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Rare earth elements in parasol mushroom *Macrolepiota procera*

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ABSTRACT

This study aimed to investigate occurrence and distribution of 16 rare earth elements (REEs) in edible saprobic mushroom *Macrolepiota procera*, and to estimate possible intake and risk to human consumer. Mushrooms samples were collected from sixteen geographically diverse sites in the northern regions of Poland. The results showed that for Ce as the most abundant among the RREs in edible caps, the mean concentration was at 0.18 ± 0.29 mg kg^{-1} dry biomass. The mean concentration for Σ16 REEs determined in caps of fungus was 0.50 mg kg^{-1} dry biomass and in whole fruiting bodies was 0.75 mg kg^{-1} dry biomass. From a point of view by consumer, the amounts of REEs contained in edible caps of *M. procera* could be considered small. Hence, eating a tasty caps of this fungus would not result in a health risk for consumer because of exposure to the REEs.

1. Introduction

The term "rare earth elements; REEs" relates to 17 elements that were considered as rare in nature, i.e. 15 lanthanides, lanthanum (La) and other lanthanides (Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) and historically also two other with scandium (Sc) and yttrium (Y) that have similar properties and are an additional elements. Further, the REEs can be also divided into light REE group (LREE; La through Eu), medium REE (Sm – Ho) and heavy REE (Gd through Lu). Radioactive promethium (Pm) is largely an artificial REE, and its content in the lithosphere is about 0.6 kg. Cerium is most common among the REEs in nature and its abundance in lithosphere is at 68 mg kg^{-1} (Tyler, 2004). The REEs that are well dispersed in lithosphere, pedosphere and hydrosphere can be absorbed via food chain by vegetation, animals and human (Ichihashi, Morita, & Tatsukawa, 1992; Pagano, Aliberti et al., 2015; Pagano, Guida et al., 2015; Schwabe, Meyer, Flachowsky, & Dänicke, 2012). Although the minerals (ores) of the REEs are very scarce but they are mined in some regions of the world, and now China become a major global producer (RRE, 2015).

The applications of the individual REE are increasing in volume and uses in new technologies, and each REE found different appliances (Migaszewski & Gałuszka, 2015; RRE, 2015). Since REEs become increasingly more and more popular in industrial and customer use there is a warning about their accumulation in the foods and environment following the anthropogenic inputs (Jijang, Yang, Zhang, & Yang, 2012). The exploitation of the ores could create an environmental problems locally and regionally because of deposition of the REEs in soil and sediment (Feng et al., 2000; Jijang et al., 2012; Li, Hong, Yin, & Liu, 2010).

The REEs can be also added to mineral fertilizers as possible plant growth promoters (Jijang et al., 2012; Li et al., 2010). Some superphosphate fertilizers made of apatite can contain by-side REEs while a continuous use of such fertilizers possibly could enhance concentration of REEs in the treated soils (Todorovsky, Minkova, & Bakalova, 1997).

A study by Schwabe et al. showed that feeding of the preruminant and growing Holstein calves with milk replacer supplemented with a mixture of citrate derivatives of the REEs (57.9% Ce, 34.0% La, 6.5% Pr and 1.6% of other REEs) not improved the performance of calves, and following this study use of the REEs as growth promoters for calves was not recommended (Schwabe et al., 2012). The REEs because of similar chemical and physical properties tend to exist together. They are chemically similar to calcium (Ca) and are supposed to be absorbed by organisms as a group, and in animals seem to be a bone seekers (Chen & Zhu, 2008; Zaichick, Zaichick, Karandashev, & Nosenko, 2011).

Mushrooms can be very effective in bio-concentration of different chemical elements from substrate in which mycelium lives to developed fruiting bodies, and depending on species a high values of bio-concentration factor (BCF) were obtained for e.g. Ag, As, Cd, Cu, Hg, Se and Zn (Bhatia et al., 2013; Brzostowski, Falandysz, Jarzyńska, & Zhang, 2011; Falandysz, Bona, & Danisiewicz, 1994; Kojta, Jarzyńska, & Falandysz, 2012; Kojta et al., 2015; Krasińska & Falandysz, 2015; Širić, Kasap, Bedeković, & Falandysz 2017).

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For example, Ag was hyper-accumulated by *Amanita strobiliformis* (up to 1253 mg kg^{-1} dry biomass, db) and related species of the section *Lepidella* (Borovička, Řanda, Jelinek, Kotrba, & Dunn 2007). Also arsenic was highly elevated, i.e. 120 mg kg^{-1} db (caps) and 55 mg kg^{-1} db (stipes) in *Boletus luridus* (Schaeff.) Murrill from the polymetallic soils in the region of Dali in Yunnan province of China, and >1000 (150–3200) mg kg^{-1} db in a hyper-accumulator *Sarcosphaera coronaria* (Jacq.) J. Schröt (Falandysz & Rizal, 2016). *Macrolepiota procera* (Scop.) Singer 1948 collected from the background areas also well sequestered Ag, Cd, Hg, Cu and Zn in fruiting bodies, and their BCFs were well above unity (Kojta et al., 2016; Stefanović, Trifković, Mutić, & Tešić, 2016; Stefanović, Trifković, Djurdjić, et al., 2016). Also Pb, which is relatively poorly transferred from the soil substrata to *M. procera* (BCF < 1), was well sequestered in fruitbodies (up to 14 mg kg^{-1} db in caps) (Stefanović, Trifković, Mutić et al., 2016).

In a controlled experiment with cultures of two filamentous fungi such as *Trichoderma atroviride* P. Karst., and *Trichoderma harzianum* Rifai, both species accumulated intracellularly and in the extracellular matrix the REEs added into a liquid medium (Aquino et al., 2009). On the other side, a bioconcentration potential of the RREs by fungi in spore-bearing fruiting bodies (mushrooms) is so far little known. In a recent study has been shown that *Lactarius pubescens* Fr. growing in the former uranium mining area in Ronneburg (Germany) absorbed the REEs from the soil substrate and could sequester them to some extent in fruiting bodies, while value of BCF was <1 (Grawunder & Gube, 2015).

In a few earlier studies has been shown that the REEs are common trace or ultra-trace constituents in wild-growing mushrooms (Aruguete, Altstad, & Mueller, 1998; Borovicka, Kubrova, Rohovec, Randa, & Dunn, 2011; Falandysz et al., 2001; Horowitz, Schock, & Horowitz-Kisimova, 1974; Marzano, Bracchi, & Pizzetti, 2001; Stijve, Andrey, Lucchini, & Goessler, 2002). This study aimed to investigate status of La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu (included are also Sc and Y) in composite samples of caps and a whole fruiting bodies of the Parasol Mushroom *Macrolepiota procera* collected across Poland (Fig. 1). Mushroom *M. procera* is saprobic species that is popular in Europe and in the regions of Asia, and its cap is edible and tasty but is without information on the REEs potentially accumulated in fruitbodies (Gucia, Jarzyńska, Kojta, & Falandysz 2012; Gucia Kojita et al., 2012; Kułdo, Jarzyńska, Gucia, & Falandysz, 2014; Kojta et al., 2011, 2016; Stefanović, Trifković, Mutić, & Tešić, 2016).

2. Materials and methods

The samples of the fresh fruiting bodies of *M. procera* were collected over a wide area from sixteen localizations in foraging season in August – September of a given year (Table 1). All individuals directly after pickup were cleaned up from any visible plant vegetation and soil debris with a plastic knife and brush and the bottom part of stipe was cut-off. To get insight into distribution of elements between two major morphological parts of the fruiting bodies, the specimens from some localizations were separated into cap and stipe (stem or stalk supporting the cap). Next, the individual cap, stipe or a whole fruiting body samples were sliced into pieces using a plastic disposable knife and pooled for each place (n = 10–30 individuals per pool) in composite samples representing each sampling place and time of collection (Table 1), and next dried and pulverized. A procedure used for preparation of dried and pulverized fungal materials and their preservation until laboratory analyses has been described in detail previously (Gucia, Jarzyńska et al., 2012; Gucia, Kojta et al., 2012).

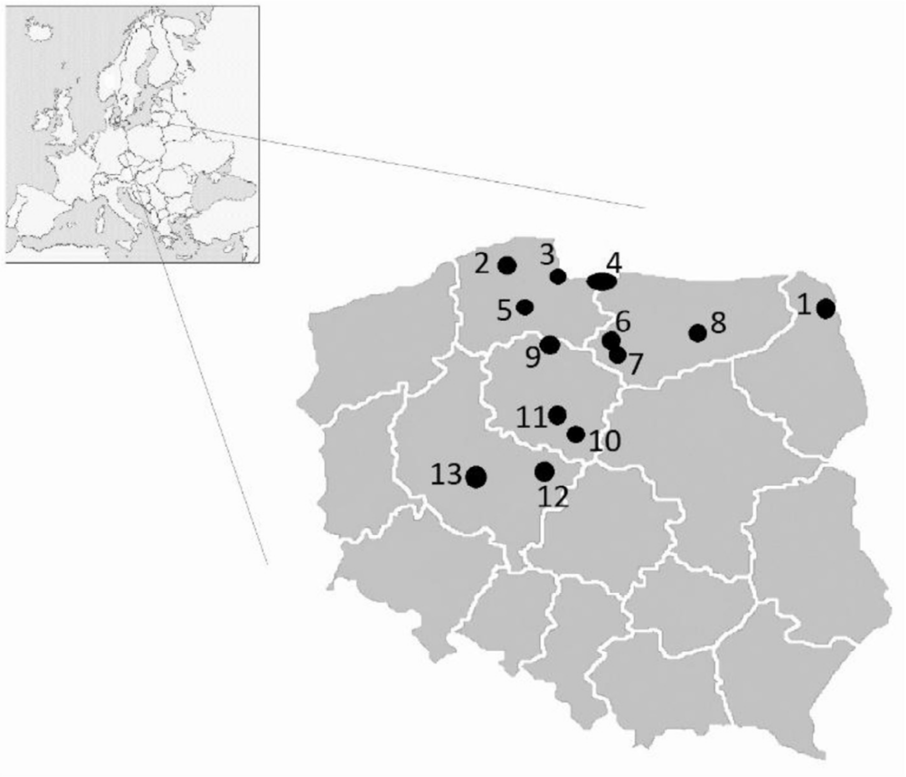

Fig. 1. Sampling sites of *M. procera* in Poland: (1) Augustów Primeval Forest; (2) Pomerania, Lębork; (3) Trójmiejski Landscape Park; (4) Vistula River Sandbar; (5) Wdzydze landscape Park; (6) Warmia region, Jeziorak, Gierszak island; (7) Warmia region, Sarnówek; (8) Olsztyn/Szczytno; (9) Tuchola Pinewoods, Łuby; (10) Kujawy region, Wloclawek – outskirts; (11) Kujawy region, Toruń – outskirts; (12) Nadwarciańska Forest; (13) Trzebiesza near Poznań.

Table 1
Rare earth elements in caps and whole fruiting bodies of parasol mushroom *Macrolepiota procera* (mg kg^{-1} dry biomass).

Place (sample ID)*, year, number of specimens and morphological part*	Sc	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
(1) Augustów Primeval Forest, 2001 (n = *15*; c)	0.063	0.14	0.17	0.34	0.035	0.11	0.018	0.0053	0.020	0.0034	0.019	0.0040	0.011	0.0018	0.012	0.0017
(2) Pomerania, Lębork, 2003 (n = *30*; c)	0.0014	0.046	0.056	0.12	0.011	0.038	0.0066	0.0015	0.0065	0.0011	0.0052	0.0014	0.0047	0.0008	0.0050	0.00088
(3) TLP, 2001 (n = *23*; c)	<0.001	0.071	0.071	0.16	0.015	0.055	0.011	0.0024	0.011	0.0018	0.0094	0.0024	0.0065	0.0011	0.0075	0.0012
(4) Vistula River Sandbar, Stegna, 2003 (n = *10*; c)	0.16	0.14	0.11	0.23	0.024	0.093	0.018	0.0033	0.018	0.0031	0.020	0.0042	0.012	0.0017	0.012	0.0017
(5) WLP, 1994/2001 (n = *21*; c)	0.0029	0.10	0.15	0.33	0.031	0.11	0.022	0.0044	0.020	0.0026	0.015	0.0032	0.0099	0.0014	0.0098	0.0018
(6) Warmia, Jeziorak, Gierszak island, 2001 (n = *15*; c)	0.087	0.067	0.060	0.13	0.013	0.047	0.010	0.0024	0.0080	0.0016	0.0087	0.0020	0.0072	0.0012	0.0076	0.0012
(7) Warmia land, Sarnówek, 2001 (n = *11*; c)	<0.001	0.060	0.067	0.15	0.015	0.058	0.010	0.0021	0.0086	0.0014	0.0096	0.0021	0.0057	0.0008	0.0059	0.00081
(8) Olsztyn/Szczytno 2002 (n = *25*; c)	<0.001	0.021	0.031	0.064	0.0057	0.023	0.0037	0.0008	0.0041	0.0007	0.0032	0.0007	0.0014	0.0002	0.0014	0.00013
(9) Tuchola Pinewoods, Łuby, 1995 (n = *15*; c)	<0.001	0.072	0.11	0.23	0.023	0.087	0.014	0.0027	0.015	0.0021	0.012	0.0023	0.0065	0.0010	0.0064	0.00094
(10) Kujawy region, Włocławek – outskirts, 2004 (n = *15*; c)	0.017	0.11	0.10	0.21	0.021	0.074	0.015	0.0047	0.014	0.0025	0.013	0.0038	0.012	0.0021	0.013	0.0017
(11) Kujawy region, Toruń – outskirts, 2000 (n = *15*; c)	0.0087	0.071	0.077	0.17	0.016	0.062	0.012	0.0029	0.010	0.0017	0.010	0.0020	0.0071	0.0009	0.0070	0.0012
(12) Nadwarciańska Forest, 1999 (n = *15*; c)	<0.001	0.015	0.014	0.030	0.0030	0.012	0.0021	0.0003	0.0020	0.0003	0.0023	0.0003	0.0012	0.0002	0.0014	0.00024
(13) Trzebiesza near Poznań, 2001 (n = *15*; c)	0.017	0.053	0.062	0.13	0.013	0.047	0.0069	0.0029	0.0059	0.0012	0.0076	0.0017	0.0057	0.0013	0.0061	0.0010
Mean	*0.028*	*0.074*	*0.083*	*0.18*	*0.017*	*0.063*	*0.012*	*0.0027*	*0.011*	*0.0018*	*0.010*	*0.0023*	*0.0070*	*0.0011*	*0.0073*	*0.0011*
±SD	*0.048*	*0.039*	*0.049*	*0.091*	*0.009*	*0.003*	*0.006*	*0.0018*	*0.0060*	*0.0009*	*0.005*	*0.0012*	*0.0035*	*0.0006*	*0.0037*	*0.0005*
(9) Tuchola Pinewoods, Osiek, 2000 (n = *15*; w)	0.025	0.13	0.17	0.37	0.036	0.13	0.021	0.0062	0.019	0.0032	0.017	0.0040	0.011	0.0017	0.013	0.0017
(11) Kujawy region, Kukawy/Goreń region, 2001 (n = *15*; w)	0.0053	0.037	0.064	0.13	0.014	0.038	0.0067	0.0015	0.0062	0.0008	0.0055	0.0012	0.0034	0.0005	0.0041	0.00053
(11) Kujawy region, Bydgoszcz – outskrits, 2001 (n = *15*; w)	0.055	0.15	0.16	0.34	0.033	0.12	0.023	0.0062	0.022	0.0036	0.020	0.0043	0.013	0.0019	0.013	0.0022
Mean	*0.028*	*0.11*	*0.13*	*0.28*	*0.028*	*0.096*	*0.017*	*0.0046*	*0.016*	*0.0025*	*0.014*	*0.0032*	*0.009*	*0.0014*	*0.010*	*0.0015*
±SD	*0.025*	*0.030*	*0.058*	*0.13*	*0.012*	*0.050*	*0.0089*	*0.0027*	*0.0084*	*0.0015*	*0.008*	*0.0077*	*0.005*	*0.0008*	*0.005*	*0.0009*

Notes: *ID (see Fig. 1); c (caps), w (whole fruiting bodies); TLP (Trójmiejski Landscape Park – Gdańsk); WLP (Wdzydze Landscape Park).

For the analysis of the REEs, about 200 mg samples of dried and pulverized fungal materials were mixed with 3 mL solution of ultrapure concentrated nitric acid (HNO_3, 65%,) and 1 mL of ultrapure hydrofluoric acid in a polytetrafluoroethylene tubes (PTFE). Then, the tubes were screw tighten in a stainless steel jackets and placed in an oven at 150 °C for 78 h. The solutions obtained were evaporated to dryness at 110 °C, to remove the excess of HF. Then, it was dissolved in about 1 mL of HNO_3 and the solution was transferred to a sample tube and the final volume was made to 50 mL. As an internal standard, rhodium (Rh) (10–20 μg per litre) was added to the samples prior to the Quadruple ICP-MS analysis. In order to achieve good analytical quality control and quality assurance, blanks and certain certified reference materials were examined. Each element was measured 3 times and the values of relative standard deviation (RSD) were within 5% in the samples and the certified values for certified reference materials (CRM). The CRMs used were citrus leafs (GBW 10020) and soil (GBW 07405) produced by Institute of Geophysical and Geochemical Exploration, China (Liang & Grégoire, 2000; Shi et al., 2011). The mean values of the REEs concentrations in the caps and a whole fruiting bodies of *M. procera* were further normalized against North American Shale Composite (NASC) and Post – Archean Australia Shales (PAAS) as reported by Dołęgowska and Migaszewski (2013).

3. Results and discussion

The results of the study showed on Ce as the most abundant among the REEs in *M. procera* with a mean value of concentration in the caps at 0.18 ± 0.09 mg kg^{-1} dry biomass (db) and range from 0.030 to 0.34 mg kg^{-1} db. For other REEs the mean concentrations in mg kg^{-1} db in descending order were: 0.083 ± 0.049 (La), 0.074 ± 0.039 (Y), 0.058 ± 0.003 (Nd), 0.017 ± 0.009 (Pr), 0.012 ± 0.006 (Sm), 0.011 ± 0.006 (Gd), 0.010 ± 0.005 (Dy), 0.0073 ± 0.0037 (Yb), 0.0070 ± 0.0035 (Er), 0.0028 ± 0.048 (Sc), 0.0027 ± 0.018 (Eu), 0.0023 ± 0.0012 (Ho), 0.0011 ± 0.0005 (Lu) and 0.0011 ± 0.0006 (Tm) (Table 1). Total concentration of Σ16 REEs determined in the caps (13 sites) was at 0.50 mg kg^{-1} db while in whole fruiting bodies (3 sites) was 0.75 mg kg^{-1} db. A higher value of Σ16 REEs obtained for the whole fruiting bodies than for the caps of *M. procera* suggests that stems of this mushroom could be richer in the REEs than caps. This can be related to reported greater concentration of Ca in stipes, which is harder than caps (see discussion below), while no data on the REEs solely in stipes were available.

Observed variation in the REEs contents in *M. procera* from a different locations (Table 1) can be related to their content in soil substratum, which was not examined. The soils in Poland are diverse in types due to high diversification of bedrocks. The brown earth dominate (brown soils and lessive soils – Eutric Cambisols, Dystric Cambisols, Albic- and Glossalbic Luvisols. – covering about 51.5% of the area of the country); podzols (podzolic soils, podzols and rusty soils – Dystric Arenosols, Haplic and Densic Podzols – covering about 26% of the area of the country) and of several other types (Niewiadowski & Tłoczko, 2014).

No other values on the absolute concentrations of REEs are available for *M. procera*. The value 0.50 mg kg^{-1} db for Σ16 REEs determined in the caps of *M. procera* is in a range of the REEs concentrations observed in fruiting bodies of King Bolete *Boletus edulis* foraged in background areas across Poland, which had Σ16 REEs at median value of 0.33 mg kg^{-1} db (unpublished, JF).

Borovička, Kubrova, Rohovec, Randa, and Dunn (2011) provided an information on concentrations of 14 REEs (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) in a bulk sample of saprobic mushrooms (Borovicka et al., 2011). In their study, the REEs in descended order (median values in mg kg^{-1} db) were at: Ce (0.042), La

(0.023), Nd (0.020), Pr (0.0056), Sm (0.0041), Gd (0.0023), Dy (0.0022), Er and Yb (0.0013), Eu (0.00068), Tb (0.00059), Ho (0.00042), Tm (0.00017) and Lu (0.00013), and the Chondric – normalized distribution of the REEs in *M. procera* showed their study a typical negative Eu-anomaly (Borovicka et al., 2011).

As this was mentioned in the introductory section, the REEs are considered by several authors as compounds that are chemically very similar to element Ca. The behaviour of REEs in the environment and fate in living organisms is considered to follow Ca, an essential macro element. *Macrolepiota procera* contained Ca in caps at 110–400 mg kg^{-1} db (median values) (Kojta et al., 2016), which is highly greater when related to the content of REEs (Table 1). More data on calcium and some other essential macro- and micro-mineral constituents in *M. procera* are available from a several articles (Falandysz et al., 2001, 2003; Falandysz, Gucia, & Mazur, 2007; Falandysz et al., 2008; Gucia, Jarzyńska et al., 2012; Gucia, Kojta et al., 2012; Kojta et al., 2011,, 2016; Jarzyńska, Gucia, Kojta, et al., 2011; Kułdo et al., 2014; Řanda, Soukal, & Mizera, 2005; Stefanović, Trifković, Mutić, & Tešić, 2016; Stefanović, Trifković, Djurdjić, et al., 2016). According to the reference papers mentioned, the element Ca is a minor macro-mineral constituent of *M. procera* and has been reported in caps at the mean concentration 60 (range 50–70) mg kg^{-1} db, and the values of the median concentrations reported by authors for many populations of this species were respectively at 87–230, 54 to 220, 130 and 230–240 mg kg^{-1} db (Falandysz et al., 2007, 2008; Gucia, Jarzyńska et al., 2012; Gucia, Kojta et al., 2012; Kojta et al., 2011; Jarzynska, Gucia, Kojta, Rezulak, & Falandysz, 2011; Kułdo et al., 2014). Calcium usually occurs in greater median concentration in stipes (stems) than in caps of mushrooms, e.g. in stipes of *M. procera* was at 70–860, 150 and 290–360 mg kg^{-1} db, and in a whole fruiting bodies was at 55 ± 18 mg kg^{-1} db (Falandysz et al. 2008; Gucia, Jarzyńska et al., 2012; Gucia, Kojta et al., 2012; Jarzynska, Gucia, Kojta, Rezulak, & Falandysz, 2011, Kojta et al., 2011; Kułdo et al., 2014; Stijve et al., 2002). The REEs as followers of calcium in biota could be more efficiently sequestered by *M. procera* in stems than caps (as is for calcium). Hence, a greater total content of Σ16 REEs in the whole fruiting bodies than caps of *M. procera* could be explained by their greater content in stems but this was not examined in this study.

An increasing demand and appliances of the REEs as well as information on their accumulation in human body raise some concern on a safety of foods possibly subjected for contamination due to anthropogenic inputs of the REEs into the ambient environment (Migaszewski & Gałuszka, 2015). The mean value of total concentration of 16 REEs determined in *M. procera* in this study when expressed on a fresh mushroom (assuming moisture content at 90%) is 0.050 mg kg^{-1} (a value suitable for calculation of intake), which is greater concentration, when related to that reported for several typical fresh foods in China but is less than in processed aquatic products there (Jijang et al., 2012). Foods in China contained Σ16 REEs (mg kg^{-1} fresh product) at 0.015 (cereals), 0.015 (vegetables), 0.016 (meats), 0.018 (eggs), 0.013 (fresh aquatic products; molluscs, crustaceans, marine fish, and freshwater fish) and 0.49 (processed aquatic products; molluscs, crustaceans, marine fish, and freshwater fish) (Jijang et al., 2012). Vegetables from the REEs mining area in Fujian Province, Southeast China contained Σ16 REEs at 0.036 mg kg^{-1} db (Li et al., 2010).

An abundance of the REEs in soil substrate could result in their greater accumulation in plants but probably also in mushrooms (topsoil beneath to fruiting bodies has not been examined in this study) (Tyler, 2004). Nevertheless, many fungi growing in background areas are much more efficient accumulators of different metallic elements when compared to other vegetation. Hence, a greater concentration of the REEs determined in *M. procera* in this study, when compared to vegetable foods from a background region in China (Jijang et al., 2012), could be related to good ability of accumulation of the lithophilic metals naturally occurring in topsoil (e.g. REEs) by some mushrooms than to an environmental pollution (Falandysz et al., 2015).

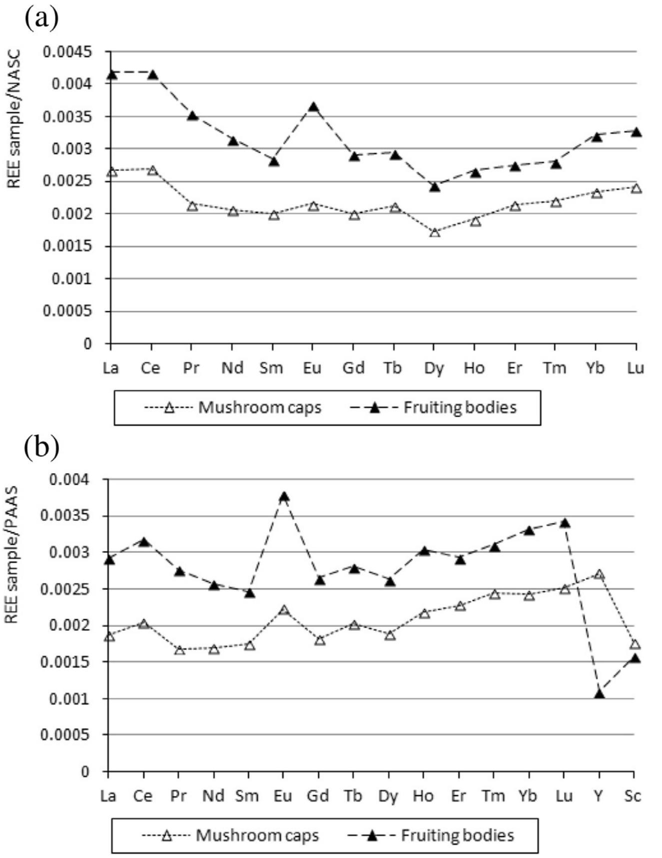

Fig. 2. NASC (a) and PAAS (b) normalized distribution of the mean values of 14 REEs contents determined in the caps and whole fruiting bodies of *M. procera* in this study (content of RRE in mushrooms in mg kg^{-1} db; content of REEs in shales in mg kg^{-1} (after Dołęgowska & Migaszewski, 2013).

The shale – normalized distribution of REEs mean contents in the caps and whole fruiting bodies of *M. procera* showed on a little different patterns, while both did indicate on the predominance of heavy rare earth elements (HREE; from La to Eu) over light rare earth elements (LREE; from Gd to Lu), and on a positive excursion into Ce, La and Eu (Eu predominated in the PASS – data set) (Fig. 2). A strong positive Eu anomaly has been observed also in the moss materials from the south-central Poland (Dołęgowska & Migaszewski, 2013).

The caps of *M. procera* are a delicacy. The caps are fragile and are usually roasted, roasted with eggs (a kind of omelette) or stuffed and broiled. They are not suitable for drying or pickling. Consumption is seasonal, rather small (one to several meals *per annum*; 100–500 g fresh biomass) and restricted only to an individual foragers. From a point of view by consumer, the amounts of REEs contained in edible caps of *M. procera* could be considered small. Hence, eating a tasty caps of this fungus would not result in a health risk for consumer because exposure to the REEs.

4. Conclusion

Mushroom *M. procera* samples collected from background areas across Poland showed on a low content of Σ16 REEs. The mean concentrations of the 16 REEs were greater in the whole fruiting bodies than caps of *M. procera*, indicating that stems could be better accumulators than caps. Hence, presence of the REEs in edible caps of *M. procera* at a trace concentrations determined seems typical for this species and does not seem to constitute any nutritional/health problem, while more data and from a diverse regions of the world could improve a very scarce database on the REEs in mushrooms.

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