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Instrumental Analysis of Metals Profile in Poison Pax (*Paxillus involutus*) Collected at Two Sites in Bory Tucholskie

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Using validated analytical procedure and applying final ICP–AES measurement, concentration profiles of 18 metallic elements were determined in Poison Pax and soil underneath the fruit bodies. Mushrooms were collected at two distant sites nearby to the Ocypel and Osiek villages in Bory Tucholskie (Tuchola forest complex). In their caps or stipes the following elements were bio-concentrated: Ag, Ca, Cu, K, Mg, Na, Rb and Zn, while Al, Ba, Co, Cr, Fe, Mn, Ni, Pb and Sr were bio-excluded. The forest soil surface layer underneath the fruit bodies at both sampling sites contained respectively „pseudo-total" and „labile" forms of Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Rb, Sr and Zn ($p > 0.05$; U Mann-Whitney test) in similar concentrations. Poison Pax from Ocypel, compared to that from the Osiek site, exhibited enhanced bioconcentration properties and showed higher concentrations of Ca and Cr in caps/stipes and of Ba and Sr in stipes ($p < 0.05$; U Mann-Whitney test). Mycelium of Poison Pax, compared to diluted (20%) HNO_3, which extracted "labile" forms of metals, was a better medium for extraction of Ag, Ca, Cu, K, Mg, Na, Rb and Zn from the surface soil horizon, but not for Al, Ba, Co, Cr or Fe.

W oparciu o sprawdzoną metodę analityczną i pomiar techniką ICP–AES określono profil stężeń 18 pierwiastków metalicznych w owocnikach krowiaka podwiniętego i w glebie spod grzybów. Grzyby pochodzące z dwóch oddalonych od siebie stanowisk w pobliżu wsi Ocypel i Osiek w Borach Tucholskich nagromadzały w kapeluszach i trzonach pierwiastki takie jak Ag, Ca, Cu, K, Mg, Na, Rb i Zn, a wykluczały Al, Ba, Co, Cr, Fe, Mn, Ni, Pb i Sr. Gleba z obu miejsc pochodzenia grzybów zawierała w warstwie powierzchniowej podobne stężenia odpowiednio „pseudo-całkowitych" i „labilnych" form Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Rb, Sr i Zn ($p > 0.05$; U Mann-Whitney test). Okazy krowiaka pod-winiętego z okolicy Ocypla w porównaniu z tymi z okolicy Osieka charaktyryzowały się wydajniejszą bioakumulacją i zawierały większe stężenie Ca i Cr w kapeluszach i trzonach oraz Ba i Sr w trzonach ($p < 0.05$; U Mann-Whitney test). Grzybnia krowiaka podwiniętego w porównaniu z rozcieńczonym (20%) roztworem kwasu azotowego jest lepszym medium ekstrahującym dla „labilnych" form Ag, Ca, Cu, K, Mg, Na, Rb i Zn z wierzchniej warstwy gleby, ale nie dla Al, Ba, Co, Cr czy Fe.

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Fungi of different types are important terrestrial biotic factors involved in biogeochemical cycling of chemical elements, especially in the forest environment. Mycorrhizal fungi take part in the cycling of nutrients in the soil as well as in mineral weathering and dissolution of insoluble metal and metalloid compounds by protonation (acidolysis), chelation (complexation) and accumulation; they may influence efficiency of translocation of metallic mineral constituents to the symbiotic plants and their accumulation there [1]. Higher fungi (macromycetes) are food to humans and many animals [2, 3]. Hence, the knowledge of the content of metallic elements and metalloids in the fruit bodies – those nutritionally vital as well as problematic ones – is important and required [4–10].

Poison Pax is a mushroom able to solubilize hardly soluble zinc phosphate and therefore it can uptake both zinc and phosphate from the soil [11]. The fruit body of this fungus can be rich in accumulated K, Mg, Ca, Na, Al and Rb, and also relatively rich in Zn (~ 100–200 μg g^{-1} of a dry weight), Cu (~ 30 μg g^{-1} dw), Mg (~ 20–50 μg g^{-1} dw) or Fe (~ 50–200 μg g^{-1} dw), but poor in many other metallic elements, including toxic Cd, Hg or Pb [12–14].

Mycelium is an absorbing medium that efficiently uptakes mineral constituents even from hardly-soluble compounds in the soil and translocate metal ions both to symbiotic plants and to its own fruit body. On the molecular level, in the process of metal detoxification fungi can employ chelation of metal ions in the cytosol with thiol-containing compounds, such as glutathione, phytochelatins (small cysteine-rich peptides), or metallothioneins [1]. Poison Pax, if exposed to cadmium, could bind this element via complexing peptides, such as metallothioneins [15]. This mushroom, as an ectomycorrhizal species, increases the tolerance of its symbiotic partner to certain metals, *e.g.* zinc, cadmium, lead [16–18]. This, in turn, can decrease metal toxicity to the plant partner and is a key feature for the use of the fungi and the accompanying plants in the process of phytoremediation of soils contaminated with metals. The symbiotic dendro-partner of Poison Pax is pine, spruce, birch, alder and/or oak [20].

Poison Pax, known also as a common roll-rim, brown roll-rim, or in-rolled paxil (*Paxillus involutus*) (Batsch ex Fr.) Fr is a poisonous fungus [19]. In Poland, before the World Word II and shortly thereafter, due to the lack of certain knowledge on toxicological risks, Poison Pax was officially considered as an edible or conditionally edible mushroom and was frequently collected by the persons fond of mushrooms. In earlier times, the fruiting bodies of Poison Pax always had to be 2–3-times boiled before cooking and eating them. Starting from early 1960's, due to the extensive education about its poisonous properties and mass-media warnings, collecting of Poison Pax became in Poland highly unpopular.

Some data on metallic elements and metalloids contained in Poison Pax have been reviewed recently [12–14, 21–34]. Apart from Hg, the information on bioconcen-

tration of Al, Cd, Cu, Fe, Mn, Pb and Zn in this fungus is limited and there is no data about the contents of other metallic elements. The studies on the accumulation potential of fungi towards metallic elements, metalloids and radionuclides as well as documentation of mineral elements contents in edible and inedible fungi are important in order to know the amounts, intake rates and time trends of chemical species related to anthropogenic emission and environmental pollution. For the purpose of this study, the specimens of Poison Pax were collected at two distant sites in Bory Tucholskie in the northern part of Poland.

EXPERIMENTAL

Poison Pax (*Paxillus involutus*) and soil (surface layer 0–15 cm; ~ 100 g) underneath the fruit bodies were collected at the Ocypel and Osiek sites in Bory Tucholskie (Tucholskie forests) in the northern part of Poland in 2001. The sampling sites were distant one from another by *ca* 25 km. 15 pooled samples (5–6 specimens in a pool) of well-grown and roughly similar size fruit bodies of Poison Pax and corresponding soil samples were collected over a relatively large area of *ca* 5–7 km^2 to avoid excessive local sampling.

The mushrooms, after cleanup from debris with a plastic knife, were air-dried for several days and further dried in an electric oven at 40°C to the constant weight, and pulverized in an agate mortar. Subsamples (*ca* ~ 1 g) of powdered mushrooms were wetted with 10 mL of concentrated HNO_3 in polytetrafluoroethylene (PTFE) vessels and left for 24 h. Afterwards, the PTFE vessels were closed and the mixture was digested in a microwave digestion system, type MARS 5 (Microwave Accelerated Reaction System, CEM Corp., Matthews, NC, USA). With each of 50 mushroom samples digested daily, 2 blank (reagents and glassware) samples were run. The digest was further diluted to 25 mL in deionised water.

Soil samples, after removal of plants, small stones and visible organisms, were dried under clean conditions for approximately 10 weeks at the room temperature and further in electrically-heated laboratory oven at 40°C for 48 h. In these soils samples both "pseudo-total" and "labile" metallic elements content was determined. The content of "pseudo-total" metallic elements in the soil was determined following the method of Sastre *et al.* [35]. Briefly, each 1.0 g-in-weight dried soil sample was initially cold-digested with 15 mL of concentrated (65%) HNO_3 solution in a Pyrex glass round-bottom flask for 16 h. Next, after fixing the flask to the partial condenser (30-cm long) and a water cooler, the mixture was heated at 140°C for 2.5 h. After the flask was cooled, the digest was filtered using a Whatman filter No. 42, diluted to 50 mL, transferred to a polyethylene bottle, and kept at 4°C until the instrumental analysis was performed. The content of "labile" metallic elements in the soil was determined following the method by Kučak and Blanuša [36]. In brief, each 2.5 g-in-weight dried soil sample was initially cold-digested using 20% HNO_3 (analytical grade) solution (2.5 mL of 65% HNO_3 and 5 mL of redistilled water) in a quartz baker for 24 h. Next, the mixture was diluted with redistilled water until HNO_3 concentration reached 10% (by completing the volume up to 25 mL with water). The digest was further filtered directly into a polyethylene bottle using a Whatman filter No. 42 and stored at 4°C until the instrumental analysis using an inductively coupled plasma atomic emission spectrometer (ICP–AES), model Optima 2000 2000™ DV Perkin–Elmer, Waltham, Massachusetts, USA, was performed. Yttrium was used as an internal standard. Each spectroscopic measurement for each individual sample was repeated in triplicate. Detection limits were as follows: K, Mg, Na – 5 $\mu g\ g^{-1}$; Rb – 1 $\mu g\ g^{-1}$; Al, Ag, Ca, Cd, Co, Cr, Fe, Ni, Zn, Pb – 0.1 $\mu g\ g^{-1}$; Ba, Cu, Mn, Sr – 0.05 $\mu g\ g^{-1}$.

pH of the soil was determined following the method of Musgrove [37]. In brief, to a 10 g-in-volume aliquot of the air-dried soil sample 50 mL of distilled water were added and the mixture was left for 1 h at 25°C. Thereafter, pH was determined using a pH meter (EC20 pH/ISE Meter, MODEL 50050, Hach Company, Ames, Iowa, USA). Organic carbon content in the soil was determined gravimetrically after combustion of the organic matter in 10 g of air-dried soil at 800°C in a laboratory furnace oven (SNOL 8,2/1100, Lithuania).

Determination method of the elements was validated and controlled on several occasions by participation in the international calibration activities and analyses, *e.g.* GESM/Food Euro proficiency testing exercise, International Atomic Energy Agency (IAEA) trials, Aquacon Project 9 "Soil Analysis", and analysis of certified plant material and within-run reproducibility control [22, 38–40].

Concentrations of the metals in the fruiting bodies did not exhibit Gaussian distribution. Statistical analyses with non-parametric tests were performed using Statistica (StatSoft) software.

RESULTS AND DISCUSSION

Mean, standard deviation, minimum and maximum (range), and median values of the concentrations of metallic elements determined in caps and stipes of Poison Pax and in soil are presented in Table 1. Concentrations data for a particular element present in the Poison Pax caps and stipes and for it "pseudo-total" and "labile" fractions in the soil lie within relatively narrow ranges for both sampling sites.

Table 1. Content of chemical elements in Poison Pax and soil [μg g^{-1} dry weight; mean, SD, range and median values] collected at the Osiek and Ocypel sites in Tuchola Forests

Element	Matrix	Site and concentration	
		Tucholskie F., Osiek, 2001 n = 15	Tucholskie F., Ocypel, 2001 n = 15
K	Cap	47000 ± 7000 (34000–57000) *48000*	56000 ± 8000 (44000–75000) *55000*
	Stipe	42000 ± 8000 (3000–62000) *41000*	41000 ± 13000 (15000–59000) *44000*
	Soil (pseudo total)	4000 ± 1100 (3000–7800) *4800*	3300 ± 2000 (1800–7300) *3400*
	Soil (extractable)	51 ± 28 (20–87) *44*	43 ± 35 (21–12) *48*
Mg	Cap	1300 ± 100 (93–1400) *1300*	1500 ± 100 (1300–1800) *1500*
	Stipe	940 ± 150 (630–1200) *920*	840 ± 210 (570–1500) *790*
	Soil (pseudo total)	4300 ± 1400 (2700–6400) *4500*	3200 ± 1600 (2200–6000) *3600*
	Soil (extractable)	100 ± 60 (38–170) *82*	110 ± 60 (70–220) *75*

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Table 1 (Continuation)

Element	Matrix	Site and concentration	
		Tucholskie F., Osiek, 2001 n = 15	Tucholskie F., Ocypel, 2001 n = 15
Ag	Cap	0.21 ± 0.10 (0.11–0.35) *0.20*	0.30 ± 0.08 (0.16–0.42) *0.30*
	Stipe	0.15 ± 0.10 (0.10–0.25) *0.16*	0.20 ± 0.08 (0.11–0.35) *0.20*
	Soil (pseudo total)	< 0.1	< 0.1
	Soil (extractable)	< 0.1	< 0.1
Al	Cap	41 ± 33 (17–150) *28*	38 ± 19 (22–99) *32*
	Stipe	35 ± 19 (18–90) *27*	150 ± 60 (62–270) *130*
	Soil (pseudo total)	35000 ± 6000 (27000–45000) *35000*	33000 ± 2000 (24000–39000) *34000*
	Soil (extractable)	1300 ± 640 (710–2000) *1200*	1400 ± 300 (600–1600) *1500*
Ba	Cap	0.32 ± 0.21 (0.12–0.91) *0.33*	0.42 ± 0.22 (0.24–1.0) *0.43*
	Stipe	0.52 ± 0.31 (0.34–1.1) *0.45*	3.3 ± 2.2 (0.7–7.2) *2.6*
	Soil (pseudo total)	95 ± 22 (48–170) *89*	91 ± 32 (49–210) *74*
	Soil (extractable)	8.0 ± 1.0 (4.0–14) *7.5*	7.6 ± 3.3 (4.3–21) *6.1*
Ca	Cap	100 ± 53 (25–210) *100*	240 ± 150 (93–580) *230*
	Stipe	230 ± 110 (85–480) *210*	680 ± 330 (260–1400) *630*
	Soil (pseudo total)	2900 ± 400 (1900–5800) *2900*	2600 ± 1100 (1900–4800) *2500*
	Soil (extractable)	150 ± 27 (54–230) *150*	180 ± 120 (87–390) *140*
Cd	Cap	< 0.1	< 0.1
	Stipe	< 0.1	< 0.1
	Soil (pseudo total)	< 0.1	< 0.1
	Soil (extractable)	< 0.1	< 0.1

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Table 1 (Continuation)

Element	Matrix	Site and concentration	
		Tucholskie F., Osiek, 2001 n = 15	Tucholskie F., Ocypel, 2001 n = 15
Co	Cap	< 0.1	< 0.1
	Stipe	< 0.1	< 0.1
	Soil (pseudo total)	7.3 ± 2.0 (4.1–12) *8.1*	6.1 ± 2.0 (5.0–11) *6.2*
	Soil (extractable)	0.41 ± 0.22 (0.23–0.80) *0.43*	0.42 ± 0.14 (0.31–0.60) *0.35*
Cr	Cap	0.18 ± 0.05 (0.11–0.28) *0.16*	0.39 ± 0.06 (0.29–0.46) *0.40*
	Stipe	0.14 ± 0.02 (0.11–0.17) *0.14*	0.29 ± 0.09 (0.12–0.41) *0.32*
	Soil (pseudo total)	19 ± 4 (15–26) *20*	14 ± 7 (9.6–33) *10*
	Soil (extractable)	1.4 ± 0.6 (0.91–2.0) *1.3*	1.2 ± 0.3 (1.0–2.9) *1.1*
Cu	Cap	50 ± 11 (29–78) *48*	70 ± 12 (52–91) *68*
	Stipe	64 ± 17 (19–94) *63*	80 ± 18 (55–120) *79*
	Soil (pseudo total)	12 ± 3 (7–18) *12*	13 ± 7 (8–27) *9*
	Soil (extractable)	0.72 ± 0.20 (0.50–1.3) *0.70*	0.71 ± 0.31 (0.51–1.5) *0.63*
Fe	Cap	53 ± 22 (31–110) *45*	70 ± 45 (43–230) *56*
	Stipe	26 ± 11 (14–54) *22*	85 ± 37 (42–170) *70*
	Soil (pseudo total)	24000 ± 5000 (18000–40000) *26000*	20000 ± 5000 (15000–31000) *20000*
	Soil (extractable)	1700 ± 480 (1300–2200) *1600*	1500 ± 160 (1100–2400) *1500*
Mn	Cap	14 ± 7 (6.0–32) *13*	15 ± 8 (6.3–39) *12*
	Stipe	35 ± 20 (12–99) *30*	120 ± 62 (35–270) *110*
	Soil (pseudo total)	62 ± 23 (43–170) *56*	53 ± 27 (28–150) *39*
	Soil (extractable)	74 ± 31 (39–210) *76*	56 ± 25 (25–150) *44*

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Table 1 (Continuation)

Element	Matrix	Site and concentration	
		Tucholskie F., Osiek, 2001 n = 15	Tucholskie F., Ocypel, 2001 n = 15
Na	Cap	410 ± 240 (14–1000) *390*	840 ± 390 (340–1800) *780*
	Stipe	990 ± 660 (20–2500) *750*	3200 ± 1500 (1200–6200) *2800*
	Soil (pseudo total)	1600 ± 100 (1500–2000) *1600*	1500 ± 300 (900–2400) *1600*
	Soil (extractable)	12 ± 1 (6.3–22) *12*	12 ± 1 (9.0–14) *12*
Ni	Cap	0.40 ± 0.21 (0.22–0.90) *0.34*	0.90 ± 0.52 (0.61–2.1) *0.75*
	Stipe	0.61 ± 0.62 (0.24–2.2) *0.54*	0.84 ± 0.31 (0.62–1.5) *0.81*
	Soil (pseudo total)	16 ± 5 (11–22) *17*	14 ± 4 (12–23) *13*
	Soil (extractable)	0.62 ± 0.21 (0.4–1.1) *0.63*	0.61 ± 0.31 (0.52–1.0) *0.55*
Pb	Cap	0.51 ± 0.42 (0.13–1.4) *0.45*	2.6 ± 1.0 (1.6–4.8) *2.3*
	Stipe	0.23 ± 0.11 (0.12–0.60) *0.21*	1.3 ± 0.3 (0.80–2.2) *1.3*
	Soil (pseudo total)	28 ± 8 (15–36) *26*	19 ± 4 (13–45) *17*
	Soil (extractable)	14 ± 3 (6.5–18) *12*	9.6 ± 1.2 (5.4–16) *9.2*
Rb	Cap	460 ± 130 (240-690) *470*	670 ± 140 (380-880) *670*
	Stipe	280 ± 71 (170-390) *260*	450 ± 96 (300-590) *440*
	Soil (pseudo total)	3.5 ± 0.7 (2.7–4.5) *3.8*	3.0 ± 1.6 (2.0–6.9) *2.2*
	Soil (extractable)	0.81 ± 0.43 (0.42–1.3) *0.72*	0.74 ± 0.32 (0.5–1.6) *0.55*
Sr	Cap	0.21 ± 0.11 (0.12–0.4) *0.15*	0.30 ± 0.11 (0.2–0.3) 0.31
	Stipe	0.32 ± 0.12 (0.23–0.6) *0.30*	1.2 ± 0.5 (0.5–2.6) *1.2*
	Soil (pseudo total)	35 ± 2 (30–42) *36*	31 ± 7 (26–49) *27*
	Soil (extractable)	0.71 ± 0.12 (0.35–0.90) *0.73*	0.81 ± 0.52 (0.44–1.5) *0.65*

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Table 1 (Continuation)

Element	Matrix	Site and concentration	
		Tucholskie F., Osiek, 2001 n = 15	Tucholskie F., Ocypel, 2001 n = 15
Zn	Cap	180 ± 30 (110–230) *180*	200 ± 35 (140–260) *190*
	Stipe	180 ± 46 (52–260) *190*	n.d.*
	Soil (pseudo total)	10 ± 2 (5.7–16) *11*	9.6 ± 2.4 (7.4–17) *8.4*
	Soil (extractable)	5.8 ± 0.8 (2.8–11) *6.2*	4.7 ± 1.4 (3.7–8.4) *4.1*

* Not determined.

The forest soil surface layer underneath the fruit bodies at both sampling sites contained respectively similar „pseudo-total' and „labile" concentrations of Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Rb, Sr and Zn ($p > 0.05$; U Mann-Whitney test). At both sampling sites the soils were acidic: their pH varied between 4.1 and 5.2 for the Ocypel site and between 4.7 and 5.2 for the Osiek site. The organic carbon content (%) in the tested soils varied, respectively, in the ranges 2.2–5.3 and 4.1–5.2. Neither the pH of nor the organic carbon content in these soils correlated with the content of metallic elements determined in them and in mushrooms.

There is no doubt that hot and concentrated (65%) HNO_3 solution was a much better extracting agent for most metallic elements determined as the "pseudo-total" forms in the forest soil when compared to the results obtained after use of a cold and diluted (20%) HNO_3 solution, which extracted "labile" forms (Tab. 1). These both extracting agents exhibited similar dissolution potency only for Mg; in case of Pb and Zn, hot and concentrated HNO_3 solution was twice as powerful as the cold and diluted one. In consequence, the differences in the extraction power between these two agents could have a large impact on the calculated BCF values (Bio-Concentration Factor of the elements) for caps and stipes of Poison Pax, and depended on whether these agents were used for „labile" or „pseudo-total" forms. Dimensionless value of BCF or of the transfer factor (TF) was calculated as a ratio of a concentration of an element in dehydrated mushroom or its morphological parts to that in a dry soil substratum.

A role of cold and diluted (20%) HNO_3 solution used as the extracting agent for the "labile" forms of metallic elements present in the surface soil horizon was to mimic the extraction potential of the plants (or mushrooms) towards mobile metal compounds present in the soil, which can be readily available during vegetation. It is known that "availability but not the total content matters". Certainly, Poison Pax is

a much better biological extraction medium for K, Ag, Cu, Rb and Zn, when compared to the chemical agents used in this study, but worse in case of Al, Ba, Co, Cr or Fe ($0.05 < p < 0.01$; U Mann-Whitney test). This fungus, when compared to the cold and diluted (20%) HNO_3 solution, was also better extraction medium for Mg, Ca and Na ($p < 0.05$; U Mann-Whitney test). Soil fungi, as mentioned in the introductory section, have developed several strategies to uptake necessary chemical elements from the soil substrate, including metals, and also to cope with these, which are toxic or present at hazardous concentrations. There is also an opinion that the uptake of the essential metallic elements could be, to some degree, regulated by mycelium [41]. Certainly, metallic elements that are toxic to fungus could be partly absorbed by mycelium along with the essential ones. That includes also the condition, when water-insoluble metallic element to be absorbed by a fungus from the soil has to be dissolved in a solubilizing agent excreted by mycelium. Cold and diluted (20%) HNO_3 solution used in this study as the extracting medium for the "labile" forms of metallic elements present in the surface soil horizon presumably differed from the Poison Pax with respect to the "metals extracting power".

Fruit bodies of Poison Pax collected at the sites nearby to the villages of Ocypel and Osiek in Bory Tucholskie were able to bioconcentrate (BCF > 1) metallic elements such as Ag, Cu (BCF for caps ~ 100), K (BCF ~ 1100), Mg (BCF ~ 20), Na (BCF ~ 50), Rb (BCF ~ 1000) and Zn (BCF ~ 40), while they bio-excluded (BCF < 1) Al, Ba, Ca, Co, Cr, Fe, Mn, Ni, Pb and Sr. Cd and Cu were bio-concentrated, BCF value for Zn was nearly 1, while Pb was excluded, according to the study of Gast *et al.* [33]. In one study [34] of Poison Pax collected in Poland, the BCF values reported for Zn were between 47 and 360, for Cd between 9.2 and 13, and for Fe between 1.3 and 1.7, while Pb, Mn and Al were bio-excluded.

In our study, the specimens of Poison Pax collected near to Ocypel, when compared to these collected at the Osiek site, showed enhanced bio-concentrating properties and exhibited higher contents of Ca and Cr in caps/stipes and of Ba and Sr in stipes ($p < 0.05$; U Mann-Whitney test) (Tab. 1). The occurrence of Ca, Ba and Sr in wild-grown mushroom species, as observed for *Suillus grevillei* [40], was in line with our findings. Higher contents of Ca, Ba, Cr, Sr and Na in the specimens of Poison Pax collected at the Ocypel site could be attributed to better bioavailability of these metals there and their lower bioavailability at the Osiek site.

A high potential of fungus to efficiently accumulate metallic elements in its fruit bodies in parallel with tolerance for their highly elevated concentrations in the soil are the features necessary for bioremediation of contaminated soil [42]. The mycorrhizal fungi, apart from sequestering metals in their fruit bodies, can also transfer a portion of the absorbed dose to the symbiotic plant. As found in this study, fruit bodies of Poison Pax have a good accumulation capacity towards large amounts of K, Mg, Ca, Cu, Fe, Na, Rb or Zn; due to relatively higher absolute concentration values

of these elements they can be considered as major metallic elements found in this fungus (Tab. 1). In the light of these data and due to high BCF values, both Cu and Zn, which are essential trace elements for humans and animals, can be considered as environmentally problematic metals in some mushroom species, which, if grown at contaminated sites, can contain these metals in the amounts hazardous to humans or big game consumers. At the same time, Poison Pax possesses a potential to bioconcentrate at least Ag, which is hazardous to mammals; it is difficult, however, to give any conclusion about Cd (Tab. 1). In case of toxicologically relevant Pb it seems that naturally grown Poison Pax, as mentioned before, does not possess a potential to bioconcentrate it and also seems to have a low accumulation capacity towards this element in the fruit body.

Mercury is a problematic metallic element – it contaminates the environment and is highly hazardous to the human health. It has not been, however, examined in this study. In another study [13] it was stated that Poison Pax collected in Bory Tucholskie contained Hg in its fruit bodies at a small concentration (*i.e.* < 0.1 μg g^{-1} dw) and, accordingly, its potential to bioconcentration of Hg was low. Nevertheless, under metal stress conditions – as observed for Cd and Hg, Poison Pax is able to absorb, translocate and sequester mercury more effectively [15, 32]. At the area contaminated with Hg – due to the neighborhood of a cinnabar mine – the soil contained 78 μg Hg g^{-1} dw and Poison Pax accumulated 10 μg Hg/g dw in its fruit body, but BCF was low, *i.e.* 0.13 [32].

At the sites contaminated with Cd and Pb (median values in soil: 0.9 and 105 μg g^{-1} dw, maximally 28 and 720 μg g^{-1} dw) Poison Pax accumulated these elements in the fruit bodies at the concentrations of 5.8 and 6.0 μg g^{-1} dw (median) and 88 and 412 μg g^{-1} dw (maximum values), respectively [33]. Hence, as concluded from this study, also under Cd and Pb-related stress mycelium of Poison Pax is able to tolerate their toxic concentrations in the soil substratum and accumulate both metals at elevated concentration (even bioconcentrates Cd; median BCF = 6.4) in the fruit bodies [33]. As indicated in the cited references, high accumulation capacity of Poison Pax towards toxic Hg, Cd and Pb leading to largely elevated concentrations of these metals seems to be related to the fungus' detoxification strategy based on its ability to the enhanced production of binding agents under metal stress conditions. These would imply a usefulness of Poison Pax as a mycorrhizal species forming an association with plants suitable for the long-term bioremediation processes of soils contaminated (at least) with Hg, Cd, Pb, Cu or Zn.

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