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Original Research

Mineral Constituents of Edible Field Parasol (*Macrolepiota procera*) Mushrooms and the Underlying Substrate from Upland Regions of Poland: Bioconcentration Potential, Intake Benefits, and Toxicological Risk

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Abstract

This paper presents analytical and scientific monitoring data on the bioconcentration potential of trace elements and mineral compounds in *Macrolepiota procera* collected from background areas in upland regions of central Poland. The contents of Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Rb, Sr, and Zn in caps and stipes (99 specimens) and underlying soil substrates were examined by ICP-OES and Hg by CV-AAS after wet digestion. The results showed that *M. procera* is a rich source of especially Ca, Cu, Fe, K, Mg, Mn, Na, and Zn. The contents of Pb, Hg, Ag, and Cd in the caps were 1.7±0.6 to 5.9±1.7, 1.8±0.8 to 5.3±0.8, 1.2±0.7 to 16±7, and 0.56±0.13 to 4.9±5.4 mg kg^{-1} dry matter, respectively. Probable dietary intake assessment showed that occasional consumption (once a week) of *Macrolepiota procera* caps could be safe, while consumption more than once a week could provide doses of toxic metals that exceed the provisionally allowed daily intake limits for humans.

Keywords: foods, mushrooms, heavy metals, minerals, *Macrolepiota procera*

Introduction

Fungi play a key role in the transfer of metals from the lithosphere into the biosphere. Within the bio-diverse world, mushrooms are the important groups that constitute edible and medicinal mushrooms. Most of the estimated 2,000 species of edible wild mushrooms that grow worldwide are poorly characterized with respect to their composition and multi-mineral constituents as well as their bio-accessibility, while such information is completely absent for numerous species. In the studies of metallic elements and metalloids in mushrooms, the pertinent question is the unraveling of the qualitative and quantitative interrelationships between the elements/

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compounds sequestered in the fruiting bodies from the soil or other substratum on which the mycelium develops [1-5].

Mushrooming is a global gourmet heritage practiced by both rural inhabitants and city dwellers. Nevertheless, sufficient knowledge of the composition and mineral constituents of many edible wild mushrooms available for picking is lacking [6-7]. Consequently, it has become common for only several of the well recognizable species to be collected and consumed. The few figures available on the annual intake rates of wild mushrooms shows that the average consumption varies by country: from 1.0 kg *per capita* in Sweden to 10 kg for fanciers in the Czech Republic, and as much as 20-24 kg *per capita* in the Liangshan Yi nationality in China. The portion of mushrooms in a single meal is usually 300 g (fresh weight), but this could sometimes be as much as 500 g [8]. Edible wild as well as cultivated mushrooms are highly popular among vegetarians and vegans.

In Poland, under a regulation issued by the Minister of Public Health and Welfare, 42 mushrooms can be purchased commercially, and these are readily available through retail sales. M. procera (common name field parasol or parasol mushroom) is on this ministerial list [9].

Edible wild mushrooms on sale in Poland are dominated by three groups or species, i.e.:

- *Boletus spp.*, or the bay bolete (*Imleria badia*)
- Yellow-cracked bolete (*Boletus subtomentosus*) and golden bolete (*Xerocomellus chrysenteron*); *Boletus* spp., and in majority the king bolete (*Boletus edulis*)
- Common chanterelle (*Cantharellus cibarius*) [10]

Mushrooms, especially wild grown mushrooms and the edible species in particular, have been reported to be rich in mineral constituents as well as in some toxic metals [11-16]. As such, mushroom studies have shown that some mushroom species are rich sources of essential trace elements. Mycelium can have a species-specific ability to absorb and trans-locate metallic elements or metalloids from the mushroom substrate into the mushroom fruiting body. This is because of the specific physiological requirement of some mushroom species that can be impacted by geogenic factors, e.g., low stable Cs (^{133}Cs) and low-radio Cs (^{137}Cs), or relatively high stable Sr and relatively high-radio Sr (^{90}Sr) [17-19], while the reason for metals absorption is note completely elucidated. A well-known example of a hyperaccumulator is the fly agaric (*Amanita muscaria*), which readily bioaccumulates vanadium found in the metalorganic compound amavadine [20]. A typical example of a mushroom species rich in As is the amethyst deceiver (*Laccaria amethystina*), which contains dimethylarsinic acid, methylarsonic acid, trimethylarsine oxide, arsenic acid, and arsenobetaine [21-22]. *Albatrellus* and *Boletus* mushrooms are rich in Se, while for some of the *Boletus* mushrooms in particular, several seleno-compounds have been identified [23]. Some mushrooms are rich in Ag, while many species are able to efficiently bioaccumulate Ag [24-27]. King bolete (*Boletus edulis*), bay bolete (*Imleria badia*), *Amanita* spp. (*A. crocea, A. muscaria, A. submembranacea*), and some other species can accumulate Ag in large amounts when they emerge at Ag-contaminated sites (e.g., a Au/Cu mine dump or Ag-rich galena smelting [11, 28]), while the species *Amanita strobiliformis* is a hyper-accumulator of Ag [29].

Similarly, Hg is efficiently bio-concentrated by numerous mushrooms, and many species are relatively abundant in mercury even at uncontaminated sites where the Hg content of the substrate/soil on which the mushroom is growing is very low [30-39]. For instance, the reported mean BCF (the ratio of the content of the element in the cap or stipe to its content in the soil) for the caps of *M. procera* varied from 16±6 to 220±110 (range 0.52 to 470) and that for *B. edulis* varied from 41±6 to 130±39 (range 13 and 170) [13, 30, 32-34].

Mushrooms are also often rich in metals that are weakly bio-available or in metals that feature as "bio-excluded" by mycelium, but which are abundant in the top layer of soils. Typical examples of this are Al, Ca, or Fe [12]. Cadmium, chromium, mercury, lead, copper, and zinc are the heavy metals most frequently studied in wild mushrooms [40-45]. There is scarcity of data on mineral composition of the same mushroom species that emerged at geographically distant places that have different soil bedrock geochemistry and environment [46-48].

The aim of this study is to examine the contents of Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Rb, Sr, and Zn in widely consumed *M. procera*, and the soil substrates beneath the mushrooms that were collected from southern and central parts of Poland, and also to assess the mineral interrelationships and bioconcentration potentials of this species. The nutritional benefits and toxicological risk of the minerals determined in *M. procera* are also discussed.

Materials and Methods

Sampling

Mature specimens of *M. procera* or parasol mushroom (*Macrolepiota procera*) and samples (ca. 100 g) of top organic and mineral soil horizon (0-15 cm) beneath the fruiting bodies were collected across the central and southern regions of Poland between 1999 and 2002. The sites surveyed (Jarocin, Lubraniec, Gostyńsko-Włocławskie Forest, Starachowickie Forest, and Poniatowa) are shown in Fig. 1. We also examined archived samples of caps and stipes of 16 fruiting bodies of parasol mushroom collected on the outskirts of the town of Gubin in western Poland in 1994 (Fig. 1), but only cadmium, manganese, lead, copper, and zinc were determined in them.

Elemental Analysis

Fresh mushrooms, after being cleaned of any visible plant vegetation and soil substrate debris using a plastic knife, were air-dried for several days. Thereafter, each

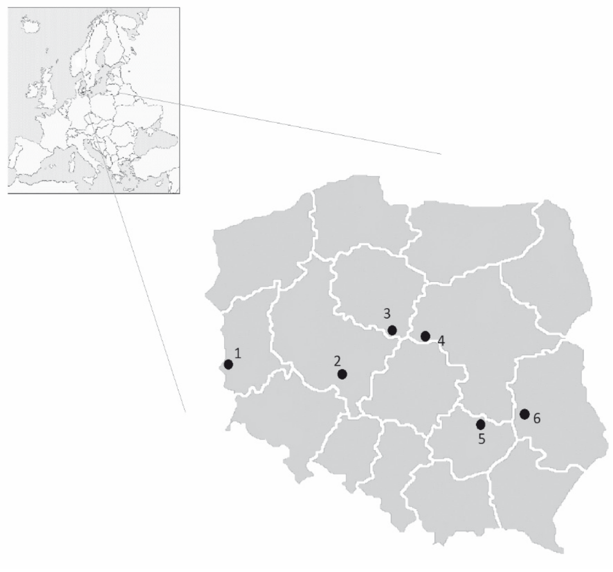

Fig. 1. Location of the sampling sites of field parasol. Abbreviations: Gubin (1), Jarocin (2), Lubraniec (3), Gostyńsko-Włocławskie Forest (4), Starchowickie Forest (5), and Poniatowa (6).

sample of the fruiting body was separated into two parts – the cap and the stipe – and dried at 65ºC to constant weight. Dried mushroom parts were pulverized in an agate mortar and kept in brand new sealed polyethylene bags under dry conditions. The pulverized sub-samples (400 mg) of caps and stipes were weighed into pressure-resistant and analytical quality pro-digestive vessels made of polytetrafluoroethylene (PTFE). The fungal materials were pre-digested for 24 hours with concentrated nitric acid (65%; Suprapure, Merck; 7 mL) at room temperature and further digested under pressure in a MARS 5 (CEM Corp. Matthews, NC, USA) automatic microwave digestion system. The digest, after the addition of an internal standard (yttrium), was diluted to 25 mL using deionized water and subjected to instrumental analysis [49-50].

The soils were air dried at room temperature under clean conditions for several weeks and next sieved through a pore size of 2 mm and further dried in an electric oven at 40ºC to constant weight. Next, the soil sub-samples (0.5 g) were cold-treated with 20% nitric acid solution (20 mL) for 24 hours in quartz vessels [49-50]. Furthermroe, after the addition of deionized water (10 mL), the extract was filtered through Whatmann No. 42 filter paper into a polyethylene bottle. After the addition of internal standard (yttrium), the extract was diluted to 50 mL using deionized water and thereafter subjected to instrumental analysis.

The trace elements (Ag, Al, Ba, Ca, Cd, Co, Cu, Cr, Fe, K, Mg, Mn, Na, Ni, Pb, Rb, Sr, and Zn) were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Optima 2000 DV, Perkin-Elmer, USA) [49-50] and Hg by cold vapor atomic absorption spectrometry (CV-AAS; Mercury analyzer type MA-2000, Nippon Instruments Corporation, Takatsuki, Japan) [51].

Quality Control/Quality Assurance

These methods of metallic element measurements were validated and controlled on several occasions by the analyses of officially certified reference materials: IAEA 359 cabbage leaves from the International Atomic Energy Agency, and from participation in international calibration trials such as the GESM/Food Euro proficiency testing exercise, the IAEA-338 Proficiency Test of Trace Elements in Lichen, the Aquacon Project 9 Soil Analysis (European Commission Environment Institute), and Oriental tobacco leaves (CTA-OTL-1), tea leaves (INTC-TL-1), and Polish herbal blend (INCT-MPH-2) by the Institute of Nuclear Chemistry and Technology in Warsaw, Poland [49-50]. Discrepancies between certified values and contents quantified were below 10%. Duplicates and blanks followed with every set of 10 mushroom or soil samples examined. For blank samples no major interferences were found for the elements quantified.

Limits of detection for Al, Ba, Ca, Cd, Co, Cu, Cr, Fe, K, Mg, Mn, Na, Ni, Pb, Rb, Sr, and Zn were between 0.01-0.10 mg kg^{-1} dry matter (dm), and 0.005 mg kg^{-1} dm for Hg. Coefficients of variation for these measurements on routine runs were well below 10%.

Multivariate Analysis

All data produced were statistically treated to find possible statistically significant differences between the variables of the Mann-Whitney *U* test, principal component analysis (PCA), and cluster analysis (CA) (Figs 2-4, Tables 2 and 3) [41]. The computer software package Statistica version 8.0 was used for statistical analysis of data.

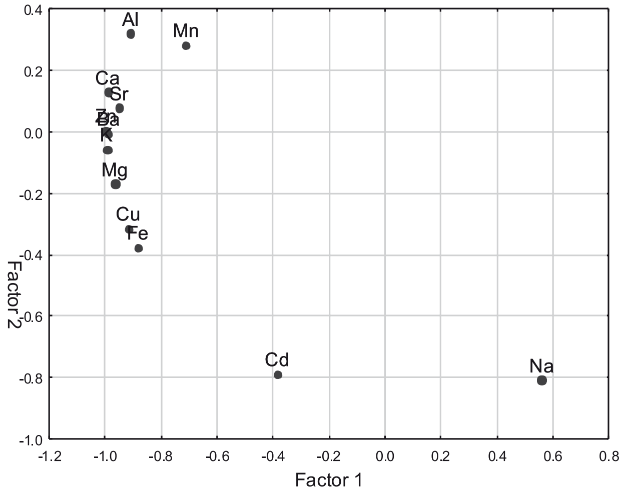

Fig. 2. Plot of loadings (unrotated) based on the content of metallic elements in caps of *Macrolepiota procera* in spaces of the first and second factors.

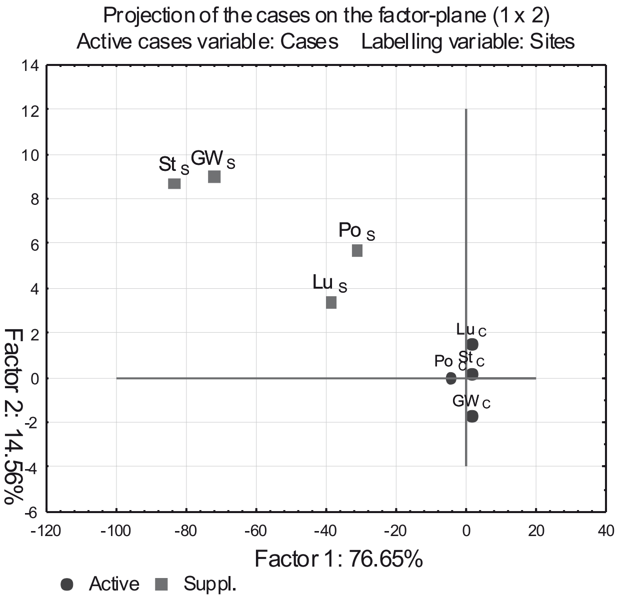

Fig. 3. Projection of the data on caps of *Macrolepiota procera* and forest soil substratum collected from four sampling sites set on the factor 1 (PC 1) and factor 2 (PC 2) planes.

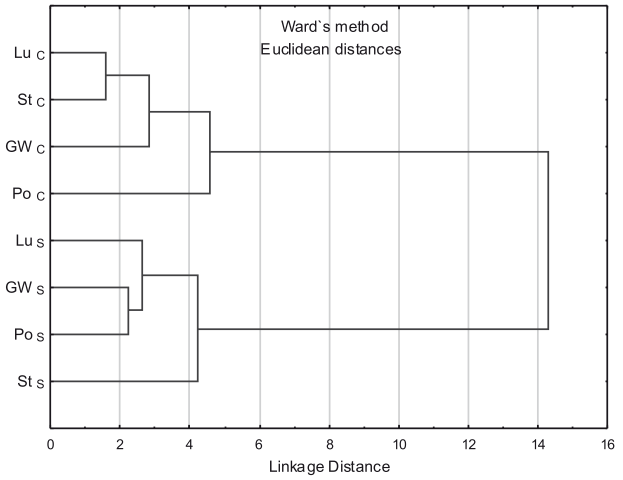

Fig. 4. Bundle diagram of similarity of minerals composition between caps of *Macrolepiota procera* and forest soil substratum from four spatially distant sites (Lu – Lubraniec, GW – Gostyńsko-Włocławskie Forest, St – Starchowickie Forest, Po – Poniatowa, C – caps, S – soil).

Results and Discussion

The metal contents in caps and stipes of *M. procera* and the soils as well as the values of the quotient of metal contents in cap to stipe ($Q_{C/S}$) and in cap or stipe to contents in soil substratum (BCF, or bioconcentration factor) are given in Table 1 and in Annexes 1 and 2. These data are for 99 specimens of *M. procera* m collected from six geographically distant regions of central, southwest, and southeast Poland. Some metallic elements in this study were determined in mushrooms and soils collected in one (Rb), two (Co, Cr, Hg, Pb), three (Ag), or four to six sites (other metals), respectively (Annexes 1 and 2).

Essential Metals: Major Minerals (K, Mg, Ca)

Among the essential macro-elements determined, potassium (K) was found in highest amounts in *M. procera*. Depending on the site, K content varied between 32,000±7,000 and 49,000±3,000 (total range 18,000-52,000) mg kg^{-1} dm. The caps were on average about two-fold richer in K than the stipes. Potassium content of caps and stipes as well as the value of $Q_{C/S}$ for this metal varied significantly between the sampling sets (year of collection) of mushrooms ($p < 0.05$; Mann-Whitney *U* test; Table 1). *M. procera* is characterized by a high potential to bioconcentrate potassium. The BCF (also called enrichment or transfer factor) of K ranged from 140 to 740 for caps and from 76-750 for stipes of 88 specimens collected from four of the sites surveyed. BCF is a unitless parameter and is calculated as a quotient of the content of the metal determined in the fruiting body or its morphological part in relation to metal content of the substratum in which the mycelium lives (soil, wood, compost). All analytical data are expressed on a dry-weight basis. The mean K content of the soil substratum to *M. procera* varied between 0.10±0.03 and 0.15±0.06 mg kg^{-1} dm.

Potassium content in caps of *M. procera* is followed by Mg and then Ca. Mean magnesium contents in caps of four sites varied from 1,000±100 to 2,500±100 (range 650-2,700) mg kg^{-1} dm, while the mean Ca content varied between 130±75 and 440±140 (range 63-850) mg kg^{-1} dm (Table 1). Calcium is usually more abundant in stipes than in caps of this fungus, while Mg is usually more in the caps (Table 1). As in the case of K, the contents of Mg and Ca in *M. procera* varied statistically, depending on the sites surveyed ($0.05<p<0.01$; Mann-Whitney *U* test). The contents of magnesium in soil (substratum to Parasol Mushroom fruiting bodies) varied from 89±20 to 190±40; and Ca from 400±58 to 1,300±190 mg kg^{-1} dm. Up-take of Mg and Ca by *M. procera*, when assessed from the BCF values, decreased as content of Mg and Ca in topsoil increased. Nevertheless, the relatively high contents of Mg and Ca in the soils – in spite of the lower availability or regulated up-take by the mushroom – resulted in the abundance of these metals in the fruiting bodies, as noted for Gostyńsko-Włocławskie Forest (Mg) and the Poniatowa site (Ca) (Table 1).

Essential Metals: Trace Constituents (Zn, Cu, Fe, Mn, Na, Cr, Co)

Among Zn, Cu, Fe, Mn, Na, Cr, and Co, which are essential to mammals, some of them (Zn, Cu, Fe, Mn, and Na) are also essential to fungi [52]. These five metals were determined in the caps in contents ranging from 10±7 mg kg^{-1} dm (Na) to 640±36 mg kg^{-1} dm (Zn), while Cr and Co

Table 1. Metals content of caps and stipes of Field Parasol and soil substrate, bioconcentration factor values from Lubraniec, Gostyńsko-Włocławskie forest, Starachowickie forest and Poniatowa (mean, SD, median value -in parentheses and range).

Element	Item	Lubraniec 2000 *n = 16*	Gostyńsko-Włocławskie forest, 2001 *n = 15*	Starachowickie forest, 2001 *n = 15*	Poniatowa, 2002 *n = 15*
g kg^{-1} dry matter					
K	Cap	34±5 (36) 18-38	37±4 (37) 29-43	32±7 (35) 20-42	49±3 (50) 42-52
	Stipe	22±4 (22) 15-32	24±7 (25) 13-33	33±11 (31) 18-62	21±4 (21) 12-28
	$Q_{C/S}$	1.6±0.4 (1.6) 0.57-2.6	1.7±0.7 (1.4) 1.1-3.9	1.1±0.4 (1.2) 0.30-2.0	2.4±0.6 (2.3) 1.8-3.9
	Soil	0.13±0.08 (0.13) 0.12-0.14	0.15±0.06 (0.15) 0.089-0.22	0.10±0.03 (0.094) 0.069-0.15	0.11±0.03 (0.13) 0.069-0.15
	BCF_C	270±43 (270) 140-310	270±87 (260) 130-410	350±130 (310) 170-590	480±170 (390) 280-740
	BCF_S	170±29 (170) 120-240	170±69 (160) 76-280	360±150 (350) 150-750	200±61 (190) 120-330
Mg	Cap	1.0±0.1 (1.1) 0.65-1.2	1.4±0.1 (1.4) 1.2-1.7	1.5±0.3 (1.5) 1.0-2.1	2.5±0.1 (2.5) 2.3-2.7
	Stipe	0.78±0.10 (0.76) 0.65-1.0	0.72±0.13 (0.74) 0.48-0.98	1.5±0.5 (1.3) 1.0-3.0	1.7±0.2 (1.7) 1.4-2.1
	$Q_{C/S}$	1.4±0.2 (1.4) 0.63-1.7	2.1±0.4 (1.9) 1.5-2.9	1.1±0.4 (1.3) 0.33-1.7	1.5±0.2 (1.5) 1.2-1.8
	Soil	0.13±0.06 (0.14) 0.12-0.14	0.19±0.04 (0.19) 0.15-0.23	0.13±0.06 (0.11) 0.080-0.24	0.089±0.020 (0.098) 0.066-0.11
	BCF_C	7.0±1.0 (7.3) 4.2-8.0	7.5±4.4 (8.5) 2.1-15	20±9 (18) 10-36	30±7 (26) 23-39
	BCF_S	5.2±0.7 (5.2) 4.2-6.7	3.8±2.5 (3.4) 0.88-7.8	19±9 (18) 8.5-39	20±4 (18) 16-30
mg kg^{-1} dry matter					
Al	Cap	85±55 (59) 32-230	48±30 (41) 14-140	42±16 (40) 24-88	99±61 (87) 24-240
	Stipe	280±400 (130) 32-1600	70±78 (41) 15-320	40±20 (36)) 19-99	110±61 (91) 37-210
	$Q_{C/S}$	1.0±1.7 (0.48) 0.031-7.0	0.88±0.38 (0.83) 0.43-1.9	1.2±0.6 (1.3) 0.43-2.5	0.96±0.57 (0.89) 0.39-2.1
	Soil	850±47 (870) 770-890	1500±540 (1200) 1100-2200	880±260 (860) 570-1200	540±70 (500) 480-620
	BCF_C	0.10±0.06 (0.068) 0.038-0.26	0.031±0.014 (0.031) 0.0070-0.062	0.053±0.035 (0.038) 0.023-0.15	0.19±0.13 (0.17) 0.038-0.50
	BCF_S	0.35±0.52 (0.15) 0.036-2.0	0.045±0.037 (0.030) 0.0075-0.14	0.049±0.027 (0.041) 0.019-0.12	0.22±0.13 (0.17) 0.075-0.44
Ba	Cap	1.2±1.3 (0.87) 0.43-6.9	0.85±0.24 (0.89) 0.52-1.3	0.95±0.42 (0.96) 0.33-1.7	1.3±0.4 (1.4) 0.82-2.1
	Stipe	3.3±3.2 (2.5) 0.86-12	1.2±0.6 (0.95) 0.50-2.6	1.2±0.7 (1.1) 0.45-3.5	4.2±2.0 (4.2) 1.4-7.6
	$Q_{C/S}$	0.65±0.64 (0.35) 0.073-3.5	0.85±0.34 (0.86) 0.32-1.8	0.94±0.53 (0.80) 0.31-2.2	0.39±0.23 (0.33) 0.20-1.0
	Soil	16±3 (16) 11-19	39±21 (35) 17-63	44±19 (48) 14-69	14±2 (13) 12-17
	BCF_C	0.080±0.079 (0.056) 0.023-0.36	0.027±0.013 (0.026) 0.0092-0.051	0.028±0.022 (0.020) 0.0065-0.075	0.098±0.038 (0.10) 0.049-0.17
	BCF_S	0.25±0.31 (0.14) 0.046-1.1	0.038±0.026 (0.029) 0.010-0.11	0.033±0.024 (0.028) 0.0089-0.094	0.31±0.17 (0.27) 0.10-0.62

Table 1. Continued.

Ca	Cap	210±170 (160) 120-850	130±49 (120) 63-220	130±75 (130) 45-310	440±140 (400) 250-760
	Stipe	780±650 (600) 130-2300	160±79 (140) 58-530	200±64 (220) 89-330	1900±1000 (2000) 600-4200
	$Q_{C//S}$	0.70±1.5 (0.31) 0.067-6.3	0.90±0.33 (0.83) 0.51-1.8	0.72±0.50 (0.54) 0.21-2.0	0.31±0.24 (0.24) 0.13-1.0
	Soil	400±58 (420) 330-470	1100±55 (1100) 460-1700	650±470 (490) 190-1400	1300±190 (1400) 1100-1500
	BCF_C	0.54±0.40 (0.45) 0.31-2.0	0.15±0.08 (0.14) 0.039-0.31	0.27±0.15 (0.21) 0.078-0.63	0.34±0.11 (0.31) 0.22-0.55
	BCF_S	2.1±1.9 (1.4) 0.32-7.0	0.18±0.11 (0.16) 0.047-0.42	0.51±0.39 (0.42) 0.11-1.4	1.5±0.7 (1.5) 0.44-2.9
Cd	Cap	0.56±0.13 (0.57) 0.19-0.75	4.9±5.4 (1.7) 0.67-17	1.5±0.8 (1.6) 0.41-2.8	1.9±1.0 (1.7) 0.60-3.9
	Stipe	0.20±0.13 (0.17) 0.10-0.68	ND[a]	1.2±0.9 (0.89) 0.40-3.3	ND
	$Q_{C//S}$	3.4±1.3 (3.8) 0.29-5.6	ND	2.0±1.5 (2.3) 0.18-6.0	ND
	Soil	0.079±0.019 (0.082) 0.050-0.10	0.13±0.04 (0.14) 0.094-0.20	0.49±0.38 (0.39) 0.12-1.1	0.17±0.02 (0.17) 0.16-0.20
	BCF_C	7.9±3.4 (6.9) 2.4-15	37±38 (15) 4.5-120	5.9±6.6 (3.8) 0.36-20	11±6 (10) 3.8-25
	BCF_S	2.7±1.7 (2.4) 1.1-8.3	ND	3.7±3.4 (2.4) 0.84-12	ND
Cu	Cap	110±20 (110) 58-150	180±87 (150) 72-340	94±24 (99) 49-120	220±33 (220) 170-320
	Stipe	63±16 (58) 45-110	120±39 (110) 53-190	86±24 (77) 62-130	110±25 (97) 70-150
	$Q_{C//S}$	1.9±0.5 (1.9) 0.53-3.0	1.5±0.5 (1.4) 0.76-2.5	1.2±0.5 (1.4) 0.40-2.0	2.2±0.4 (2.3) 1.4-2.7
	Soil	2.7±0.5 (2.8) 2.0-3.1	3.8±3.1 (3.8) 0.71-8.3	2.0±1.0 (1.8) 1.1-3.9	1.8±0.1 (1.8) 1.6-1.9
	BCF_C	44±13 (42) 22-70	85±71 (67) 21-220	57±25 (57) 13-100	120±18 (130) 100-170
	BCF_S	24±7 (23) 16-42	60±47 (46) 14-160	49±22 (46) 31-120	60±13 (60) 44-89
Fe	Cap	65±30 (53) 38-140	90±22 (87) 60-150	49±18 (44) 27-91	120±29 (130) 69-160
	Stipe	180±260 (94) 31-1000	69±51 (53) 28-240	44±21 (40) 22-100	150±64 (140) 73-270
	$Q_{C//S}$	1.4±1.0 (1.2) 0.19-3.5	1.6±0.5 (1.5) 0.62-2.4	1.3±0.6 (1.5) 0.38-2.3	0.93±0.42 (0.77) 0.45-1.8
	Soil	1300±0 (1300) 1200-1300	1800±1100 (1200) 960-3600	1100±240 (1100) 860-1400	780±310 (730) 510-1300
	BCF_C	0.051±0.023 (0.041) 0.031-0.11	0.063±0.029 (0.060) 0.017-0.12	0.046±0.023 (0.040) 0.021-0.11	0.18±0.08 (0.20) 0.053-0.31
	BCF_S	0.15±0.21 (0.073) 0.024-0.84	0.044±0.025 (0.039) 0.011-0.10	0.040±0.023 (0.036) 0.018-0.072	0.23±0.14 (0.19) 0.057-0.51
Mn	Cap	39±35 (31) 21-170	15±4 (15) 8.8-22	86±54 (69) 19-180	85±15 (84) 60-120
	Stipe	120±67 (120) 27-280	17±13 (14) 5.5-48	120±74 (85) 36-270	290±17 (240) 86-630
	$Q_{C//S}$	0.68±1.5 (0.28) 0.090-6.1	1.1±0.5 (1.3) 0.47-1.8	1.1±1.2 (0.44) 0.22-3.5	0.42±0.25 (0.34) 0.16-1.0
	Soil	38±6 (39) 31-45	160±75 (150) 83-240	700±200 (770) 330-890	210±130 (240) 62-340

Table 1. Continued.

	BCF_C	1.0±0.9 (0.84) 0.54-4.4	0.12±0.07 (0.10) 0.042-0.27	0.13±0.07 (0.13) 0.027-0.29	0.69±0.54 (0.32) 0.17-1.7
	BCF_S	3.3±2.2 (2.9) 0.71-9.1	0.14±0.15 (0.081) 0.030-0.58	0.22±0.21 (0.13) 0.044-0.75	2.3±2.4 (1.4) 0.28-7.5
Na	Cap	20±11 (18) 6.4-43	130±170 (67) 36-690	32±14 (27) 9.7-58	10±7 (8.1) 3.2-26
	Stipe	24±11 (25) 7.9-46	250±190 (200) 49-740	34±16 (29) 13-69	21±17 (12) 4.9-68
	$Q_{C//S}$	0.92±0.56 (0.79) 0.26-2.4	0.49±0.22 (0.44) 0.17-0.93	1.3±1.1 (0.79) 0.33-3.8	0.79±0.64 (0.64) 0.11-2.1
	Soil	8.0±0.8 (7.9) 7.2-9.3	21±19 (12) 7.3-53	3.8±2.1 (4.1) 1.1-6.2	15±2 (14) 13-18
	BCF_C	2.5±1.4 (2.2) 0.80-5.5	10±15 (5.0) 0.68-59	13±13 (7.7) 2.8-45	2.0±1.2 (1.9) 0.59-4.0
	BCF_S	3.1±1.4 (2.8) 0.85-5.9	19±16 (16) 0.92-63	13±2 (8.0) 2.4-44	5.4±6.1 (2.3) 0.89-25
Sr	Cap	0.74±0.61 (0.56) 0.37-3.0	0.50±0.15 (0.48) 0.24-0.74	0.29±0.13 (0.32) 0.10-0.54	1.2±0.3 (1.1) 0.90-1.9
	Stipe	2.2±1.7 (1.8) 0.47-6.4	0.64±0.29 (0.57) 0.27-1.3	0.36±0.14 (0.31) 0.15-0.67	4.4±2.2 (3.8) 1.6-9.0
	$Q_{C//S}$	0.75±1.5 (0.35) 0.079-6.9	0.88±0.40 (0.75) 0.51-2.0	0.90±0.48 (0.74) 0.39-1.9	0.34±0.18 (0.28) 0.15-0.75
	Soil	3.1±0.6 (3.2) 2.3-3.7	5.0±1.7 (5.8) 3.0-6.7	2.6±1.6 (2.1) 0.86-4.9	4.9±0.7 (5.3) 4.0-5.6
	BCF_C	0.24±0.19 (0.21) 0.12-0.93	0.11±0.03 (0.11) 0.037-0.16	0.14±0.08 (0.12) 0.039-0.30	0.25±0.06 (0.24) 0.19-0.41
	BCF_S	0.77±0.71 (0.53) 0.15-2.8	0.14±0.06 (0.12) 0.057-0.27	0.20±0.14 (0.20) 0.050-0.58	0.90±0.40 (0.86) 0.29-1.7
Zn	Cap	86±13 (87) 51-120	100±29 (96) 70-180	140±45 (130) 90-230	640±36 (650) 580-710
	Stipe	54±12 (51) 41-94	51±18 (52) 28-100	150±69 (130) 79-260	310±48 (290) 250-400
	$Q_{C//S}$	1.6±0.3 (1.7) 0.55-2.2	2.1±0.4 (2.0) 1.6-2.8	1.1±0.5 (1.3) 0.40-1.8	2.1±0.3 (2.1) 1.5-2.5
	Soil	9.4±0.7 (10) 8.3-10	19±9 (24) 7.0-28	25±16 (20) 10-56	11±1 (11) 9.7-12
	BCF_C	9.3±1.6 (9.1) 5.3-12	6.9±4.0 (4.0) 2.9-14	8.1±5.1 (7.5) 1.9-16	59±7 (58) 51-70
	BCF_S	5.8±1.3 (5.7) 4.6-9.8	3.4±1.9 (2.2) 1.2-6.5	7.5±3.5 (6.1) 3.8-14	28±4 (28) 21-40

[a] Not determined

ANNEX 1. Metals contents of caps and stipes of field parasol and soil substrate, bioconcentration factor values from Lubraniec and Poniatowa (mg kg^{-1} dry mass; mean, SD^{a}, median value – in parentheses and range).

Element	Item	Site	
		Lubraniec 2000 *n = 16*	Poniatowa, 2002 *n = 15*
Ag	Cap	3.8±1.3 (3.2) 1.3-5.9	16±7 (13) 5.4-31
	Stipe	1.6±0.8 (1.4) 1.0-4.1	5.7±2.8 (5.3) 1.5-10
	$Q_{C//S}^{b}$	2.4±0.8 (2.4) 0.31-3.2	2.9±0.7 (2.9) 1.6-4.5
	Soil	0.0084±0.0011 (0.0086) 0.007-0.010	0.031±0.020 (0.036) 0.011-0.056
	BCF_{c}	420±130 (410) 190-680	730±630 (470) 130-2600
	BCF_{S}	200±120 (160) 110-610	260±210 (190) 28-890
Co	Cap	0.050±0.033 (0.05) 0.01-0.10	0.22±0.07 (0.20) 0.10-0.40
	Stipe	0.14±0.11 (0.10) 0.025-0.49	0.36±0.12 (0.37) 0.20-0.65
	$Q_{C//S}$	0.50±0.41 (0.34) 0.026-1.2	0.66±0.31 (0.61) 0.23-1.6
	Soil	0.39±0.03 (0.41) 0.34-0.42	0.34±0.14 (0.35) 0.21-0.56
	BCF_{c}	0.13±0.09 (0.13) 0.0054-0.27	0.71±0.29 (0.82) 0.18-1.2
	BCF_{S}	0.37±0.35 (0.25) 0.062-1.5	1.2±0.6 (1.2) 0.35-2.2
Cr	Cap	0.26±0.16 (0.24) 0.13-0.78	1.3±0.1 (1.3) 1.0-1.6
	Stipe	0.58±0.67 (0.30) 0.12-2.7	0.98±0.22 (0.94) 0.75-1.6
	$Q_{C//S}$	0.87±0.69 (0.78) 0.10-2.7	1.4±0.3 (1.4) 0.74-1.8
	Soil	1.4±0.1 (1.5) 1.3-1.5	0.76±0.14 (0.69) 0.63-0.92
	BCF_{c}	0.18±0.11 (0.16) 0.088-0.52	1.8±0.4 (1.8) 1.1-2.5
	BCF_{S}	0.41±0.50 (0.20) 0.083-2.0	1.3±0.2 (1.3) 0.94-1.7
Hg	Cap	2.1±0.6 (2.0) 1.3-3.5	3.1±0.9 (2.9) 1.8-4.6
	Stipe	0.98±0.28 (0.87) 0.67-1.7	1.5±0.4 (1.5) 0.63-2.7
	$Q_{C//S}$	2.2±0.7 (2.1) 1.5-4.5	2.1±0.5 (2.1) 1.3-2.9
	Soil	0.13±0.02 (0.13) 0.11-0.16	0.047±0.010 (0.046) 0.035-0.063
	BCF_{c}	16±6 (15) 8.3-31	69±23 (62) 29-120
	BCF_{S}	7.6±2.8 (6.8) 4.3-15	34±9 (34) 10-46
Ni	Cap	0.86±0.22 (0.84) 0.60-1.5	0.29±0.47 (0.01) 0.01-1.3
	Stipe	1.3±0.6 (1.2) 0.52-2.9	0.39±0.95 (0.05) 0.01-3.6
	$Q_{C//S}$	0.81±0.60 (0.68) 0.23-2.8	0.84±0.64 (1.0) 0.41-2.6
	Soil	1.0±0.1 (1.0) 0.85-1.1	0.80±0.06 (0.79) 0.75-0.89
	BCF_{c}	0.87±0.21 (0.83) 0.57-1.4	0.36±0.59 (0.01) 0.0056-1.6
	BCF_{S}	1.4±0.8 (1.2) 0.50-3.4	0.49±1.2 (0.067) 0.0056-4.4
Pb	Cap	2.3±0.7 (2.2) 1.6-4.4	5.9±1.7 (5.2) 3.6-8.9
	Stipe	1.9±1.3 (1.4) 0.50-5.1	3.7±0.9 (3.8) 1.9-5.5
	$Q_{C//S}$	1.9±0.9 (1.9) 0.44-4.0	1.7±0.7 (1.6) 0.81-3.1
	Soil	12±1 (12) 10-13	8.8±1.4 (7.9) 7.4-11
	BCF_{c}	0.20±0.06 (0.20) 0.13-0.37	0.70±0.20 (0.65) 0.38-1.1
	BCF_{S}	0.17±0.14 (0.13) 0.041-0.52	0.43±0.12 (0.44) 0.21-0.59

ANNEX 1. Continued.

Rb	Cap	ND[c]	38±7 (37) 27-53
	Stipe	ND	21±37 (12) 6.8-150
	QC//S	ND	3.3±1.5 (3.3) 0.22-6.4
	Soil	ND	0.45±0.21 (0.39) 0.25-0.71
	BCF_c	ND	100±48 (110) 44-180
	BCF_S	ND	45±50 (36) 12-220

[a] SD standard deviation

[b] $Q_{c/s}$ values of the quotient of metal contents in cap to stipe

[c] Not determined

(determined for samples from two sites only) were below 1 mg kg^{-1} dm, on average (Annex 1).

The mean content of Zn in caps of *M. procera* is 200 mg kg^{-1} dm, followed by 170, 81, 48, and 45 mg kg^{-1} dm for Cu, Fe, Na, and Mg, respectively. The corresponding values (arithmetic mean) for stipes are 120 mg kg^{-1} dm for Zn and 110 mg kg^{-1} dm for Cu, Fe, and Mn, and 81 mg kg^{-1} dm for Na. The contents of Mn, Na, and Fe showed that they appeared to be more abundant in the stipes than in the caps of *M. procera*. Nevertheless, the $Q_{C/S}$ values of these metals varied significantly depending on the site surveyed (Table 1).

These metals occurred in caps in contents that were comparable (p>0.05) for some sites while there were significant differences for some other sites (p<0.05; Mann Whitney *U* test), e.g., higher content of Cu, Fe, Mg, and Zn, but lower for Na in individuals from the Poniatowa site (Table 1). Considering the mean values for caps at the sites surveyed, the contents varied for Zn from 86±13 to 640±36 (range 51-710) mg kg^{-1} dm followed by Cu with mean values ranging from 94±24 to 280±73 (range 49-440) mg kg^{-1} dm. The caps were about twice more abundant in Zn and Cu than the stipes (Table 1). The soil substrates from Gostyńsko-Włocławskie Forest and Starachowickie Forest contained twice more Zn than the soils from the Lubraniec and Poniatowa sites, while those from Gostyńsko-Włocławskie Forest were also about twice richer in Cu (0.05<p<0.01). There is an apparent variation in the availability of Zn and Cu to *M. procera* between the sites surveyed, as can be seen from the values of the BCF. Both Zn and Cu are well bioconcentrated by *M. procera*, and their BCF values varied between the sites, on average from about 5 to 60 for Zn and from 40 to 120 for Cu. At the Poniatowa site Zn and Cu were more bioavailable from soil than elsewhere in this study.

The mean Fe content in caps varied from 49±18 to 120±29 (range 27-160) mg kg^{-1} dm, followed by Mn, which varied from 15±4 to 86±54 (range 8.8-180), and then Na, which varied from 10±7 to 130±170 (range 3.2-690) mg kg^{-1} dm (Table 1). Iron was abundant in the soils, and also to some degree in the caps and stipes – and Fe can be considered to be bioexcluded by this fungus since its BCF values were <0.5. Similar to Cu and Zn, Fe was more bioavailable at the Poniatowa site, where the soil Fe contents were significantly lower (p<0.01; Mann-Whitney *U* test) when compared to other sites. Mn and Na content of the upper soil layer varied highly between the sites (0.001<p<0.01; Table 1), and Mn was usually bioexcluded, while Na was bioconcentrated in fruiting bodies (median BCF of 1.9-7.7 for caps).

Cobalt and chromium were determined only in soils and mushrooms from the Lubraniec and Poniatowa sites (Annex 2). The median contents of these metals in caps varied significantly (p<0.01) between the sites. The mean Co content values ranged from 0.050±0.033 to 0.22±0.07 (range 0.01-0.40) mg kg^{-1} dm, and for Cr between 0.26±0.16 and 1.3±0.1 (range 0.13-1.6) mg kg^{-1} dm, while the corresponding values for stipes were smaller. The availability of these two trace metals to *M. procera* also varied between the sites investigated. The results of this study indicate that Co is bioexcluded by the parasol mushroom. For Cr, the BCF for specimens from the Poniatowa site was around 1.5, while the corresponding value for the Lubraniec site is much lower.

Some other Metals: Trace Constituents (Al, Ba, Ni, Rb, Sr)

Aluminum is bio-excluded by *M. procera* (BCF<0.5). Nevertheless, Al was found to be relatively abundant in the caps with the mean values of the sites investigated ranging from 42±16 to 99±61 (total 14-240) mg kg^{-1} dm. In spite of the low BCF, the relative abundance of Al in the fruiting bodies may be a result of its abundance in the soil (between 500 and 1,200 mg kg^{-1} dm, on average; Table 1), and there may be a competitive co-absorption of Al with other metals.

Barium and strontium contents in the soils for the four sites varied from 14±2 to 44±19 (range 11-69) mg kg^{-1} dm, and from 2.6±1.6 to 5.0±1.7 (range 0.86-67) mg kg^{-1} dm, respectively (Table 1). Barium and strontium are bioexcluded by *M. procera*. In caps they occurred in contents between 0.85±0.24 and 1.3±0.4 (range 0.33-6.9) mg Ba kg^{-1} dm m, and between 0.29±0.13 and 1.2±0.3 (range 0.10-3.0) mg Sr kg^{-1} dm. Both metals were usually more enriched in stipes than in the caps of fruiting bodies of this fungus.

ANNEX 2. Metals contents of caps and stipes of field parasol and soil substrate and bioconcentration factor values (mg kg^{-1} dm; mean ± SD and median value – in parentheses and range).

Element	Item	Site	
		Gubin, 1994 *n=16*	Jarocin, 1999 *n=12*
Ag	Cap	ND[a]	1.2±0.7 (1.2) 0.40-3.1
	Stipe	ND	1.5±0.6 (1.4) 0.52-2.5
	$Q_{C//S}$[b]	ND	0.77±0.42 (0.70) 0.49-2.0
	Soil	ND	0.062±0.007 (0.063) 0.054-0.069
	BCF_C	ND	19±14 (19) 6.6-57
	BCF_S	ND	25±10 (26) 8.6-41
Ba	Cap	ND	0.57±0.19 (0.58) 0.11-0.83
	Stipe	ND	0.98±0.70 (0.90) 0.16-2.2
	$Q_{C//S}$	ND	0.92±0.55 (0.75) 0.18-2.5
	Soil	ND	47±1 (47) 47-48
	BCF_C	ND	0.012±0.004 (0.012) 0.0047-0.018
	BCF_S	ND	0.021±0.015 (0.019) 0.0033-0.047
Cd	Cap	3.2±2.4 (2.2) 1.3-11	1.1±0.5 (0.97) 0.57-2.3
	Stipe	1.2±1.0 (0.50) 0.50-3.3	0.47±0.33 (0.37) 0.23-1.4
	$Q_{C//S}$	4.6±5.0 (3.7) 0.69-22	2.5±0.7 (2.6) 1.6-4.4
	Soil	ND	0.20±0.01 (0.20) 0.20-0.21
	BCF_C	ND	5.6±2.6 (4.7) 2.8-12
	BCF_S	ND	2.3±1.7 (1.8) 1.1-7.4
Co	Cap	ND	0.044±0.020 (0.040) 0.020-0.090
	Stipe	ND	0.049±0.024 (0.048) 0.024-0.11
	$Q_{C//S}$	ND	0.94±0.29 (0.92) 0.54-1.5
	Soil	ND	0.80±0.01 (0.80) 0.78-0.81
	BCF_C	ND	0.055±0.025 (0.047) 0.021-0.11
	BCF_S	ND	0.061±0.029 (0.069) 0.030-0.14
Cr	Cap	ND	0.14±0.03 (0.13) 0.10-0.21
	Stipe	ND	0.16±0;07 (0.18) 0.088-0.32
	$Q_{C//S}$	ND	0.96±0.41 (0.74) 0.42-1.7
	Soil	ND	2.0±0.01 (2.0) 1.9-2.0
	BCF_C	ND	0.070±0.017 (0.066) 0.049-0.11
	BCF_S	ND	0.083±0.035 (0.081) 0.044-0.16
Cu	Cap	280±73 (240) 210-440	120±38 (120) 60-210
	Stipe	220±110 (200) 120-590	81±23 (76) 37-130
	$Q_{C//S}$	1.4±0.4 (1.5) 0.66-2.0	1.6±0.3 (1.6) 1.2-2.2
	Soil	ND	2.7±0.09 (2.7) 2.6-2.8
	BCF_C	ND	46±15 (45) 21-80
	BCF_S	ND	30±9 (28) 13-51

ANNEX 2. Continued.

Hg	Cap	5.3±0.8 (5.3) 3.2-6.6	1.8±0.8 (1.9) 0.05-2.8
	Stipe	3.2±0.9 (3.1) 2.3-4.9	0.97±0.50 (1.0) 0.05-2.0
	$Q_{C//S}$	1.7±0.4 (1.7) 1.2-2.4	1.8±0.4 (1.9) 1.0-2.6
	Soil	0.067±0.068 (0.053) 0.017-0.27	0.10±0.00 (0.10) 0.095-0.10
	BCF_C	160±100 (180) 33-390	18±8 (19) 0.52-29
	BCF_S	100±77 (99) 19-290	10±5 (11) 0.52-21
Mn	Cap	22±9 (20) 8.1-47	21±8 (20) 13-41
	Stipe	42±24 (33) 12-92	35±24 (23) 11-84
	$Q_{C//S}$	0.65±0.33 (0.63) 0.14-1.3	0.88±0.54 (0.86) 0.23-2.2
	Soil	ND	670±59 (650) 620-750
	BCF_C	ND	0.032±0.011 (0.029) 0.021-0.061
	BCF_S	ND	0.052±0.037 (0.031) 0.018-0.13
Pb	Cap	4.7±3.0 (4.0) 0.64-11	1.7±0.6 (1.5) 0.58-2.6
	Stipe	7.3±6.6 (5.7) 1.4-27	0.75±0.40 (0.61) 0.37-1.7
	$Q_{C//S}$	1.2±1.8 (0.54) 0.15-6.3	2.4±1.2 (2.2) 1.3-6.0
	Soil	ND	14±0 (14) 14-14
	BCF_C	ND	0.12±0.05 (0.19) 0.041-0.18
	BCF_S	ND	0.053±0.028 (0.044) 0.027-0,12
Rb	Cap	ND	22±12 (17) 9.4-42
	Stipe	ND	13±7 (11) 5.6-26
	$Q_{C//S}$	ND	1.7±0.4 (1.6) 1.4-2.7
	Soil	ND	1.4±0.4 (1.4) 1.4-1.5
	BCF_C	ND	16±8 (12) 6.4-30
	BCF_S	ND	9.0±4.2 (7.8) 3.8-19
Sr	Cap	ND	0.40±0.17 (0.35) 0.18-2.5
	Stipe	ND	0.46±0.23 (0.36) 0.20-0.86
	$Q_{C//S}$	ND	0.99±0.45 (0.91) 0.35-1.9
	Soil	ND	6.2±0.1 (6.2) 6.1-6.3
	BCF_C	ND	0.064±0.028 (0.057) 0.030-0.12
	BCF_S	ND	0.074±0.038 (0.058) 0.049-0.11
Zn	Cap	96±23 (90) 67-140	110±10 (110) 85-130
	Stipe	77±51 (60) 35-210	74±15 (75) 52-97
	$Q_{C//S}$	1.5±0.5 (1.7) 0.62-2.2	1.5±0.2 (1.6) 1.2-1.7
	Soil	ND	19±0 (19) 19-20
	BCF_C	ND	5.7±0.8 (6.1) 4.3-7.1
	BCF_S	ND	3.9±0.8 (4.0) 2.6-6.1

[a] Not determined

[b] $Q_{c/s}$ values of the quotient of metal contents in cap to stipe

Nickel was determined only in mushrooms and soils from the Lubraniec and Poniatowa sites. The soil Ni contents were similar ($p>0.05$) for the two spatially distant sites. Caps contained Ni in content varying between 0.29±0.47 and 0.86±0.22 (range 0.01-1.5) mg kg^{-1} dm, while the stipes were a little more abundant in Ni than the caps ($p<0.05$; Mann-Whitney *U* test). From the data summarized in Annex 1 it can be concluded that the *M. procera* bioexcludes Ni. Laboratory studies showed that Ni (from $NiCl_2$) was absorbed by mycelium and accumulated by *M. procera*, but the mushroom growth was impaired as the Ni content increased in the substrate medium from 0.05 to 0.8 mM [53]. Rubidium is effectively bioconcentrated by *M. procera* in caps (BCF ~100) and stipes (BCF~40). Rubidium contents in the soil were approximately 0.40 mg kg^{-1} dm, while the mean content in the caps was 38±7 (range 27-53) mg Rb kg^{-1} dm (Annex 1).

Toxic Metals (Cd, Pb, Hg, Ag)

These metals (Cd, Pb, Hg, and Ag) are highly toxic to mammals. In the caps of *M. procera* from the Lubraniec and Poniatowa sites, the mean Pb content varied from 1.7±0.6 to 5.9±1.7 (range 0.58-11) mg kg^{-1} dm. The mean Hg contents in caps from the Lubraniec, Poniatowa, Jarocin, and Gubin sites are 2.1±0.6, 3.1±0.9 (range 1.3-4.6), 1.8±0.8, and 5.3±0.8 mg kg^{-1} dm, respectively (Annex 1). Silver content in caps (for three sites including the Jarocin site) varied from 1.2±0.7 to 16±7 (range 0.40-31) mg kg^{-1} dm, and for Cd (for six sites) the mean values varied from 0.56±0.13 to 4.9±5.4 (range 0.07-17) mg kg^{-1} dm (Annex 1 and 2). The abundance of these metals in the caps varied significantly ($0.01<p<0.05$; Mann-Whitney *U* test) for some of the sites.

Cadmium, mercury, and silver were accumulated by *M. procera* while Pb was bioexcluded. The ranges of Cd, Ag, Hg, and Pb contents in the soils were 0.1-0.4, 0.01-0.06, 0.05-0.15, and 8-15 mg kg^{-1} dm, respectively, while the range of their BCF values for caps were 4-15, 20-470, 15-160, and 0.2-0.7 (for Cd, Ag, Hg, and Pb), respectively (Annexes 1 and 2). For some of the sampled sites, Ag and Hg were highly bioconcentrated by *M. procera* and their BCF values reached more than 100. Earlier studies reported that *M. procera* has the ability to efficiently bioconcentrate Hg [54-57]. This species, apart from being rich in the essential elements (including Cu and Zn), is also rich in Cd, Pb, Hg, and Ag [2, 14, 26, 40, 56].

The occurrence of essential and toxic trace elements in edible mushrooms collected in the wild as well as their intake rates, nutritional benefits, and likely risks, are of primarily concern to consumers. The traditional cooking recipes describe that caps of freshly collected *M. procera* are especially good for stuffing (filling) and then broiling, or for frying in a pan with some oil or butter after being dipped in whisked egg and breadcrumbs. In Slovakia, for example, baked *M. procera* caps are stuffed with ground pork, oregano, and garlic. In these culinary treatments the possible leaching of the mineral contents of the mushroom cap is small, if any, and can therefore be ignored when estimating toxic metals intake rates and the likely risks to consumers.

Mercury

For the assessment of possible risks due to intake of Hg accumulated in caps of *M. procera*, the reference dose (RfD; 0.0003 mg kg^{-1} body mass daily) and established value of provisionally tolerable weekly intake (PTWI; 0.004 mg kg^{-1} bm) were applied [58-59]. From a toxicological point of view, Hg content of caps of *M. procera* is of concern. A meal cooked using 300 or 500 g of caps collected at the Jarocin, Lubraniec, Poniatowa, or Gubin sites will result in the intake of Hg ranging from 0.9 to 2.7 or 1.5 to 4.4 µg kg^{-1} body mass for consumption of 300 g or 500 g of caps, respectively. These doses were derived from the minimum and maximum median values of 0.18 (Jarocin) and 0.53 (Gubin) mg Hg kg^{-1} caps, wet weight (assuming 90% water content in caps and an adult individual of 60 kg body mass). The weekly consumption of 500 g of caps of *M. procera* from Gubin, which showed the highest Hg content, will result in the intake of 0.26 mg Hg, which is above the 0.24 Hg recommended dose of PTWI (assuming that no Hg from other foods is ingested).

Selenium plays a protective role against Hg contained in foods. It is presumed that the co-occurrence of Se and methyl mercury or total Hg in stoichiometric ratio could prevent the toxic effects of Hg [60]. Among fungi *M. procera* is relatively rich in Se. As reviewed recently, Se content of the caps of *M. procera* usually varies around 5 mg kg^{-1} dm [61]. In a recent study, the average Se content of *M. procera* (n=11) collected from Spain were 16±9 mg kg^{-1} dm for the hymenophores and 5.5±2.3 mg kg^{-1} dm for the rest of the fruiting body (including inedible stipes) [39].

The PTWI for methylmercury is 0.0016 mg kg^{-1} body mass [62]. The methylmercury content of *M. procera* were not determined in this study. Bargagli and Baldi reported 0.06% of the total mercury content parasol mushroom as methyl Hg in a single specimen of Parasol Mushroom from a cinnabar mining area near Siena, Italy (4 mg Hg kg^{-1} dm) [63].

Cadmium

Cadmium is known for its high toxicity to the mammalian body systems and especially because of its nephrotoxicity and accumulation in kidneys. The PTWI of 7 µg Cd kg^{-1} body mass (equivalent to 1 µg kg^{-1} bm daily) was set earlier by the World Health Organization [64]. The provisional tolerable monthly intake (PTMI) for this metal is 25 µg kg^{-1} bm [65]. The median values of Cd in caps from six of the sites surveyed varied from 0.57 (Lubraniec) to 2.2 (Gubin) mg kg^{-1} dm (0.057 and 0.22 mg kg^{-1} fresh weight; assuming 90% water content). In the European Union the maximum level of cadmium allowed for mushrooms is 1.0 mg kg^{-1} fresh product (10 mg kg^{-1} dm; assuming 90% moisture content) [66]. The median and arithmetic mean content values in this study were below the acceptable limit, and only a few specimens collected from Gostyńsko-Włocławskie Forest and from the Gubin region contained Cd in caps at levels >10 mg kg^{-1} dm (Table 1, Annex 1).

A meal made with 300 or 500 g of caps of *M. procera* from these sites will result in Cd intake of between 17 and 28 or 66 and 110 µg Cd, respectively, assuming no Cd intake from other foods is ingested. These amounts correspond to doses of 0.28 to 0.47 (for consumption of 300 g of caps of *M. procera* or 1.1 to 1.8 µg Cd kg^{-1} bm (for consumption of 500 g of caps). These estimated values show that a tolerable weekly intake (TWI) dose of 150 µg Cd (for an individual of 60 kg bm) will not be exceeded when eating a large meal (500 g) made of caps of *M. procera* collected from the Gubin region once weekly, or two to three meals weekly for caps from the other investigated sites. The estimated TWI value of 150 µg for Cd is equivalent to 21 µg daily intake and this value is close to the estimated Cd intake from a 300 g portion of fresh caps of parasol mushrooms from the other sites surveyed.

The caps of some *M. procera* samples from four of the six sites surveyed showed substantially higher Cd contents (Annex 1 and 2) compared to the maximum median values used in these estimations. This suggests that consumers could be exposed to relatively higher doses of Cd if they consume larger meals of *M. procera* often – especially during the mushrooming season each year.

Lead

The median Pb values of caps from four of the sites surveyed were 1.5 (Jarocin), 2.2 (Lubraniec), 4.0 (Gubin), and 5.2 (Poniatowa) mg kg^{-1} dm (between 0.15 and 0.52 mg kg^{-1} fresh product; assuming 90% moisture content). In the EU regulation, the permitted content of Pb in cultivated mushrooms (champignon, oyster, and shiitake) is 0.3 mg kg^{-1} fresh mushrooms (3.0 mg kg^{-1} dm; assuming 90% moisture) [67]. For *M. procera* specimens from the Poniatowa and Gubin sites, the median Pb contents for caps exceeded the EU limit (also for some specimens from the Lubraniec site) (Annexes 1 and 2). A meal made with 300 or 500 g of caps from these sites will result in the intake of between 45 and 75 or 156 and 260 µg Pb, respectively. These values correspond to doses of 0.75 to 1.2 or 2.6 to 4.3 µg Pb kg^{-1} bm, assuming no Pb intake from other foods.

The FAO/WHO JECFA established a PTWI for lead of 1.5 mg for person of 60 kg body mass, which is equivalent to 214 µg daily, 25 µg kg^{-1} bm weekly, or 3.6 µg kg-^{1} bm daily [68]. Nevertheless, because of the relatively elevated Pb levels accumulated in *M. procera*, the frequent eating of caps during the mushrooming season can result in intake doses close to or exceeding the allowed intake value. This can be of special toxicological concern since a significant proportion of the caps in specimens studied showed Pb content exceeding the median values determined (Tables 1 and 2).

Table 2. Eigenvalues of correlation matrix and related statistics[a]

Factor	Eigenvalue	Percent of total variance	Cumulative eigenvalue	Cumulative percent
1	9.20	76.65	9.20	76.65
2	1.75	14.56	10.95	91.25

[a] Minimum Eigenvalue = 1

Silver

Unlike Hg, Cd, or Pb, there are no intake limits or tolerance values set for Ag in foodstuffs. Silver ion is very reactive and quickly bonds to the surface of epithelium cells of the alimentary tract and hence is largely excreted (not absorbed) [69], thereby raising questions about the absorption rates of Ag from the food chain — especially for Ag in edible wild mushrooms.

Many mushroom species are relatively rich in silver, and this metal is efficiently taken-up from contaminated substratum by the champignon mushroom (*Agaricus bisporus*) or in the wild by the meadow mushroom (*A. campestris*), while the warted amanita (*Amanita strobiliformis*) is a hyperacumulator of silver [11, 24-26]. Plant foods such as cereals, vegetables, pulses, and fruits are known to contain much lower Ag when compared to many mushroom species. The chemical species of Ag in mushrooms were identified recently as the metallothionein isoforms involved in intracellular sequestration of Ag by *A. strobiliformis* [24]. The availability and toxicity of Ag bonded to fungal peptides from a mushroom meal is unknown.

The median values of silver in caps at three of the sites surveyed varied widely: 1.2 for Jarocin, 3.2 for Lubraniec, and 13 for Poniatowa mg kg^{-1} dm (range 0.12 to 1.3 mg kg^{-1} fresh productt; assuming 90% water content). Hence, a meal made with 300 or 500 g of fresh caps of *M. procera* collected from these sites (Jarocin, Lubraniec, and Poniatowa) will result in intake of silver of between 36 and 60 or 390 and 650 µg Ag, respectively, assuming no silver intake from other foods is ingested. These amounts correspond to doses of 0.6 to 1 or 6.5 to 10.8 µg Ag kg^{-1} bm.

Principal Component Analysis

In order to examine the profiles of metals sequestered in the caps from four sites (Table 1), the principal component analysis (PCA) was used. The contents of K, Mg, Al, Ba, Ca, Cd, Cu, Fe, Mn, Na, Sr, and Zn were used as variables. The correlation matrixes resulting from the PCA are displayed in Tables 2 and 3. The number of components was chosen with the ordinary rule of selecting eigenvalues >1. The PCA of the data matrix gave a two-dimensional model that explained 91% (77% and 15%, respectively) of the total variance in the data set. The loading plot (Fig. 2) shows that the first principal component

Table 3. The values of the coefficients of principal components (factor loadings)[a]

Metal	Factor 1	Factor 2
K	**-0.9930**	-0.0569
Mg	**-0.9641**	-0.1692
Al	**-0.9117**	0.3204
Ba	**-0.9896**	-0.0055
Ca	**-0.9899**	0.1298
Cd	-0.3861	**-0.7875**
Cu	**-0.9177**	-0.3143
Fe	**-0.8818**	-0.3769
Mn	**-0.7131**	0.2808
Na	0.5587	**-0.8062**
Sr	**-0.9319**	0.0775
Zn	-0.9983	0.0049

[a] Number of factors = 6; factor loadings >**0.7**

(eigenvalue = 9.2) is influenced by negatively correlated variables such as K, Mg, Al, Ba, Ca, Cu, Fe, Mn, Sr, and Zn. The second factor (eigenvalue = 1.75) presented a negative correlation with Cd (coefficient -0.79) and Na (coefficient -0.81).

In the two-dimensional factor space spanned by vectors PC1 and PC2, K, Ba, Ca, and Zn are the least correlated to PC1 (coef. -0.99). Cadmium and sodium are negatively correlated to PC1 and PC2, respectively. The loading plot (Fig. 2) shows graphically the association existing among metals in the factor matrices. Other metals are not apparently associated with a definite-factor axis.

A projection of *M. procera* trace metals data set on the PC plane allows us to visualize the contribution of the particular groups of metallic elements to the specimens' spread (Fig. 3). First, the principal component showed a strong separation of Poniatowa site from Lubraniec site, Starachowickie Forest, and Gostyńsko-Włocławskie Forest, which clustered close together with respect to PC1. Metals such as K, Mg, Al, Ba, Ca, Cu, Sr, and Zn introduced the greatest variance in the mineral constituents' composition, while only caps collected at the Poniatowa site could be separated based on these criteria (Fig. 3). PC2 indicated that the caps collected from Gostyńsko-Włocławskie Forest are low in Cd and Na contents (Fig. 3). This configuration of cluster inter-correlations could be explained by considering that the contents of macro and trace metals in higher mushrooms depends mainly on several factors, including biological ones (which are to some degree species- or genera-dependent), as well as the soil bedrock and other environmental factors [46-48, 70].

In order to demonstrate possible spatial variations in *M. procera* mineral composition, the we conducted a cluster analysis (CA) based on the cap and soil data set (Fig. 4). The CA diagram divided all cases into two main fractions, and this apparently reflected interdependent relationships occurring between them. The first fraction separated caps of *M. procera* collected from four stands in south-central Poland while the second fraction separated soil collected from the same sites. In the first cluster the strongest similarity occurred between caps collected from the Lubraniec site and Starachowickie Forest. In the second case, up to two subclusters could be recognized related to soils from Gostyńsko-Włocławskie Forest and the Poniatowa site.

Conclusions

M. procera from wild forest sites are efficient absorbers and accumulators of certain metals (Cd, Cu, K, Mg, Na, Zn) that naturally occur in the substrates on which the mushroom mycelium develop. Though delicious and rich in many minerals, when cooked the caps of *M. procera* can provide to the consumer/fanciers substantial quantities of several essential trace elements but also such toxic metals as cadmium, lead, and mercury. Considering the contents of cadmium, lead, and mercury observed in the caps (which varied spatially) and the recommended intake limits for these toxic metals, the frequency of eating *M. procera* by people fond of this species (or people with easy access to it during the mushrooming season) should be limited to one or two meals weekly. The frequent eating of *M. procera* collected from unpolluted forests within the investigated sites while providing the unique taste of a juicy dish will not expose consumers to doses of cadmium, lead, and mercury above recommended limits.

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