SIMULTANEOUS UPTAKE OF RARE EARTH ELEMENTS, ALUMINIUM, IRON, AND CALCIUM BY VARIOUS MACROMYCETES

Tjakko Stijve, Sentier de Clies no 12, CH-1806 St-Légier, Switzerland
Daniel Andrey, Nestlé Research Centre, P.O. Box 44, CH-1000 Lausanne 26, Switzerland
GianFelice Lucchini, Via S. Abbondio 31, CH-6925 Gentilino, Switzerland
Walter Goessler, Institut für Analytische Chemie, Karl-Franzens-Universität, A-8010 Graz

Abstract

A multi-element study of 12 cultivated and 35 wild-growing mushroom species from various origins revealed the presence of significant quantities of lanthanides, also known as rare earth elements, in most of the samples. Cultivated mushrooms generally contained less than 0,1 mg/kg dry weight of the sum of cerium, lanthanum and neodymium, which make up 80 per cent of the 14 lanthanide metals. Nineteen wild-growing mushrooms had more than 1 mg/kg of the three metals, but this feature is probably not species-dependent. Specimens collected near a former mining area in the Malcantone region of the Swiss canton Ticino were rather rich in lanthanides. Those rare earth metals were invariably accompanied by 500–1000 times higher amounts of aluminium and iron, and often by high levels of calcium as well. Interestingly, simultaneous presence of thorium in concentrations amounting to 5–10 per cent of that of the total lanthanides was also noted. The metals are taken up as a group. No bioconcentration or – exclusion was observed. Mushrooms developing partly underground, *e.g.* *Agaricus bitorquis*, *A. geesterani*, *Gyrophragmium dunalii*, and *Podaxis pistillaris* tend to have higher concentrations of aluminium, iron, calcium and lanthanides. The highest value for total lanthanides (75 mg/kg dry weight) was measured in edible *P. pistillaris*. The food safety aspects are briefly discussed: although no legal limits for lanthanides in mushrooms are needed, it is disturbing to note that intake of aluminium from modest consumption of some species readily exhausts the Provisional tolerable weekly intake (PTWI). In view of their high content of potentially toxic metals, regular consumption of wild-growing mushrooms should be discouraged.

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Introduction

During the last 30 years many macromycetes have been recognised as potent accumulators of various trace elements, including heavy metals, arsenic and selenium. Reviews on this subject have been published (Kalac & Svoboda 2000, Mjestrik & Lepsová 1992, Seeger 1982, Stijve 1993) Understandably, much of this research has been focussed on the occurrence of notoriously toxic metals as mercury, cadmium and lead in edible wild-growing (Collet 1977, Seeger 1976, Stijve & Besson 1976) and cultivated mushrooms (Haldimann *et al.* 1995, Overstijns & Verloo 1982), although a number of species have also found application as indicators of environmental pollution with heavy metals (Mjestrik & Lepsová 1992, Rauter 1976, Turnau 1991).

Still, there are no reports in the open literature about the occurrence of less common elements, such as the rare earth elements (also known as lanthanides), except for the paper by Aruguete *et al.* (1998) who studied the differences in metal uptake by three ectomycorrhizal mushroom species from an industrial and residential area. Metals found at significant levels included the three lanthanides cerium, neodymium and lanthanum. Concentration ranges (in mg/kg dry weight) in the edible mushroom *Amanita rubescens*, gathered near a residential area, were cerium (0,21–2,98), lanthanum (0,086–1,77) and neodymium (<0,01–0,52). It was observed that the lanthanides acted as a group and were apparently absorbed together into the mushrooms.

Lanthanides are increasingly used in industry. Cerium is a component of pyrophoric alloys in cigarette lighters, and its oxide is used in glass manufacture and as a catalyst in petroleum refining. Samarium and neodymium compounds are contained in the powerful magnets that are used in CD players, lanthanum and yttrium are used in the manufacture of high temperature superconductors, whereas others find application as catalysts or in the production of laser crystals. Europium and samarium are good neutron absorbers and used as such in nuclear reactors. Moreover, in China where rare earth metals occur widely, mixed nitrate salts as well as amino acid complexes of these metals are applied to such food crops as wheat, apple, beet and sugarcane, both as a fertiliser and growth stimulant (Chua *et al.* 1998, Zichang *et al.* 1991). As a result, these metals enter the food chain and are increasingly polluting the environment (Xinde *et al.* 2000). In Europe and in the U.S.A. lanthanides do not seem to be an environmental problem, although an investigation conducted by MAFF (1998) has shown that many food items contain trace amounts of these rare metals. During the last 10 years the use of ICP-MS as a technique for the simultaneous determination of many elements has become a well established technique in many laboratories involved in the quality control of food and agricultural products. This method also allows analysis of lanthanides with little additional effort (Cao *et al.* 1998, Zbinden & Andrey 1998).

Since it may be reasonably expected that many mushroom species will take up these metals, it was decided to investigate this matter. The present paper reports results obtained for lanthanides and associated metals in various species of cultivated and wild-growing mushrooms.

Materials and methods

Most cultivated mushrooms for our study were purchased at markets in the Lake of Geneva region in Switzerland. Some species were supplied by American growers. Herbarium collections of wild-growing mushrooms were mainly obtained from the Cantonal Museum of Natural History in Lugano (Ticino, CH), whereas some secotioid agarics were made available by the H.D. Thiers Herbarium of the San Francisco State University in California, U.S.A. Other species of interest were gathered in The Netherlands, Germany, France, Switzerland, Thailand and the Paraná State of Brazil. In all cases identification was performed by competent mycologists. Mushrooms were either freeze dried or dehydrated in an air stream at 50°C. Subsequently, the material was ground to a fine powder and stored in glass at 5°C until analysis. Multi-element analyses including the lanthanides were carried out by the ICP-MS method (Zbinden & Andrey 1998). Since quantitative analysis of some elements, *e.g.* phosphorus, iron, calcium is somewhat problematic by this method, we also used the ICP-AES technique (AOAC 1996).

Results and discussion

Since in most countries consumption of cultivated mushrooms largely exceeds that of wild-growing species, we decided to concentrate our efforts on commercially available cultivated species available both in Europe and the U.S.A. The following mushrooms were bought or directly obtained from Swiss and American growers: *Agaricus bisporus*, *A. bitorquis*, *A. blazei*, *Agrocybe aegerita*, *Grifola frondosa*, *Lentinula edodes* (Shiitake), *Lepista nuda*, *Pholiota nameko*, *Pleurotus ostreatus*, *P. eryngii*, *P. citrino-pileatus* and *Volvariella volvacea*.

Results obtained for heavy metals and arsenic were reassuringly low, *i.e.* in the same order of magnitude as reported earlier (Haldimann *et al.* 1995). Only cadmium in the gourmet and medicinal mushroom *Agaricus blazei* often exceeded the Swiss legal limit. The screen for lanthanides included cerium, lanthanum and neodymium, which are geochemically the most abundant. In all samples the sum of these three metals was below 0,1 mg/kg dry matter with the notable exception of *A. bitorquis*, which contained 0,35 mg/kg. Since the concentrations found were even less than those of ordinary heavy metals, and therefore unlikely to present a risk to the consumer, we did not pursue this matter. However, chance would have it that soon afterwards we were confronted once again with the occurrence of these unusual metals, but this time in wild-growing mushrooms.

In our study of selenium and heavy metals in the genus *Albatrellus*, we had repeatedly observed that *A. pes-caprae*, a choice edible fungus was extremely rich in selenium, but rather poor in heavy metals and arsenic (Stijve *et al.* 1998). When screening several samples of this mushroom, we found that three collections from Malcantone, an area in the Swiss Canton of Ticino contained not only unusually high levels of aluminium and iron, but also at least nine lanthanides (Table 1). One sample marked F1073 from the Lugano herbarium, collected in 1983 in Malcantone, without further indication of the origin, proved unexpectedly rich: it contained not only more than 2000 mg/kg of both Al and Fe, but also 6,7 mg/kg of the sum of cerium, neodymium, and lanthanum plus appreciable concentrations of the rarer lanthanides. In addition, other trivalent metals, *i.e.* gallium and yttrium were also high, as was thorium, a tetravalent metal that often occurs together with cerium in minerals. All these metals, and the presence of significant levels of lead, silver, vanadium and even molybdenum suggested that this particular sample must have been collected at a mining site. There are indeed several in the area; some of them are even a minor tourist attraction. Interestingly, two other collections from Malcantone/Mendrisiotto were also relatively rich both in lanthanides and the accompanying metals, whereas the concentrations in *A. pes-caprae* from the Canton Bern, and Bavaria in Germany were virtually negligible. Only in a collection of Skamania County in the American State of Washington we found levels of Fe, Al and the lanthanides comparable to those measured in the Mondini and Murgala collections.

Table 1 : Concentrations of chemical elements in six collections of *Albatrellus pes-caprae* from different origins

Chemical element	Canton Ticino, CH Malcantone and Mendrisiotto area			Baräu, Bern, CH	Simbach-Leiten, Bavaria, Germany	Skamania County, WA, U.S.A.
	Fl 1073	Bedigliora Mondini 0713	Meride Murgala 3086			
Phosphorus	5370	n.a.	n.a.	3430	3770	3290
Aluminium	2230	664	837	87	54	609
Iron	2080	541	699	56	51	680
Calcium	472	180	225	118	121	264
Lead	~5	2,4	1,9	0,51	0,55	~1
Silver	~4	1,21	0,49	0,49	0,34	0,38
Vanadium	~4	0,86	1,45	<0,05	<0,05	~1
Cobalt	~3	0,60	0,43	0,27	0,51	0,25
Cerium	~4	0,81	0,58	0,074	0,17	0,47
Neodymium	~2	0,30	0,17	<0,05	0,068	0,24
Praseodymium	0,44	0,08	<0,05	<0,05	<0,05	0,061
Yttrium	~2	0,25	0,17	<0,05	0,061	0,27
Lanthanum	0,73	0,14	0,08	<0,05	0,074	0,11
Thorium	0,99	0,12	0,06	<0,05	<0,05	<0,05
Gallium	0,64	0,24	0,24	<0,05	<0,05	0,19
Gadolinium	0,31	0,052	<0,05	<0,05	<0,05	<0,05
Samarium	0,30	0,056	<0,05	<0,05	<0,05	<0,05
Erbium	0,087	<0,05	<0,05	<0,05	<0,05	<0,05
Dysprosium	0,21	<0,05	<0,05	<0,05	<0,05	<0,05
Molybdenum	0,14	<0,05	<0,05	<0,05	0,052	0,083

All values in mg/kg on dry matter; n.a. = not analysed.

Since *A. pes-caprae* is a rare species, the occurrence of lanthanides in more common mushrooms from Malcantone was further studied by analysis of herbarium material collected at various sites in that area. For comparison, mushrooms from widely different origin were also investigated. The results for a total of 34 species are listed in Table 2.

Table 2. Concentrations of aluminium, calcium, major lanthanides, iron and thorium in various wild-growing mushrooms (in mg/kg dry weight)

Species	Aluminium	Calcium	Sum of Cerium Lanthanum Neodymium	Iron	Thorium
Sepultaria sumneriana	850–2010	2220–6210	1,26–3,85	637–2190	0,088–0,28
Boletopsis grisea	947	270–330	1,13	454–650	0,13
Boletopsis leucomelaena	432	132–173	1,07	95–235	0,086
Suillus placidus	597	480	0,83	399	0,11
*Tricholoma imbricatum**	3160	377	5,0	1715	0,68
Lepista nuda	936	730	1,09	705	0,14
Mycena pura	270	2110	0,64	244	0,056
*Entoloma caccabus**	676–3580	244–778	0,50–5,1	519–2580	0,068–0,44
Entoloma lividoalbum	384	281	0,31	292	0,034
Phaeolepiota aurea	159	448	0,35	128	<0,05
Limacella guttata	124	724	0,34	127	<0,05
Agaricus bisporus	48	200	~0,05	91	0,006
Agaricus arvensis	615	503	0,88	440	0,073

*Agaricus geesterani**	2060	1470	4,1	1870	0,32
Agaricus bitorquis	1080	1250	3,8	945	0,21
Agaricus silvicola	75	286	0,16	99	~0,01
Gyrophragmium dunalli	930	4140	9,0	1510	0,60
Longula texensis	525	395	3,2	897	0,14
Endoptychum depressum	1530	357	1,33	1060	0,12
E. agaricoides	71	462	~0,1	89	<0,01
*Podaxis pistillaris**	6730	2370	62	6570	6,0
Panaeolus retirugis	396	2010	1,56	466	0,20
Psilocybe subcubensis	6,4	139	~0,05	144	<0,01
Psilocybe cubensis	1890	713	22,2	881	n.a.
Psilocybe semilanceata	1504	408	0,91	798	0,11
*Inocybe haemacta**	4190	6700	9,7	2550	n.a.
Russula amoena	741	89	0,95	542	0,14
Russula velenovskyi	90	232	~0,1	74	<0,01
Bovista plumbea	674–867	225–2540	0,92–1,67	486–695	0,15–0,33
*Geastrum triplex**	986–2300	3710–5400	3,0–6,7	804–1670	0,34–0,72
Phallus impudicus Gelatinous layer	321	1500	0,69	201	0,06
Laterna pusilla	844	n.a.	1,20	1600	0,081
Protubera maracuja	125	600	~0.1	97	<0,01
Clathrus crispus	674	252	5,9	848	0,32

* More detailed results for these collections are given in Table 3; n.a. = not analysed

The presence of significant amounts of cerium, lanthanum and neodymium was by no means limited to mushrooms from Malcantone. Appreciable levels were even observed in two *Agaricus* species from a Dutch polder! The highest value, *i.e.* 62 mg/kg of combined Ce, La, and Nd was found in the secotioid mushroom *Podaxis pistillaris* from the Californian desert. Uptake of these trivalent metals does not seem to be species-dependent. The results obtained rather suggest that many mushroom species can take up lanthanides, provided there are sufficient amounts of aluminium, iron, and, to a lesser extent, calcium in assimilable form in the substratum. Indeed, mg/kg levels of lanthanides are accompanied by enormous amounts of these metals. Most important are undoubtedly trivalent aluminium and iron, but at high concentrations calcium has also some influence on lanthanide uptake, as suggested by the results for—among others—*Mycena pura*, *Panaeolus retirugis*, and *Phallus impudicus*.

It is interesting to note that Al, Fe, Ca and the lanthanides tend to be higher in mushrooms developing partly underground, such as *Agaricus bitorquis*, *A. geesterani*, *Gyrophragmium dunalii* and *Podaxis pistillaris*. In species having a preference for stony substrata, the said metals may also be abundant. For example, *Sepultaria sumneriana*, the Cedar cup, growing in soil had less Al, Fe, Ca and lanthanides than a collection gathered amidst the gravel of a foot path.

Information on aluminium in mushrooms is relatively scarce. Tyler (1980) studied the Al content of 130 species of basidiomycetes, and found a median value of only 30 mg/kg dry weight. The highest concentration, 427 mg/kg, was found in a *Hymenochaete* species. Müller *et al.* (1997) reported 14–112 mg/kg Al on dry weight in wild-growing mushrooms. In cultivated mushrooms there was even less. The mean value for 15 samples of *Agaricus bisporus* was 14 ±7 mg/kg. In an impressive study on heavy metals and aluminium in mushrooms gathered in the Bielefeld area in Germany, Günther *et al.* (1989) analysed 493 collections of 145 mushroom species, and found that Al concentrations fluctuated between 10 and 3890 mg/kg with the following distribution: 16,2% of the collections had less than 63 mg/kg, 79% 63–398 mg/kg, whereas Al levels over 1000 mg/kg were only measured in 4,8% of the samples. No taxonomic trends were observed. The highest values were found in *Agaricus bitorquis*: five collections had more than 1000 mg/kg, and two exceeded 2000 mg/kg, which agrees with what we measured in a Dutch collection (Table 2). Moreover, the German scientists observed that the Al content of the mushrooms depended clearly on the soil concentration. In two collections of *Boletus edulis* the concentration of this metal was found to be 800 and 150 mg/kg, reflecting the oxalate-soluble amounts of 3,3 and 0,6% measured in the soil at their respective sites of collection. The transfer factor mushroom/soil was therefore about 0,025.

Studies on the iron content of mushrooms suggest that uptake of this metal is species- and even genus-dependent (Lupper 1988, Tyler 1980). Both authors observed more than average concentrations in *Coprinus* spp., and lower amounts in Russulaceae. In an even earlier study Schmitt *et al.* (1977) measured more than 1200 mg/kg in both *Agaricus bitorquis* and *Cystoderma amianthinum* and up to 5000 mg/kg in some wood-destroying Tremellales.

In mushrooms having an appreciable level of lanthanides we measured invariably a quantity of thorium amounting to 5–10 per cent of the total concentration of Ce+La+Nd. Literature data for thorium in higher fungi are scarce indeed, but scattered results for a number of species (Horovitz *et al.* 1974, Latiff *et al.* 1996) are in the same order of magnitude as ours. Interestingly, the latter authors found not less than 3,50 mg/kg Th in an edible *Termitomyces* sp., accompanied by an equally high concentration of lanthanum and 1200 mg/kg iron! These results strongly suggest that the mushroom must have been loaded with other lanthanides and with aluminium as well, but these metals were not included in the neutron activation analysis used in the study.

A more detailed analysis of six selected mushroom species is given in Table 3. Clearly, as was already observed by Aruguete *et al.* (1998), lanthanides are taken up together. The ratio between the individual metals is a constant one: cerium makes up about 50 per cent of the total lanthanide content, and together with lanthanum and neodymium approximately 80 per cent. There is no evidence of a preferential uptake or exclusion of any of these metals. The staggering levels of lanthanides and other metals in *Podaxix pistillaris* cannot be explained by simple absorption. Since desert sands have virtually no humus, it is highly probable that *Podaxis* solubilizes metals by exuding organic acids from the hyphal tips of its mycelium. In this way it could even dissolve granitic bedrock (Jongmans *et al.* 1997). As a matter of fact, a South African handbook (Bottomley 1948) has a photograph showing it growing on red gravel!

Table 3. Lanthanides in selected mushrooms from Table 2 (in mg/kg dry weight)

	Tricholoma *imbricatum* No. 6523 Astano, Monte Lema TI, CH	***Entoloma caccabus* No. 5549 Astano, Laghetto TI, CH**	***Geastrum triplex* No. 976 Novaggio Parco Clinica TI, CH**	**Agaricus *geesterani* Zuid Beveland NL, 1990**	***Inocybe haemacta* Bellaria, La Tour de Peilz VD, CH**	***Podaxis pistillaris* S. Bernardino County, Granite Pass, CA, U.S.A.**
Cerium	3	3	2–4	2,5	4,9	34
Lanthanum	0,83	0,89	0,32–1,1	0,47	2,50	15
Neodymium	1,2	1,2	0,77–1,6	1,1	2,28	13
Praseodymium	0,31	0,32	0,20–0,42	0,29	0,60	4,1
Samarium	0,21	0,21	0,14–0,30	0,21	0,47	1,71
Europium	0,013	0,017	0,011–0,023	0,018	0,092	0,21
Gadolinium	0,19	0,21	0,14–0,30	0,19	0,43	2,25
Terbium	0,025	0,028	0,018–0,042	0,026	0,061	0,31
Dysprosium	0,13	0,15	0,10–0,23	0,14	0,32	1,74
Holmium	0,020	0,025	0,016–0,038	0,022	0,062	0,29
Erbium	0,055	0,073	0,04–0,11	0,061	0,16	0,94
Thulium	0,007	0,01	<0,005–0,013	0,008	0,02	0,12
Ytterbium	0,047	0,067	0,036–0,093	0,052	0,12	0,79
Lutetium	0,007	0,01	<0,005–0,014	< 0,008	0,039	0,11
TOTAL	**6,04**	**6,15**	**3,80–8,28**	**5,10**	**12,05**	**74,6**

Food safety aspects

Among the mushrooms containing mg/kg amounts of lanthanides some are of good *(Agaricus bitorquis)* and some of mediocre edibility, *e.g. Tricholoma imbricatum*. As a matter of fact, young specimens of *Podaxis pistillaris*, in which we found the highest content of these metals, are eaten in India and considered choice (Gupta & Singh 1991). Among the cultivated mushrooms analysed in this study, only *A. bitorquis* contained a more than average level of Ce+La+Nd.

There are, at the moment, not yet any legal limits for lanthanides in foods. It is questionable whether we need any for cultivated edible mushrooms, since the low levels reported here are in all probability without toxicological significance. However, lanthanides in wild-growing mushrooms are always accompanied by far more aluminium which could, at concentrations ranging from 1000–6000 mg/kg, be potentially harmful to the consumer. There are few maximum or action levels for aluminium in foods, but a provisional weekly intake (PTWI) of 7 mg/kg body-weight has been established (FAO/WHO JECFA 1988). This means that a person weighing 60 kg is allowed to ingest $60 \times 7 = 420$ mg aluminium per week. A moderate weekly consumption of 300 g of a mushroom containing 1200 mg/kg Al on fresh weight would already exhaust the PTWI by about 85 per cent. In this calculation no allowance is made for intake from other food items, beverages, drinking water, and migration from aluminium cooking utensils. Since many wild-growing mushrooms also concentrate notoriously toxic metals as cadmium, mercury and lead, their regular consumption is a potential health risk and should therefore be discouraged.

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