the substrate and was increased in the case of K, Mg, Na, and P when fertilizer was added. Such a trend was not often the case for essential trace elements, while the observed differences in their content in the fruit bodies were not significant enough from a dietary perspective. Importantly, the study highlighted that some *Pleurotus* species might tend to accumulate toxic elements despite their low substrate content—this was confirmed in the case of Pb for *P. ostreatus* and in the case of Ni for *P. cystidiosus*, *P. djamor*, *P. o. var. florida*, *P. pulmonarius* and *P. sajor-caju*. Selected species revealed a Cd content exceeding the maximum allowance level in the European Union. All in all, the present study concludes that using inorganic sources of N, such as ammonium and nitrates, may not result in significant growth enhancement or lead to beneficial changes in the mineral content of fruit bodies, while in some instances it may support the accumulation of selected toxic elements.

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Compliance with ethical standards

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

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

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