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Substrate role in the accumulation of heavy metals in sporocarps of wild fungi

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Abstract The distribution of neodymium, lead, thorium and uranium was investigated in about 100 samples of 12 different species of common, edible and non-edible mushrooms collected in unpolluted areas in the province of Ciudad Real, Central Spain. The quantitative analysis of heavy metals was performed by X-ray fluorescence spectrometry (a simple, accurate and non-destructive method). The concentration of these elements was related to three factors: mushroom specie, life style/substrate and study area. The results reveal considerable amounts of the four metals in all species analyzed as well as significant differences on the capability to accumulate these elements. The maximum absorption of Nd and Pb was found in the ectomycorrhizal *Cantharellus cibarius*, reaching values of 7.10 and 4.86 μg g^{-1}, respectively. Thorium and uranium were mainly accumulated (3.63 and 4.13 μg g^{-1}, respectively) in *Hypholoma fasciculare* although it is an epiphyte species, isolated from the mineral particles of soil. The distribution patterns of these metals in sporocarps of different habitats and locations showed no significant differences, except for thorium, mainly accumulated in mushrooms living on wood regarding these living on soil organic matter. The species-specific is therefore the determining factor for accumulation of Nd, Pb, Th and U, more than substrate, in this study.

Keywords Epiphitic · Saprotrophic · Ectomycorrhizal · Lead · Neodymium · Thorium · Uranium

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Introduction

The presence of heavy metals and toxic elements in the environment, which has increased markedly in the recent decades, concerns to scientists owing to its clear negative impact on wild life. It is known that wild-growing mushrooms can accumulate high concentrations of toxic metallic elements, metalloids and radio nuclides (Kalac 2001; Vetter 2004; Svoboda et al. 2006). Most reports have focused on the ability of mushrooms, mainly edibles ones, to accumulate metals and other elements (Kalac and Svoboda 2000; Falandysz et al. 2008) and on the possibility of using fungi, either as indicators of soil contamination, either as agents of bioremediation (García et al. 2005). In addition, other studies related pollution of metallic elements to human activity, urban waste, pollution of air and water in areas of industry, agriculture, etc.(Demirbas 2001; Svoboda et al. 2006).

The mycelium of mushrooms is designed to accumulate all kinds of elements, including heavy metals, in its sporocarps and reach much higher concentrations than those of the substrate where they live. The polysaccharic components of the cell walls of mushrooms, such as chitin, have the property of fixing metallic elements on functional groups (phosphate, carboxyl, amine, etc.). These elements are quickly transported to the cell interior and then circulate throughout the entire mycelium. This translocation is favoured for the fungal special form of osmotrophic nutrition and highly efficient cellular communication.

The uptake and presence of heavy metals in sporocarps of some fungi depend on environmental factors and on the particular genetic characteristics of each species. The soil determines the mobility and availability of the metals and the fungi, in relation with the specific substrate on which the mycelium develops, define the major or minor accumulative capacity according to their nutritional necessities (Gadd 2003, 2004). The presence of specific proteins and other macromolecules, implied in the reception and transport of metals, is a factor linked to genetics of the fungus that will be responsible for the accumulation capacity of each species. All biological and ecological processes involved and their interactions will determine the capacity of reception and accumulation of metals in the mycelium. Many of these factors are still not well known and the importance both biological and ecological that can have on the entire ecosystem is currently an incognito (Alonso et al. 2004).

Although concentrations of heavy metals in mushrooms (edible and non-edible) have been recently related with the mineral substrate (Falandysz et al. 2008) or with high pollution areas like big cities or industrial sites (Svoboda et al. 2006), a connection between single-species, single-area populations and single-life style/substrate has rarely been studied. The aim of our study was to compare concentrations of neodymium (Nd), lead (Pb), thorium (Th) and uranium (U) in different mushrooms species from unpolluted areas so as to relate these levels with location, species and life style. To our knowledge no quantitative or qualitative studies on the composition of these metals in Spanish mushrooms have been previously reported.

Materials and methods

Study areas

The study was carried out in three unpolluted areas inside the county of Ciudad Real, in the centre of the Iberian Peninsula (Table 1). The mushrooms were collected during the extraordinary humid autumn of 2006 (months of October and November). The samples were taken from an area of 250 roughly around the point of the coordinates expressed in the Table 1.

Sampling and analytical procedure

The complete fruiting bodies, cap and stalk, of twelve mushrooms species (Table 2) were collected from the study areas and the youngest were selected, rejecting

Table 1 Data of coordinates, average height, more representative vegetation, bedrock and pH in three zones of mushrooms sampling

Study areas	Coordinates/Height (m)	Vegetation	Bedrock/Soil pH
Los Valles	38° 50′ 03,13″ N 4°32′ 00,26″ W /650	Mainly: *Quercus ilex*, *Quercus pyrenaica*, *Quercus coccifera*, *Cistus ladanifer* Scattered: *Pinus pinaster*, *Eucaliptus camaldunensis*, etc.	Quartzitic/5.8
Puente Retama	39° 00′ 12,71″ N 4° 29′ 26,42″ W /600	Mainly: *Quercus ilex*, *Quercus pyrenaica*, *Cistus ladanifer* Scattered: *Pinus pinaster*, *Pinus pinea*, etc.	Quartzitic/5.6
Río Frío	39° 06′ 15,82″ N 4° 28′ 45,36″ W /700	Mainly: *Quercus ilex*, *Quercus pyrenaica*, *Quercus súber*, *Cistus ladanifer* Scattered: *Pinus pinaster*, *Eucaliptus camaldunensis*, etc.	Quartzitic/5.5

Table 2 List of mushrooms species tested and habitat

Order/family	Species	Habitat
Agaricales/Agaricaceae	*Macrolepiota procera* (Scop.: Fr.) Singer[a]	Soil organic matter. Meadows and places with scattered vegetation
	Agaricus campestris (L.: Fries) Quélet[a]	Soil organic matter. Meadows and places with scattered vegetation
Agaricales/Strophariaceae	*Hypholoma fasciculare* (Huds.: Fr.) Kummer	Oak stumps (*Quercus* sp.)
Boletales/Omphalataceae	*Omphalotus olearius* (DC: Fr.) Singer	Oak stumps and trunks weakened. (*Quercus sp.*)
Cortinariales/Cortinariaceae	*Hebeloma sinapizans* (Paulet) Gillet	Ectomycorrhiza (*Quercus* sp.)
	Gymnopilus spectabilis (Fr.: Fr.) Smith	Oak stumps and trunks weakened. (*Quercus sp.*)
Poriales/Cantharellaceae	*Cantharellus cibarius* Fr.[a]	Ectomycorrhiza (*Quercus* sp.)
Russulales/Russulaceae	*Lactarius controversus* (Pers.: Fr.) Fr.	Ectomycorrhiza (*Populus* sp.)
Sclerodermatales/ Sclerodermataceae	*Pisolithus tinctorius* (Scop.: Pers.) Raus.	Soil organic matter. Meadows and places with scattered vegetation
Tricholomatales/ Tricholomataceae	*Tricholomopsis rutilans* (Sch.: Fr.) Singer	Saprobe on dead wood of pines.
	Tricholoma ustaloides Romagesi	Ectomycorrhiza (*Quercus* sp.)
	Clitocybe geotropa (Bull.: Fr.) Quélet[a]	Soil organic matter. In forest clearings forming rings witch

[a] Edible species

those very mature or rotten. All species were investigated in every zone. Each sample, three per specie and area, contained about eight to twelve sporocarps. Usually more than 500 g fresh weigh of each species was picked up in order to have enough dry material for testing. The mushrooms were brushed and washed with distilled water and then dried at 60°C for 48 h, powdered and sieved (100 μm mesh.). The resulting powder was stored in hermetic plastic recipient.

The analytical procedure for determination of elements was made through X-rays fluorescence spectrometry (PHILIPS-PW2404 Pananalytical, Magix-Pro model, with automatic loader PW2540), equipped with logging data software (Mino and Yukita 2005). This is one of the simplest, most accurate and most economic analytical methods for the determination of the chemical composition of many types of mineral and organic substrates. A total of 5 g of resulting mushroom samples powder was mixed and homogenized with 0.5 ml methyl methacrylate (Vacite) and pressed (150 kj) into 40 mm diameter aluminium cylinder with a layer of H_3BO_3. This same procedure was applied to standard samples which contained neodymium, lead, thorium and uranium. The cylinder-samples were exposed to X-ray and the resulting data, related to the percentage calcination of each sample, were expressed in μg g^{-1} (dry weight basis).

Soil pH

The soil pH was measured with PCE-PH20S calibrated in laboratory. 30 measures of pH scattered throughout each sampling area were taken at a depth of no more than 5 cm from the surface.

Statistical analysis

Data are represented as the sample mean and standard error (S.E.). Response variables included element concentrations (Nd, Pb, Th, U); experimental effects included single-species, single-study areas and single-habitats (life style/substrate). Differences were tested with an analysis of variance (ANOVA) using a Statgraphics 5.0 (Statistical Graphics Corp., Rockville, MD, USA).

Results

The coordinates, average height and more representative vegetation of the three zones used in the present study are shown in Table 1. These areas, of about

250 ha each one, were located in Central Spain in a zone with large well-preserved forest on acid quartzite soils, normally used for deer hunting and with a great variety and abundance of mushroom species.

The samples of mushrooms analyzed, about 10 fruiting bodies of each species and each sampling area, belonged to 12 species of 8 different Families, including edible and non-edible mushrooms (Table 2).

In general, concentrations of neodymium (Nd) in samples of mushrooms were high and differed significantly among the species tested (Table 3). Values oscillated between 7.10 μg g^{-1} in the ectomycorrhizal species *Cantarellus cibarius* and 2.80 μg g^{-1} in *Pisolithus arrhizus* and *Lactarius controversus*. *Gymnopilus spectabilis*, a species that live on wood without contact with mineral particles of soil, reached a concentration of 5.53 μg g^{-1}.

Lead (Pb) is a highly toxic element and its presence in the mushrooms is well known, including the edibles (García et al. 1998; Cocchi et al. 2006; Yamaç et al. 2007). The values given for these authors, and others, oscillated between 1 and 20 μg g^{-1} in dry matter, including fruiting bodies collected in polluted areas (Svoboda et al. 2006). In our study the values fluctuate between 4.86 μg g^{-1} in *Cantharellus cibarius* and 1.33 μg g^{-1} in *Macrolepiota procera*.

Thorium is a tetravalent metal found in appreciable amount in all mushrooms species we sampled (Table 3). The species that accumulate more Th are *Hypholoma fasciculare* with 3.63 μg g^{-1} and *Gymnopilus spectabilis* with 3.53 μg g^{-1}, two very common mushrooms that appear on wood dead or alive. Most of species studied showed concentrations of thorium around 1.50 μg g^{-1} as *Agaricus campestris*, *Hebeloma sinapizans*, *Omphalotus olearius*, *Pisolithus arrhizus*, *Tricholomopsis rutilans* and *Tricholoma ustaloides*. In our work we have found thorium in all examined species and in appreciate amounts.

The highest uranium concentration determined was 4.13 μg g^{-1} in *Hypholoma fasciculare*. This is a common fungus living on wood without contact with the ground, and that usually occurs in tight clusters of numerous sporocarps. The values of U in others species were in a moderate range among 1.50 μg g^{-1} and 2.50 μg g^{-1}. *Clitocybe geotropa*, a saprophytic species that grow on soil organic matter, reached the lowest concentration of uranium.

Discussion

Distribution of element between species

Neodymium is a rare element of which there are still few reports in the open literature about its occurrence in mushrooms. Aruguete et al. (1998) found low

Table 3 Element concentrations (μg g^{-1}, dry weight basis) in different mushroom species (means ± standard error) growing in unpolluted areas[a]

Mushrooms species	Neodymium	Lead	Thorium	Uranium
Agaricus campestris	4.46 ± 0.40[bc]	2.53 ± 0.11[e]	1.57 ± 0.21[ce]	4.10 ± 0.39[d]
Cantharellus cibarius	7.10 ± 0.49[d]	4.86 ± 0.47[d]	2.40 ± 0.33[b]	2.30 ± 0.44[b]
Clitocybe geotropa	4.20 ± 0.56[bce]	4.73 ± 0.69[d]	1.80 ± 0.37[bce]	0.80 ± 0.35[c]
Gymnopilus spectabilis	5.53 ± 0.27[b]	3.63 ± 0.15[bc]	3.53 ± 0.11[d]	1.63 ± 0.21[bc]
Hebeloma sinapizans	4.56 ± 0.30[bc]	2.76 ± 0.23 [ce]	1.60 ± 0.25[ce]	2.36 ± 0.33[b]
Hypholoma fasciculare	3.33 ± 0.22 [ce]	3.50 ± 0.30[bce]	3.63 ± 0.42[d]	4.13 ± 1.05[d]
Lactarius controversus	2.80 ± 0.14[e]	4.06 ± 0.57[bd]	2.33 ± 0.35[b]	1.53 ± 0.33[bc]
Macrolepiota procera	5.43 ± 0.82[b]	1.33 ± 0.35[f]	2.10 ± 0.18[bc]	1.80 ± 0.07[b]
Omphalotus olearius	3.26 ± 0.53[cd]	3.60 ± 0.21[bce]	1.57 ± 0.18[ce]	1.43 ± 0.04[bc]
Pisolithus arrhizus	2.80 ± 0.65[e]	3.93 ± 0.40[bd]	1.53 ± 0.21[ce]	1.63 ± 0.29[bc]
Tricholoma ustaloides	5.17 ± 0.66[b]	3.33 ± 0.96[bce]	1.47 ± 0.28[ce]	2.06 ± 0.21[b]
Tricholomopsis rutilans	5.70 ± 0.25[bd]	3.23 ± 0.28[bce]	1.43 ± 0.15[e]	1.56 ± 0.40[bc]

[a] Autumn harvest dates occur between October and November of 2006

[b–f] Means followed by the same letter within a column do not differ ($P \leq 0.05$) using the LSD test concentrations in ranges of <0.01–0.52 μg g^{-1} for three species of ectomycorrhizal fungi from an industrial and residential area. Likewise Stijve et al. (2001) reported amounts of lanthanides (sum of cerium, lanthanum and neodymium) which fluctuated between <0.10 and 0.35 μg g^{-1}, although *Podaxis pistillaris*, a species from the Californian desert, was able to accumulate until 62 μg g^{-1} of these three metals.

In our work we detected significant differences of Pb among the different species studied, being *Cantharelus cibarius* the most accumulative with a mean of 4.86 ± 0.47 μg g^{-1}. For this same species, it has been reported mean values of 3.1 μg.g^{-1} (Svoboda et al. 2006) and 1.13 μg g^{-1} (Cocchi et al. 2006). High values of Pb were also reached in *Clitocybe geotropa* (4.73 ± 0.69 μg g^{-1}), an edible mushroom picked up, consumed and very appreciated in our zone. Other species such as *Gymnopilus spectabilis*, *Hypholoma fasciculare*, *Omphalotus olearius*, *Pisolithus arrhizus*, *Tricholomopsis rutilans* and *Tricholoma ustaloides* showed Pb concentrations of about 3.50 μg g^{-1}. The species with lower Pb content were *Agaricus campestris*, *Hebeloma sinapizans* and *Macrolepiota procera*.

We believe it is important to underline that information relating thorium with mushrooms is scarce (Baeza and Guillén 2006; Turhan et al. 2007) and there are no conclusive data on Th concentration in fungal carpophores. It was quite interesting to observe that the highest values were detected in two species (*H. fasciculare* and *G. spectabilis*) that live on wood. In the same order of magnitude of our results Latiff et al. (1996) found not less than 3.50 μg $g^{-1}$of Th in an edible *Termitomyces* species.

Despite the fact that some authors have previously reported the presence of radionuclides in mushrooms (Kalac 2001; Turhan et al. 2007), they have generally investigated isotopes of cesium, lead, radium, thorium and others, but not uranium. Only the papers of Baeza and Guillén (2006) relate the content of U to mushrooms with the bioavailability of the soil mineral substrate or with the degree of maturity of mushrooms. These last authors found low transfer factor values for $^{234, 238}$U in all species they studied, except for *Hebeloma cylindrosporum* that it is recommended as an indicator of a soil radioactive content.

Influence of the substrate and the sampling zone

Our data on heavy metals in mushrooms for different habitats (life style/substrate) and for different study areas are shown in Fig. 1. We did no find significant differences in metal concentrations among the three study areas or among the different habitats studied. Just for thorium, we have observed significant

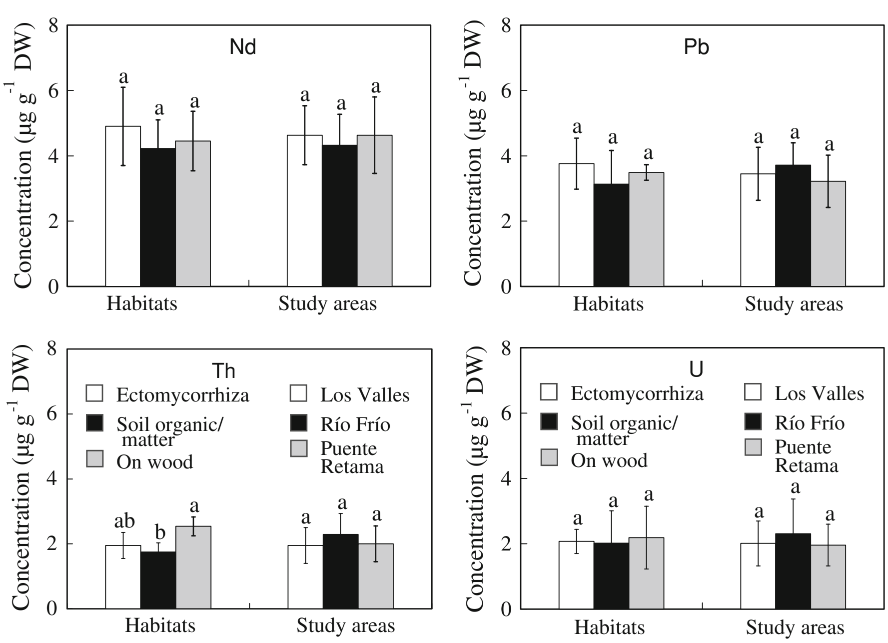

Fig. 1 Mean concentration of neodymium (Nd), lead (Pb), thorium (Th) and uranium (U, μg g^{-1}) in mushroom, from unpolluted zones, differing in habitats (life style/substrate) or in area of study. Values followed by the same letter within localities or ways of life do not differ ($P \leq 0.05$) using the LSD test. Vertical bars represent ± S.E. of the mean

differences between the mushrooms that live on wood (average content of 2.54 μg g^{-1}), and those who live on the soil organic matter (average content of 1.75 μg g^{-1}). Surprisingly, in the mushrooms that live on the soil organic matter, thorium accumulation was lower. In this regard, several authors have indicated that substrate composition is an important factor to be considered (Cocchi and Vescovi 1997; Kalac and Svoboda 2000; Stijve et al. 2004).

Our results show that the capacity of the mushrooms studied to accumulate heavy metals, such as Nd, Pb, Th and U, is independent of the area where the mushrooms were located and of the substrate on which they have grown, at least in unpolluted areas. On the other hand, it appears that accumulation of these specific metals could be species-dependent, as has already been noticed by Kalac and Svoboda (2000).

Conclusions

The species of mushrooms analyzed showed significant differences in the capability to accumulate neodymium, lead, thorium and uranium. Appreciable amounts of these metals were incorporated into wild-growing mushrooms (some of them edible), even though they were collected in uncontaminated areas of forests very well preserved. *Cantharellus cibarius* was the species that reached the highest levels of Nd and Pb in addition to significant amounts of Th and U. However, this is not surprising if we consider that it is an ectomycorrhizal fungus that lives directly in contact with the soil mineral particles. It is important to underline the high incorporation of thorium and uranium in *Hypholoma fasciculare* although this species live on wood, without contact with the ground. The comparison of distribution patterns of these metals in sporocarps of different habitats and locations showed no significant differences, neither among the three areas studied, nor among life style/substrate, except for thorium. Only for this element, the values of accumulation were higher in the fungi that live on wood compared with those living on soil organic matter. The results of our work highlight the importance of the physiological aspects of each species and minimized the role of the substrate and habitat. The species-specific is, therefore, the determining factor of heavy metals accumulation in the mushrooms investigated.

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