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Ústav geochemie, mineralogie a nerostných zdrojů

Studijní program: Aplikovaná geologie

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Interakce velkých hub a stopových prvků v půdách

Interactions of macrofungi and trace elements in soils

Disertační práce

Vedoucí disertační práce: RNDr. Jan Borovička, Ph.D.

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ABSTRAKT

Tato disertační práce navazuje na mou diplomovou práci, ve které jsem se zaměřila především na problematiku obsahu a stanovení uranu v plodnicích velkých hub (výsledky byly publikovány, Příloha 1). Závěr práce, tedy že plodnice hub neakumulují uran, mě vedl k úvaze, že prvky jako uran mohou být akumulovány v ektomykorhizách, protože některé publikované práce naznačovaly významnou roli hub v environmentální geochemii uranu. Proto jsem se rozhodla na mou diplomovou práci navázat a zabývat se obsahem stopových prvků v ektomykorhizách obecně. Vzhledem k tomu, že jsem měla možnosti využít řadu analytických metod, podílela jsem se i na jiných studiích z oboru geomykologie.

V reakci na poplašné zprávy šířící se v českých médiích jsem se zaměřila na obsah a distribuci radiocesia v plodnicích hřibu hnědého. Jak je patrné z Přílohy 2, plodnice hřibu hnědého v České republice nepředstavují pro konzumenty zdravotní riziko.

Pomocí molekulárních metod (PCR se specifickými primery) jsme zkoumali distribuci mycelia saprotrofní houby pečárky Bernardovy v půdním profilu na lokalitě v Praze (Příloha 3). Naše výsledky ukázaly, že mycelium tohoto druhu zasahuje i do hloubky 30 cm a že izotopické složení olova v plodnicích tohoto druhu naznačuje transport tohoto kovu z hloubky minimálně okolo 13-17 cm.

Hlavní část mé práce byla věnována studiu stopových prvků v ektomykorhizách. Jako hlavní analytickou metodu jsem použila neutronovou aktivační analýzu. Vzorky ektomykorhiz, jemných kořenů a půdních vzorků byly sbírány především v okolí Příbrami, která je znečištěna činností kovohutě. Téměř všechny vzorky ektomykorhiz se podařilo identifikovat do druhu pomocí molekulárních metod (sekvenace DNA). Výsledky z této části výzkumu lze nalézt v Přílohách 4 a 5. Podobně jako je tomu u plodnic, akumulace prvků v ektomykorhizách záleží na druhu prvku a druhu houby, koncentrace prvků se vyznačují mimořádnou variabilitou. Dále jsme kvantifikovali biomasu houby v ektomykorhizách hřibu hnědého a muchomůrky červené pomocí metody qRT-PCR.

Hlavní cíle této práce byly naplněny a podařilo se rozšířit naše znalosti z oboru geomykologie. Největším přínosem této práce bylo: 1) zjištění koncentrací 14 prvků v ektomykorhizách z kontaminovaných lokalit; 2) zjištění distribuce mycelia saprotrofní pečárky Bernardovy v půdním profilu; a 3) determinace koncentrace houbové biomasy v etomykorhizách dvou druhů makromycetů.

ABSTRACT

This PhD thesis follows my master‘s thesis, which I focused on the problem of uranium determination and content in macrofungal fruit-bodies (the results have been published, Appendix 1). Macrofungi apparently do not accumulate uranium in fruit-bodies but as other studies suggested major roles of fungi in environmental geochemistry of uranium, I hypothesized possible accumulation of uranium and other elements in ectomycorrhizae. I therefore decided to continue the research and focus on investigation of trace elements in ectomycorrhizae. As I had opportunity to use a variety of analytical methods, I also participated in other studies in the field geomycology and the results are included in this thesis.

In response to alarmist reports in Czech media, I focused on activity and distribution of radiocaesium in fruit-bodies of *Boletus badius*. As demonstrated in Appendix 2, the fruit-bodies of this species do not represent a health risk for mushroom consumers.

Distribution of mycelium of saprotrophic *Agaricus bernardii* in a soil profile in Prague was investigated by use of molecular methods (PCR with specific primers). The results have shown that the mycelium reaches the depth of 30 cm. Lead isotopic composition of fruit-bodies suggests lead can be accumulated from soil depth of 13-17 cm (Appendix 3).

However, the main aim of my thesis was investigation of trace elements in ectomycorrhizae with instrumental neutron activation analysis as the principal analytical method. Ectomycorrhizal roots, fine roots and organic soil samples were collected mainly in the smelter-polluted area in the region of Příbram (Central Bohemia, Czech Republic). Almost all samples of ectomycorrhizae were identified at species level by molecular methods (DNA sequencing). The results of this research are presented in Appendix 4 and Appendix 5. Similarly as observed in the fruit-bodies, trace element accumulation in ectomycorrhizae depends on particular element and fungal species; very high concentration variability was observed. Furthermore, fungal biomass was quantified in ectomycorrhizae of *Boletus badius* and *Amanita muscaria* by use of qRT-PCR.

The aims of thesis were fulfilled and the knowledge of the field of geomycology deepened. The greatest outputs of this study were: 1) determination of 14 elements in ectomycorrhizae from polluted sites; 2) identification of distribution of mycelium of saprotrophic *Agaricus bernardii* in a soil profile; and 3) determination of fungal biomass concentration in ectomycorrhizae of two macromycete species.

Poděkování:

Ráda bych touto cestou poděkovala všem, kteří mi během psaní této práce a mých studií pomohli. Za velmi cenné rady, připomínky a hlavně notnou dávku trpělivost svému školiteli RNDr. Janu Borovičkovi, Ph.D., z Ústavu jaderné fyziky AV ČR a Geologického ústavu AV ČR. Můj velký dík patří i doc. RNDr. Milanu Gryndlerovi, CSc., z Mikrobiologického Ústavu AV ČR za poskytnutí laboratorního zázemí, konzultace metodiky a návrh specifických primerů. Tato práce vznikla též s finanční podporou Grantové agentury Univerzity Karlovy v Praze (projekt č. 535 112).

Na tomto místě nemohu opomenout ani svojí rodinu, které bych touto cestou ráda poděkovala za podporu během studií a pochopení, kterého se mi dostalo především od mé maminky, manžela a freťáka Dárwina. Děkuji především za jejich trpělivost a porozumění, které se mnou měli během mých příprav na zkoušky, konference a přednášky.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce, s výjimkou Přílohy 1, nebyla předložena k získání jiného nebo stejného akademického titulu. Příloha 1 obsahuje data získaná a publikována již v průběhu magisterského studia a byla využita k získání magisterského titulu. Protože však představovala základní východisko pro zvolené téma, je přirozenou součástí této disertační práce.

V Praze, 16.02.2016

SEZNAM PŘÍLOH

Příloha 1
Borovička J., Kubrová J., Rohovec J., Řanda Z., Dunn C. E. (2011): Uranium, thorium and rare earth elements in macrofungi: what are genuine concentrations? Biometals 24: 837-845.
Počet citací podle WOS: 10

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Borovička J., Kubrová J., Řanda Z. (2012): K radioaktivitě hřibu hnědého. Mykologický sborník 89 (4): 92-98.

Příloha 3
Borovička J., Mihaljevič M., Gryndler M., Kubrová J., Žigová A., Hršelová H., Řanda Z. (2014): Lead isotopic signatures of saprotrophic macrofungi of various origins: Tracing for lead sources and possible applications in geomycology. Applied Geochemistry 43: 114-120.
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Příloha 4
Kubrová J., Žigová A., Řanda Z., Rohovec J., Gryndler M., Krausová I., Dunn C. E., Kotrba P., Borovička J. (2014): On the possible role of macrofungi in the biogeochemical fate of uranium in polluted forest soils. Journal of Hazardous Materials 280: 79-88.
Počet citací podle WOS: 1

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Příloha 5
Cejpková J., Gryndler M., Hršelová H., Kotrba P., Řanda Z., Synková I., Borovička J., Bioaccumulation of heavy metals, metalloids, and chlorine in ectomycorrhizae from smelter-polluted area, Manuscript, Under Review (Janaury 2016)

Poznámka: Během studia došlo u autorky disertační práce ke změně příjmení z rodného příjmení Kubrová na Cejpkovou

PROHLÁŠENÍ ŠKOLITELE

Slovně a procentuálně vyjadřuji míru účasti předkladatelky doktorské disertační práce Mgr. Jaroslavy Cejpkové (roz. Kubrové) na níže uvedených publikačních výstupech. Nemám však k dispozici metodiku, jak procentuálně stanovit podíl na publikaci pro jednotlivé spoluautory, a proto jsou uvedená procenta jen mým subjektivním hodnocením.

1. **Borovička J., Kubrová J., Rohovec J., Řanda Z., Dunn C. E. (2011): Uranium, thorium and rare earth elements in macrofungi: what are genuine concentrations? Biometals 24: 837-845.**

Tento článek vychází z výsledků získaných v rámci diplomové práce. Studentka postupovala pod mým vedením a provedla většinu manuální laboratorní práce. Její podíl na výstupu: 30 %.

2. **Borovička J., Kubrová J., Řanda Z. (2012): K radioaktivitě hřibu hnědého. Mykologický sborník 89: 92-98.**

Studentka se podílela na pracovním postupu i přípravě článku. Její podíl na výstupu: 40 %.

3. **Borovička J., Mihaljevič M., Gryndler M., Kubrová J., Žigová A., Hršelová H., Řanda Z. (2014): Lead isotopic signatures of saprotrophic macrofungi of various origins: Tracing for lead sources and possible applications in geomycology. Applied Geochemistry 43: 114-120.**

Tento článek je zaměřen na izotopy olova a distribuci mycelia saprotrofní houby v půdním profilu. Studentka přispěla ke splnění cílů druhé části této studie, když provedla qRT-PCR. Její podíl na výstupu: 15 %.

4. **Kubrová J., Žigová A., Řanda Z., Rohovec J., Gryndler M., Krausová I., Dunn C. E., Kotrba P., Borovička J. (2014): On the possible role of macrofungi in the biogeochemical fate of uranium in polluted forest soils. Journal of Hazardous Materials 280: 79-88.**

Tento článek je přímým a zásadním výstupem práce studentky v rámci doktorského studia. Její podíl na výstupu: 60 %.

5. **Cejpková J., Gryndler M., Hršelová H., Kotrba P., Řanda Z., Synková I., Borovička J., Bioaccumulation of heavy metals, metalloids, and chlorine in ectomycorrhizae from smelter-polluted area. Soli Biology & Biochemistry, rukopis v recenzním řízení (únor 2016).**

Tento článek je přímým a zásadním výstupem práce studentky v rámci doktorského studia. Její podíl na výstupu: 75 %.

V Řeži u Prahy dne 1. února 2016,

RNDr. Jan Borovička, Ph.D.

ÚVOD

Zájem lidí o houby je starý téměř tak jako lidstvo samo. Když byla v roce 1991 na ledovci v Alpách objevena mumie člověka stará více než 5 000 let, která byla později pojmenována Ötzi, nalezli spolu s ním něco, co bychom dnes nazvali lékárničkou nebo krabičkou první pomoci. Tato krabička obsahovala mimo sušeného ovoce i plodnice březovníku obecného – *Piptoporus betulinis* a troudnatce kopytového – *Fomes fomentarius*. Oba tyto druhy řadíme mezi léčivé houby s antivirálními a antibakteriálními účinky. Zda je měl Ötzi s sebou pro léčebné účely anebo pro jiné praktické využití, se již asi nedozvíme. Je ale všeobecně známo, že v jihovýchodní Asii jsou houby využívány právě pro své léčivé účinky; léčivé schopnosti lesklokorky lesklé – *Ganoderma lucidum* jsou v Číně známé více než 2 000 let. V tomto kontextu je pozoruhodné, jak málo toho dnes o houbách, a především o jejich významu a funkcím v životním prostředí vlastně víme.

Nezanedbatelným důvodem je sběr hub pro účel konzumace. Češi se s oblibou nazývají národem houbařů a o tomto označení svědčí i fakt, že ¾ obyvatel u nás vyráží alespoň jednou za rok do lesa na houby. Se sběrem hub však vyvstala i otázka, zda je jejich konzumace bezpečná a jaké prospěšné či rizikové látky obsahují. První práce zabývající se obsahem stopových prvků v houbách je více než 100 let stará. Další analýzy zaměřující se na obsahy prvků v plodnicích pokračovaly ve 30. letech 20. století, ale zásadní rozvoj nastal později spolu s vývojem analytických metod až v 70. letech.

V současné době již máme k dispozici hrubý přehled o obsazích prvků v plodnicích hub. Ale s tím jak se naše znalosti rozšiřují, vyvstává řada nových otázek. Ve své diplomové práci jsem se zaměřila na obsahy uranu, thoria, stříbra a olova v plodnicích hub z čistých a kontaminovaných lokalit a spolu se školitelem jsme na jejich základě publikovali článek, který je součástí této disertační práce. Zjistili jsme, že uran se v plodnicích hub neakumuluje, ale že obsahy tohoto prvku ze vzorků z kontaminované lokality jsou zvýšené oproti těm z čistých lokalit.

Akceptovaný fakt, že ektomykorhizní houby zlepšují růst svých rostlinných partnerů na kontaminovaných lokalitách, nás vedl k úvaze, zda nejsou prvky jako uran akumulovány právě v ektomykorhizách, tedy společných orgánech rostlin a hub, kde je realizována výměna živin. Prací zabývající se tímto tématem bylo poskrovnu, ale jejich závěry naznačovaly, že ochranný mechanismus ektomykorhizních hub by mohl spočívat právě v zadržení prvků hyfovým pláštěm.

Proto jsem se rozhodla navázat na diplomovou práci a rozšířit toto téma o obsahu uranu (a dalších prvků) v ektomykorhizách se zaměřením na kontaminované lokality. Protože byla možnost využít i rozličné analytické metody a také metody molekulární genetiky, podílela jsem se i na dalších studiích z oblasti geomykologie, které jsou součástí této práce, a svým způsobem jsem tak snad alespoň nepatrně přispěla k rozvoji znalostí o houbách, které lidstvo zná už tak dlouho, ale pořád o nich neví dost.

1. MYKOLOGIE A GEOMYKOLOGIE

1.1 Říše hub

Přesto, že houby nepatří mezi autotrofní organismy a neobsahují ve svých buňkách chloroplasty s fotosyntetickými barvivy, byly v minulosti řazeny k rostlinám. Díky své heterotrofní výživě stojí spíše na pomezí mezi rostlinami a živočichy (Holec et al. 2012) a nyní jsou klasifikovány do samostatné říše *Fungi*; patří mezi nejpočetnější, nejvýznamnější a nejpopulárnější skupinu heterotrofních organismů (Kalina et Váňa 2005).

Přesný počet druhů hub není znám a v roce 1991 byl odhadován na 1,5 milionů druhů, tento odhad byl s rozvojem molekulárně genetických metod rozšířen až na 5,1 milionů druhů (Blackwell 2011). Kirk et al. (2008) uvádějí 97 861 celosvětově popsaných druhů hub.

Tato práce je zaměřena na zástupce z oddělení vřeckovýtrusých hub - *Ascomycota* a stopkovýtrusých hub – *Basidiomycota*. Jedná se především o tzv. velké houby – makromycety, které formují plodnice viditelné pouhým okem (Holec 2006a). Podle Senn-Irlet et al. (2007) se v Evropě vyskytuje nejméně 75 000 druhů hub z čehož 15 000 se řadí do kategorie makromycetů. Ani v České republice není přesný počet velkých hub znám, ale odhaduje se na 3 000 – 4 000 druhů (Holec 2006a). Dle Hawskworth (2009) bylo vědci doposud popsáno jen asi 7% druhů hub, což je i jedním z důvodů, proč bývá mykologie označována za „opomíjenou megavědu“, a to především v porovnání s botanikou, kde je dle odhadů popsáno již 90% druhů rostlin (zhruba 270 000 druhů).

1.2 Funkce hub v přírodě

Houby hrají klíčovou roli při rozkladu organické hmoty, patří mezi významné rostlinné patogeny, podílí se na celé řadě typů mykorhizních symbióz a díky houbovým vláknům – hyfám zásadně ovlivňují půdní strukturu (Gadd 2008). Dighton (2003) publikoval rozsáhlý přehled o interakci hub a jednotlivých složek životního prostředí.

Stručný přehled o ekologických skupinách hub a jejich rozdělení z hlediska výživy, které bylo využito i v této práci, přinesl Holec (2006b). **Saprotrofní houby** (SAP) disponují bohatou enzymatickou výbavou (především celulolytické a ligninolytické enzymy), kterou využívají k rozkladu organické hmoty, především dřeva, jehličí či listí, zbytků rostlinných těl a humusových látek v půdě. Jejich činností vzniká humus a v některých případech (např. u dřevomorky domácí – *Serpula lacrymans*) mohou být finálními produkty rozkladu voda a

oxid uhličitý. Tato činnost hub je nesmírně důležitá, protože umožňuje koloběh látek a živin v ekosystémech. Některé saprotrofní houby jsou vázané na speciální substráty, např. na rašeliník (sfagnikolní druhy), spálenou dřevní hmotu (antrakofilní druhy), šišky jehličnanů (strobilikolní druhy) atd.

Parazitické houby získávají organické látky z živých buněk jiných organismů. Mezi parazitické makromycety patří zejména parazité rostlin (stromů a keřů) či parazité živočichů (rostou na larvách nebo kuklách), a dokonce i parazité hub. Druhově nejpočetnější a nejvýznamnější jsou dřevožijné (lignikolní) houby, především tzv. choroše.

V České republice jsou **lichenizované houby** zastoupeny jen několik málo stopkovýtrusými druhy (např. z rodu kalichovka – *Lichenomphalia*). Tyto houby žijí v symbióze se sinicí nebo řasou a jejich spojením vzniká symbiotický organismus se speciálními fyziologickými a ekologickými vlastnostmi.

Uvnitř těl svých hostitelů (rostlin) žijí **endofytické houby**, které svojí přítomností nijak nepoškozují hostitele. Z makromycetů jsou zástupci této skupiny především dřevnatky – *Xylaria* či spálenky – *Ustulina*.

Speciální skupinou hub jsou **houby mykorhizní**, které žijí v oboustranně prospěšném vztahu s rostlinami, především stromy. Tomuto typu se podrobně věnuje následující podkapitola.

1.3 Mykorhizní symbióza

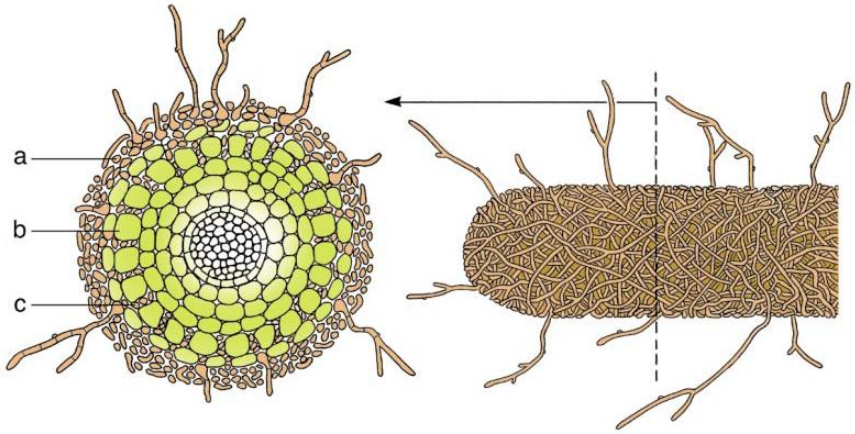
Charakterem mykorhizní symbiózy je oboustranný tok živin. Rostliny dodávají svým houbovým partnerům především produkty fotosyntézy, tedy jednoduchý cukr – glukózu a houby naopak poskytují minerální látky (např. dusík a fosfor) a vodu (Smith et Read 2008). Podrobný popis jednotlivých druhů mykorhizní symbiózy lze nalézt v přehledné práci Gryndler et al. (2004). Mykorhizní symbiózu (MS) lze členit na dvě základní skupiny: **endomykorhizu**, kdy mykorhizní houba proniká do vnitřního prostoru buněk hostitelova kořene, a na **ektomykorhizu (ECM)**, kdy se mykorhizní houba nachází v mezibuněčném prostoru hostitelova kořene. Mezi endomykorhizní typy patří arbuskulární mykorhizní symbióza, erikodiní MS a orchideoidní MS. **Arbuskulární MS** je rozšířena u 95 % druhů cévnatých rostlin a pro člověka je významná tím, že se nachází u většiny kulturních druhů rostlin. Tento typ MS formují houby z oddělení spájivých hub – *Zygomycetes*. Předpokládá se, že arbuskulární MS se vyvinula souběžně s první kolonizací půdy rostlinami před 450-500

milióny let (Cairney 2000). Pro svoji starobylost a dlouhodobé soužití s rostlinami jsou arbuskulární houby nejvíce vzdálené saptrofnímu způsobu života. **Erikoidní MS** je charakteristická pro rostliny z řádu vřesovcotvaré – *Ericales* a pro houby z třídy vřeckovýtrusých. Tento typ MS je příznačný pro kyselé biotopy, které jsou velice chudé na dusík a fosfor. Erikoidní houby jsou schopné žít saprotrofně a přetrvat na stanovišti bez rostlinného partnera i 20 let. Tento typ MS pravděpodobně vznikl před 140 milióny let a centrum jeho diverzity se nachází na jižní polokouli (Cairney 2000). **Orchideoidní MS** je typická pro rostliny z čeledi vstavačovitých – *Orchidaceae* a houby z rodu *Rhizoctonia* (patří do oddělení stopkovýtrusých hub). Čeleď vstavačovitých s 30 000 druhy patří mezi nejbohatší skupinu v rostlinné říši, z nichž zhruba 100 druhů neobsahuje chlorofyl (Finlay 2008). Původně se předpokládalo, že houby na orchidejích parazitují, ale testy DNA (deoxyribonukleová kyselina) naznačují, že se jedná o houby mykorhizní, které zároveň kolonizují jiné autotrofní rostliny a orchideje jsou vlastně „podvodníci“, kteří získávají uhlík skrze houbu od jiných rostlin (Leake 2004). Dále existují ještě přechodné typy MS a to arbutoidní MS a monotropoidní MS.

1.3.1 Ektomykorhizní symbióza

Na ektomykorhizní symbióze se podílí zhruba 10 000 rostlinných druhů (zejména stromů a keřů, byliny jsou výjimkou) a 8 000 druhů stopkovýtrusých a vřeckovýtrusých hub; z hlediska makromycetů jde o nejdůležitější typ MS vůbec. Fosilní záznamy pochází z doby před 50 milióny let, ale předpokládá se, že vývoj začal již před 200 milióny let souběžně s evolucí stopkovýtrusých hub (Cairney 2000, Finlay 2008). První záznam pozorování MS je datován do roku 1841, kdy byla považována za parazitizmus; pravý význam byl správně vyhodnocen o několik desetiletí později (Gryndler et al. 2004). Pro ektomykorhizní symbiózu je charakteristické formování speciálního útvaru, který nazýváme ektomykorhiza nebo ektomykorhizní kořen (Obr. 1.), což je společný orgán rostliny a houby. Jedná se o modifikovaný kořen, který je obalen hyfovým pláštěm; schematické zobrazení je znázorněno na Obr. 2.

Obr. 1. Ektomykorhiza holubinky hlínožluté – *Russula ochroleuca* na smrku. Fotografoval Jan Borovička.



Obr. 2. Struktura ektomykorhizy: a – houbový plášť, b – buňky kořene hostitele, c – Hartigova síť (Landeweert et al. 2001).

Již od roku 1885 je známo, že se jednotlivé ektomykorhizy liší svojí anatomií. Agerer (2001) rozdělil ektomykorhizy na 5 typů a 3 subtypy. Na ektomykorhizní symbióze se podílejí především stromy z čeledí borovicovité – *Pinaceae* (borovice, smrky, jedle, modřín), bukovité – *Fagaceae* (dub a buk), břízovité – *Betualceae* (bříza, habr, olše) a lískovité – *Corylaceae* (líska). Další dřeviny jsou schopny tvořit nejen ektomykorhizní symbiózu, ale i arbuskulární MS: zástupci čeledi vrbovité – *Salicaceae* a javorovité – *Aceraceae* (Lepšová 2003a). Existují ektomykorhizní houby, které jsou schopné kolonizovat stovky druhů dřevin

(*Cenococcum graniforme*), ale i houby, které se specializují pouze na jednu dřevinu – typickým představitelem je klouzek sličný - *Suillus grevillei* obligátně vázaný na modřín (Gryndler et al. 2004). U některých hub není doposud s jistotou známé, zda patří mezi ektomykorhizní či saprotrofní druhy.

Izotopy uhlíku a dusíku nám mohou poskytnout řadu důležitých informací i v geomykologie. První poznatky přinesli Gebauer et Dietrich (1993), kteří zjistili, že ektomykorhizní houby jsou nabohacené ^{15}N v porovnání s jejich symbiotickými rostlinami. V tomto případě se jedná pravděpodobně o izotopickou frakcionaci, kdy se všechen dusík, který se dostává do rostliny, prochází přes houby. Hobbie et al. (2001) odhalili, že mykorhizní houby jsou oproti saprotrofním obohaceny ^{15}N a ochuzeny ^{13}C, což je účinný nástroj ke zjišťování potravní strategie řady druhů hub, čehož bylo využito v práci Agerer et al. (2010), kteří se zabývali potravní strategií rodu kuřátka – *Ramaria*. Ke zjištění stáří organického materiálu hub bylo využito ^{14}C. Hobbie et al. (2002) ukázali, že saprotrofní druhy obsahují uhlík, který byl navázán před více než 6 lety, oproti ektomykorhizním houbám, které obsahovali uhlík starý pouze zhruba 0-2 roky. Dále se v práci Hobbie et al. (2012) podařilo prokázat vyšší obsah izotopů ^{13}C a ^{15}N v klobouících hub oproti třením.

1.3.2 Role ektomykorhizních hub v životním prostředí

Les můžeme vnímat jako ektomykorhizní kořenový systém, protože jednotlivé jeho složky (dřeviny a houby) jsou propojeny extramatrikálním myceliem ektomykorhizních hub, které umožňují výměnu látek a informací (Selosse et al. 2006). Základní funkcí ektomykorhizních hub je příjem živin a vody z většího objemu půdy, který je jinak pro rostlinného partnera nedostupný (Antibus et al. 1997, Chalot et Brun 1997, Smith et al. 2008, Smith et al. 2012). Houbová vlákna jsou schopna proniknout i do velice nepatrných půdních prostorů, které jsou jinak pro kořeny dřevin nedosažitelné (Landeweert et al. 2001). Simard et al. (1997) ukázali, že mycelium ektomykorhizních hub propojuje dospělé stromy se semenáčky, které rostou v jejich zástinu, přičemž dospělé stromy jim poskytují výživu v podobě uhlíkatých látek. Jak naznačují van de Heijden et Horton (2009), tento princip nefunguje vždy a byly zaznamenány i případy, kdy naopak docházelo k redukci výživy u semenáčků na úkor dospělých jedinců. Struktura, vazby a vztahy mezi rostlinami a houbami v ekosystémech jsou zjevně velice složité a doposud ne zcela objasněné.

Jak referovala Lepšová (2003d), ektomykorhizní houby mají i velký bioindikační význam. Studium diverzity ektomykorhizních hub je nejjednodušší a ekonomicky nejméně náročný způsob popisu ektomykorhizního společenstva. I přes množství limitů této metody (nutné opakované návštěvy v průběhu několika sezón) a dnes již stále dostupnější molekulárně genetické metody, posloužily především v 80. letech 20. století k zaznamenání poklesu počtu ektomykorhizních hub v západní Evropě. Jejich úbytek byl dán do souvislosti s kyselými dešti. Podrobné informace o vlivu acidifikace na ektomykorhizní houby a následných opatřeních lze nalézt v práci Lepšová (2003b,c).

Již delší dobu je známo, že ektomykorhizní houby zlepšují růst svého rostlinného partnera a chrání je před toxicitou těžkých kovů (Jones et Hutchinson 1986, Jentschke et al. 1999, Jentschke et Godbold 2000). Ektomykorhizní houby vyvinuly řadu mechanismů zvyšující jejich toleranci k těžkým kovům, které lze dělit na vnitrobuněčné a mimobuněčné. Vnitrobuněčná detoxifikace spočívá např. ve vazbě kovů na proteiny (např. metalothioneiny), v akumulaci kovů ve vakuolách či snížení nebo naopak zvýšení toku látek z a do buňky. Mimobuněčná detoxifikace spočívá ve vylučování chelatačních látek (hlavně citrátů a kyseliny šťavelové), které reagují s kovy v půdním prostředí v bezprostředním okolí hyf (Jentschke et Godbold 2000, Bellion et al. 2006). Tolerance k těžkým kovům je závislá nejen na druhu houbového partnera a koncentraci kovu v substrátu (Hartley et al. 1997, Godbold et al. 1998), ale i na ekotypu (Adriaensen et al. 2003, 2006, Colpaert et al. 2011, Ruytinx et al. 2013).

1.4 Interakce hub s půdním prostředím

V půdě se vyskytuje celá řada organismů (bakterie, řasy, prvoci, bezobratlí živočichové aj.), houby však tvoří dominantní složku půdní mikrobioty (Giri et al. 2005). Aktivita saprotrofních i ektomykorhizních hub ovlivňuje chemické a biochemické procesy v půdách a je součástí koloběhu živin i toxických těžkých kovů v ekosystémech (Gadd 2008, Baldrian 2009).

1.4.1 Geomykologie

Geomykologie je vědní obor, který se zabývá vlivem hub na geologické a geochemické procesy, zejména mykorhizním zvětráváním hornin a minerálů, akumulací kovů houbami, rolí hub v koloběhu živin a jejich vlivem na mikrobiální komunitu. Otázky

geomykologie byly podrobně rozebrány v řadě přehledných prací (Burford et al. 2003, Gadd 2004, 2007, 2008, 2013).

Houby se aktivně podílí na zvětrávání hornin a minerálů a to biomechanickým a biochemickým způsobem. **Biomechanické zvětrávání** působí přímo a nepřímo. Přímé působení se děje prostřednictvím mechanického tlaku hyf, které jsou schopny pronikat tuhým substrátem, včetně minerálního materiálu, kdy dochází k narušení struktury, např. podél krystalových ploch. Nepřímý způsob je spojen s produkcí extracelulárních látek, které formují biofilm na povrchu substrátu. Smršťování a bobtnání biofilmu vede k mechanickému tlaku na substrát. Houby produkují celou řadu organických látek (např. kyselinu šťavelovou a citrónovou), které rozpouští minerály a interagují s kationty vázanými na povrch jílových minerálů, čímž způsobují tzv. **biochemické zvětrávání.** Tím se houby podílejí na koloběhu celé řady prvků v půdách.

V podzolech byly nalezeny tunely, které svým průměrem 3-10 μm odpovídají průměrům hyf (van Breemen et al. 2000). Předpokládá se, že jsou tyto hladkostěnné tunely formovány hyfami hub, které produkují nízkomolekulární organické kyseliny (van Schøll et al. 2008). Sverdrup (2009) však poukázal na to, že ne všechny struktury viditelné na minerálních zrnech jsou výsledkem činnosti hub, protože se vyskytují i na materiálu z Antarktidy, kde více než 10 milionů let nebyla přítomná aktivita ektomykorhizních druhů hub a přisuzuje je abiotickým chemickým zvětrávacím procesům. Další důkaz o schopnosti hub vytvářet tunely, přinesli Thorley et al. (2014), kteří publikovali obrázky ze skenovacího elektronového mikroskopu, které odhalily rýhy na dolomitu podél houbových hyf. Příspěvek činnosti hub k celkovému zvětrávání je stále neznámý a odhady se velice liší: pohybují se v rozpětí od 2 do 50 %.

Houby mají také schopnost vytvářet na hyfách krystalické agregáty, které označujeme jako mykogenní minerály. Nejčastěji to bývají šťavelany whewellit a weddelit, které vykazují vysokou variabilitu krystalických forem (Fomina et al. 2010). Experimentálně byla prokázána také schopnost hub srážet sekundární karbonáty (Gadd 2007). Na základě přítomnosti či absence krystalů a jejich morfologie v plodnicích jsou dokonce založeny taxonomické klasifikace makromycetů, např. kuřátek (Christan 2008) nebo hvězdovek – *Geastrum* (Zamora et al. 2015).

1.5 Stopové prvky v plodnicích velkých hub

První přehledná práce, která se zabývala analýzou plodnic hub, je již více než 100 let stará (Zellner 1907). Na ní navázaly další studie z 30. let 20. století (Friese 1929, 1932; Ramage 1930). Podrobnější studie se objevily v 70. letech, což souviselo s rozvojem analytických metod (Stijve et Cardinale 1974, Stijve 1977; Byrne et al. 1976, 1979). V české literatuře té doby lze najít informace populární formou o obsahu kadmia, manganu, mědi, olova, rtuti a zinku (Babička 1973, Macků 1977, Šebek 1979).

1.5.1 Akumulace stopových prvků houbami

Stopovým prvkům v houbách se podrobně věnovali Kalač (2009), Kalač et Svoboda (2000), Kalač (2010) a Falandysz et Borovička (2013), kteří publikovali rozsáhlé přehledy o prvcích v houbách: některé velké houby jsou známé svojí schopností akumulovat stopové prvky ve svých plodnicích. Na rozdíl od rostlin, které kumulují vysoké koncentrace (polo)kovů především na (polo)kovy bohatých půdách, houby mohou akumulovat vysoké koncentrace (polo)kovů i v prostředích s jejich normálními koncentracemi. Poměr obsahu prvku v houbě k obsahu prvku v půdě je tzv. bioakumulační faktor (BAF). Prvky typicky akumulované v houbách (BAF>1) jsou: Au, Ag, As, Br, Cd, Cl, Cs, Cu, Hg, Rb, Se, V a Zn. Prvky s typicky nižší koncentrací v houbě (BAF<1) jsou: Co, Cr, F, I, Ni, Sb, Sn, Th, U a prvky vzácných zemin (REE). Pokud je BAF u některého druhu houby výrazně vyšší (zhruba 100krát), mluvíme o hyperakumulaci. Prvky se v plodnici hromadí dvojím způsobem – transportem přes mycelium anebo atmosférickou depozicí, která má význam především u chorošovitých hub (Borovička 2007).

Faktorů, které ovlivňují akumulaci prvků v houbách, je celá řada a některé z nich jsou velmi málo prozkoumány. Jedná se především o přírodní faktory (geologické podloží a půdní typ), environmentální znečištění nebo přirozené anomálie koncentrace prvků v substrátu, životní cyklus hub (saprotrofní houby akumulují některé prvky více než ektomykorhizní, ale i naopak) a akumulace je i druhově a prvkově specifická (Falandysz et Borovička 2013).

První případ hyperakumulace byl pozorován u muchomůrky červené – *Amanita muscaria*, v jejíchž plodnicích byly nalezeny stovky ppm[1] vanadu, přičemž v houbách se vanad obvykle nachází v koncentracích pod 1 ppm (Koch et al. 1987). Vzhledem k tomu, že

[1] ppm – parts per million (10^{-6}), v geovědách běžně používaná jednotka, kterou lze technicky správně vyjádřit jako $mg \cdot kg^{-1}$

vyšší obsahy vanadu byly nalezeny v dolní části třeně, což je část blízká hostiteli, uvažuje se o možné roli vanadu v regulaci spolupráce s hostitelem (da Silva et al. 2013).

Navzdory relativně nízké koncentraci arzenu v půdě publikovali Stijve et al. (1990) vysoké obsahy tohoto prvku v baňce velkokališné – *Sarcosphaera coronaria*, více než 1 000 ppm. Nejvyšší koncentraci arsenu v baňce velkokališné (7 090 ppm) publikoval Borovička (2007). Obsahy stříbra v ektomykorhizní muchomůrce šiškovité – *Amanita strobiliformis* uveřejněné v práci Borovička et al. (2007) dosáhly hodnoty 1 253 ppm, což je 2 500krát více než v půdě.

Hyperakumulace u hub bývá často vysvětlovaná tzv. „teorií obrany“, která byla popsána u cévnatých rostlin jako způsob obrany před přirozenými škůdci, jako jsou např. larvy hmyzu či bakterie (Boyd 2007), ovšem u hub toto nebylo doposud testováno. Gryndler et al. (2012) ukázali, že houbová biomasa bohatá na Ag silně ovlivňuje půdní bakteriální komunitu a působí podobně jako ionty stříbra (Ag ve formě $AgNO_3$).

Chorošovité houby, které vytrvávají delší dobu na lokalitě, byly předmětem řady biomonitorovacích studií. Analýza chorošů z NP Šumava a z Prahy ukázala vyšší koncentrace zkoumaných prvků (Al, Be, Cd, Cu a Pb) ze vzorků pocházejících z Prahy. Tato odlišnost byla vysvětlena nepřítomností velkých zdrojů znečištění v národním parku (Gabriel et Baldrian 1995, Gabriel et al. 1997). Při pokusech *in vitro* byla pozorována velká variabilita tolerance ke kadmiu u březovníku obecného (Gabriel et Baldrian 2002). Toxické prvky mohou u hub zpomalovat růst a způsobovat morfologické změny (Baldrian et Gabriel 1997). Některé houby vyžadují pro svůj růst těžké kovy, jako jsou např. kadmium, mangan, nebo zinek, v nadbytku jsou však tyto kovy toxické. Měď a mangan u hub bílé hniloby se podílejí na degradaci ligninu (Baldrian 2003).

1.5.2 Speciace (chemická forma) prvků v houbách

K posouzení zdravotního rizika konzumace volně rostoucích hub nestačí znát pouze koncentraci daného prvku v plodnici, ale je nutné posoudit i chemickou formu prvku (Niedzielski et al. 2013). Např. toxicita arsenu se odvíjí od jeho chemické formy a za nejtoxičtější se považují anorganické formy As (III) a As (IV) (Schoof et al. 1999, Zavala et al. 2008, Sun et al. 2009). Organokovové sloučeniny jsou považovány za méně toxické a aresenobetain dokonce za netoxickou formu arsenu (Kaise et al. 1985, Adair et al. 2005). Jak ukázala rozsáhlá práce Nearinga et al. (2014), arsen se nachází v plodnicích hub ve směsi řady

forem (anorganické formy, arsenobetain či metylované sloučeniny arsenu). Dále bylo zjištěno, že převládající forma arsenu odpovídá fylogenetické skupině hub, např. u čeledi pýchavkovité – *Agaricaceae* převládá arsenobetain.

Muchomůrky z okruhu m. červené jsou známé svojí schopností akumulovat vanad. Ten se v muchomůrkách nachází v organokovové sloučenině nazývané amavadin. Jedná se o komplex čtyřmocného vanadu s dvěma molekulami kyseliny N-hydroxyimino-2,2´-dipropionové (Garner et al. 2000).

Metalothioneiny a fytochelatiny jsou odlišné třídy na cystein bohatých molekul, které udržují homeostázu a slouží k detoxifikaci kovů u celé řady organismů (Gadd 1993, Cobbet et Goldsbrough 2002). Metalothioneiny mají vysokou schopnost se slučovat s esenciálními (např. Cu a Zn), ale i s neesenciálními kovy (např. Ag, Cd a Hg) (Loebus et al. 2013).

Jako klíčový mechanismus pro toleranci čechratky podvinuté – *Paxillus involutus* ke kadmiu označili Jacob et al. (2004) komplexaci s metalohioneiny. Oproti tomu Collin-Hansen et al. (2007) zjistili, že se kadmium v klobouku hřibu smrkového – *Boletus edulis* váže na peptidy patřící do skupiny fytochelatinů.

Osobová et al. (2011) objevili, že i stříbro se v muchomůrce šiškovité váže na metalothioneiny. Ke stejným závěrům došli i Sácký et al. (2014) u slzivky opásané – *Hebeloma mesophaeum*. Na metalothioneiny se váže i zinek v holubince černonachové – *Russula atropurpurea* (Leonhardt et al. 2014).

1.5.3 Antropogenní radionuklidy v houbách

Houby jsou známé svojí schopností efektivně akumulovat ve svých plodnicích i některé radionuklidy, a proto se staly předmětem intenzivního zájmu řady studií (Horyna et Řanda 1988, Kalač 2001, Mietelski et al. 2002, Guillén et Baeza 2014). Z hlediska radioaktivity a případné radiotoxicity hub jsou nejdůležitějšími přírodními radionuklidy ^{40}K, ^{235}U, ^{238}U, ^{232}Th (Skwarzec 2012). Přeměna izotopu ^{238}U produkuje ^{210}Pb a ^{210} Po, které lze také v houbách detekovat (Mietelski et al. 2002).

Kromě přirozených radionuklidů se do životního prostředí dostaly i antropogenní radionuklidy, které se uvolnily během havárií jaderných elektráren (např. Černobyl v roce 1986 a Fukušima v roce 2011) či testů jaderných zbraní, které probíhaly v 60. letech 20. století. Jedná se především o ^{241}Am, ^{134,137}Cs, ^{131}I, ^{238,239,240}Pu a ^{90}Sr (Mietelski et al. 2004, 2010; Trappe 2014).

Ektomykorhizní houby akumulují více cesia než houby satrotrofní (Smith et al. 1993) a tato akumulace je druhově závislá (Gillett et Crout 2000, Mascanzoni 2009). Vinichuk et al. (2010, 2013) naměřili vyšší aktivitu ^{137}Cs (radiocesium) v ektomykorhizních houbách než v saprotrofních druzích a aktivita hub byla vyšší než okolní vegetace. Guillén et al. (2012) se zabývali přenosem ^{60}Co, ^{134}Cs a ^{85}Sr do saprotrofní hlívy máčkové – *Pleurotus eryngii* a přenos ^{134}Cs byl nejvyšší ze zkoumaných radionuklidů. Podrobně se akumulací a aktivitou cesia v plodnicích hub zabývali Duff et Ramsey (2008).

Hlavním zdrojem radiocesia je pro houby půdní, organický horizont, kam se radiocesium dostává atmosférickou depozicí (Steiner et al. 2002). V případě radiocesia hrála dominantní úlohu mokrá depozice a předpokládá se, že až 90% radiocesia se dostalo do půdy pomocí mokré depozice; suchá depozice hrála minoritní roli (Kinser 2001). Území Evropy bylo spadem radiocesia zasaženo nerovnoměrně (Dubois et De Cort 2001), což se odráží i v hodnotách naměřených v plodnicích hub. Aktivitou hub v České republice a na Slovensku se v posledních letech zabývali Dvořák et al. (2006) a Škrkal et al (2013): aktivita naměřená v houbách z České republiky byla vyšší než ze Slovenské republiky a mnohdy překračovala povolený hygienický limit (vyhláška č. 307/2002 Sb.), který stanovuje nejvyšší přípustnou úroveň radioaktivní kontaminace potravin radiocesiem hodnotu 600 Bq/kg, což při přepočtu na sušinu, která u hub představuje 90 %, činí 6 000 Bq/kg sušiny. Nejvyšší aktivita naměřená Dvořákem et al. (2006) byla 6 263 Bq/kg sušiny v hřibu hnědém – *Boletus badius* a Škrkal et al. (2013) naměřili nejvyšší aktivitu v hřibu smrkovém 11 800 Bq/kg sušiny. Řanda et al. (1988a,b) naopak považují hřib smrkový za nízký akumulátor cesia.

1.6 Molekulárně genetické metody v geomykologii

Dlouhou řadu let bylo pro studium druhové diversity makromycetů na lokalitách možné využívat prakticky jen sběr a identifikaci plodnic. Houby však fruktifikují pouze sporadicky, za příhodných klimatických a meteorologických podmínek, a skutečná diverzita na lokalitách tak nebyla známa, stejně jako např. distribuce mycelia v půdním profilu. Významný posun představují aplikace molekulárně genetických metod, které v posledních přibližně 10 letech prodělaly obrovský rozvoj.

V geomykologii nacházejí molekulární metody uplatnění především jako nástroj pro přímou identifikaci druhů hub v ektomykorhizních kořenech, což nahradilo velmi omezené možnosti morfologické identifikace (Agerer 2001). Dále je např. možné přímo detekovat

mycelium vybraného druhu houby v půdě (Borovička et al. 2014) nebo v jiných environmentálních vzorcích, např. v kořenech (Halbwachs et al. 2013). Kromě toho je možné mycelium cílených druhů hub v půdě nejenom detekovat, ale také kvantifikovat (Landeweert et al. 2003, Parladé et al. 2007, Hortal et al. 2008, De la Varga et al. 2011, Kurth et al. 2013).

1.6.1 Polymerázová řetězová reakce

Základní molekulárně genetickou metodou pro studium hub je polymerázová řetězová reakce – PCR (z angl. Polymerase Chain Reaction). Principem této reakce je metoda rychlého zmnožení (amplifikace) vybraného úseku DNA principem opakované denaturace dvouřetězové molekuly DNA a následná renaturace řetězců se specifickými oligonukleotidy (primery), které se nachází v reakční směsi. Reakční směs obsahuje oligonukleotidy, dvojici primerů, vodu a enzym DNA polymerázu. Podrobné informace o PCR lze nalézt v knize Brown (2007).

V prvním kroku – **denaturace** se molekula DNA zahřeje na teplotu 95^{o}C, což vede k rozpadu vodíkových můstků mezi dvěma řetězci DNA a vznikají nám dvě jednořetězové molekuly DNA (tzv. templát). Ve druhém krok – **hybridizace** (annealing) dochází k nasednutí primerů (což jsou oligonukleotidy komplementární s templátem). Teplota, při které dochází k hybridizaci, je kritická a specifická pro jednotlivé primery. Třetím krokem je – **elongace**, kdy dochází k syntéze nového řetězce komplementárního s templátovou DNA. Tento krok probíhá za teploty 65-75^{o}C. Po prvním cyklu se počet řetězců DNA v reakční směsi zdvojnásobí. Při opakování cyklu počet řetězců DNA exponenciálně roste a teoreticky z původní jedné molekuly DNA po 32 cyklech získáme 1 miliardu molekul DNA.

Nejvyužívanějším molekulárním markerem je úsek označovaný jako ITS rDNA (ITS1-5.8S-ITS2), který je v závislosti na druhu houby dlouhý zhruba 650-900 párů bází. Pro amplifikaci se obvykle využívají univerzální primery ITS1 a ITS4, nebo specifické primery pro houby (ITS1F) či bazidiomycety (ITS4B) (White et al. 1990, Gardes et Bruns 1993). Získané amplifikované úseky DNA je možné osekvenovat a získat tak pořadí nukleotidů v molekulárních markerech, což umožňuje identifikaci organismů izolovaných z environmentálních vzorků, např. determinaci druhu houby v ektomykorhizní špičce (Nieto

et Carbone 2009). Identifikace se obvykle provádí porovnáním s veřejně přístupnými databázemi GenBank[2] nebo UNITE[3] (Kõljalg et al. 2005).

Molekulární metody se stávají stále oblíbenějšími, jak dokazují tisíce prací publikované v posledních letech – na heslo „fungi and PCR“ poskytl vyhledávač Web of Science společnosti Thomson Reuters v srpnu roku 2015 více než 41 000 odkazů na studie, z nichž okolo 90% bylo publikováno v letech 2000-2015.

1.6.2 Kvantitativní Real-time PCR

Kvantitativní Real-Time PCR (qRT-PCR) je založena na klasické PCR s tím rozdílem, že se v průběhu PCR kontinuálně zaznamenává na konci každého cyklu množství DNA. To je detekováno např. pomocí fluorescenčního substrátu, který se váže na dvouřetězcovou DNA a fluoreskuje až po navázání. Zdrojem fluorescence je např. nespecifická sonda SybrGreen či specifická sonda Taqman. qRT-PCR má 2 typy – realtivní a absolutní. Při **relativní** qRT-PCR se množství fluorescence testovaného vzorku porovná s množstvím fluorescence jiného vzorku, při **absolutní** qRT-PCR se množství DNA odečte přímo z kalibrační křivky, kterou získáme analýzou ze vzorků o známé koncentraci. Podrobné informace o kvantitativní Real-time PCR lze nalézt v řadě prací, např. Heid et al. (1996), Raeymaekers (2000) a Fierer et al. (2005).

[2] http://www.ncbi.nlm.nih.gov; http://blast.ncbi.nlm.nih.gov/Blast.cgi

[3] http://unite.ut.ee

2. DISTRIBUCE MYCELIA HUB V PŮDNÍM PROFILU

2.1 Distribuce mycelia ektomykorhizních hub v půdním profilu

Nejvyšší hustota kořenů stromů se v lesních půdách nachází v organických horizontech a v minerálních horizontech, které na něj bezprostředně navazují (Makkonen et Helmisaari 1998), ale kořeny mohou zasahovat i hlouběji do půdy (Jackson et al. 1996).

Cairney (2005) ukázal, že diverzita ECM hub určená na základě sběru plodnic neodpovídá jejich skutečné diverzitě v půdách, což vede k přímé potřebě studovat mycelium v půdě. Landeweert et al. (2003) publikovali přehled metod, které lze využít ke studiu mycelia v půdě. Běžně používané metody k určení biomasy mycelia v půdách jsou studium délky hyf s měřením specifických houbových biomarkerů jako jsou ergosterol či fosfolipidové mastné kyseliny; tyto metody však neumožňují specifickou identifikaci. Proto jsou využívány také molekulárně genetické metody, které nám umožňují nejen identifikovat mycelium houby do druhu, ale také jej kvantifikovat.

K ekologickým studiím, které jsou zaměřené na kvantifikaci mycelia ECM hub v půdě, se hojně využívá kvantifikační Real-Time PCR (Parladé et al. 2007, Hortal et al. 2008, Kurth et al. 2013). Mycelium ECM hub představuje 30-80% mikrobiální biomasy (De la Varga et al. 2011) a v půdě přežívá i 11 let (Landeweert et al. 2003). Erland et Taylor (2002) ukázali, že pouze několik běžných druhů může kolonizovat většinu kořenů (50-70 %) a zbytek připadá na vzácnější druhy. Dále bylo zjištěno, že druhy, které dominují při tvorbě plodnic, nebývají ty, které dominují na kořenech (Horton et Bruns 2001).

Přestože se mycelium ECM hub nachází ve všech půdních horizontech, většina studií zabývajících se distribucí ECM hub v půdě je zaměřena pouze na svrchní organické horizonty. Rosling et al. (2003) a Rosling et Rosenstock (2008) ukázali, že nejvyšší hustota ECM špiček v podzolu se nachází v organickém horizontu a klesá s hloubkou (výjimkou je iluviální horizont, kde dochází k nárůstu počtu ECM špiček). Vzhledem k rozsahu minerálního horizontu se ale 2/3 ECM špiček nachází v minerálním horizontu a polovina druhů je omezena na minerální horizont. Tedersoo (2003) odhalil, že druhová variabilita ECM špiček je velká i v malém prostorovém měřítku. Rosling et al. (2003) a Luis et al. (2005) ukázali, že poloha mycelia, a tedy i ECM špiček souvisí s půdním profilem, druhem mykorhizní houby a symbiotickým partnerem. Druhy jako klouzek obecný – *Suillus luteus* a holubinka osmahlá – *Russula adusta* byly omezeny pouze na minerální horizont, zatímco

např. vatovečka měkká – *Tomentellopsis submollis* byla nalezena pouze v organickém horizontu.

2.2 Distribuce mycelia saprotrofních hub v půdním profilu

Předpokládá se, že SAP houby kolonizují organický horizont, ovšem existuje jen velmi málo prací zabývajících se vertikální distribucí mycelia těchto hub v půdě (Robinson et al. 2009). Jelikož SAP houby získávají živiny z mrtvé organické hmoty, jsou omezeny na vyšší půdní horizonty (Luis et al. 2005). Ovšem již v roce 1917 uveřejnili svou práci Shantz a Piemeisel, kde je z obrazové dokumentace patrné, že mycelium pečárky označené jako *Agaricus tabularis* rostoucí na travnatých plochách v Coloradu (USA) zasahuje hluboko do půdy, zhruba do 30 cm, ale i hlouběji. V té době pochopitelně nešlo s jistotou ověřit, zda se jedná opravdu o mycelium studované pečárky.

Součástí studie o využití izotopických poměrů olova v saprotrofních houbách byla detekce mycelia v půdním profilu (Borovička et al. 2014, Příloha 3). V této práci byly využity molekulárně genetické metody k detekci mycelia pečárky Bernardovy – *Agaricus bernardii* v půdě. S pomocí specifického primeru pro p. Bernadovu byla provedena relativní kvantifikační Real-time PCR. Přítomnost sledovaného druhu byla potvrzena v celém půdním profilu, který byl na lokalitě studován, tedy v hloubce 0-30 cm. Nejvyšší hustota mycelia byla zjištěna v hloubce 4-6 cm a s hloubkou víceméně klesala. Obsah organického uhlíku byl nejvyšší v povrchové vrstvě (~5 %) a od hloubky 6 cm a níže se jeho koncentrace pohybovala okolo 1 %.

3. AKUMULUJÍ HOUBY URAN?

3.1 Geochemie uranu

Uran je radioaktivní těžký kov, který se vyskytuje přirozeně ve 3 izotopech ^{234}U, ^{235}U a ^{238}U. Nejvíce hojným izotopem je ^{238}U (99,3%), následuje ^{235}U (0,72%) a ^{234}U (0,01%) (Pluskal 1971). Hlavní uranovou rudou je uraninit neboli smolinec s chemickým složením UO_2, kvůli oxidaci však obvykle představuje směs oxidů s variabilním zastoupením (U_3O_8).

Nejvyšší koncentrace uranu lze nalézt v granitoidních horninách (2,5 – 6,0 ppm), dále pak v břidlicích (3,0 – 4,1 ppm), vápencích (2,2 – 2,5 ppm), pískovcích (0,45 – 0,59 ppm), v bazických horninách (0,3 – 1 ppm) a nejnižší hodnoty v ultrabazických horninách (0,003 – 0,01 ppm) (Kabata-Pendias 2001). Ložiska uranu lze rozdělit na 5 hlavních typů (Cuney 2008): uranová ložiska související s povrchovými procesy, synsedimentární ložiska, uranová ložiska související s hydrotermálními procesy, uranová ložiska související s částečným tavením a ložiska související s krystalickou frakcionací. Příbramské uranové ložisko souvisí s hydrotermálními procesy (Růžička 1993) a je vázáno na rozhraní dvou velkých geologických jednotek: Středočeského plutonického komplexu a Barrandienu.

Téměř 90% uranu lze v životním prostředí nalézt v hexavalentním mocenství (U^{VI}) jako uranylový kationt $[UO_2]^{2+}$, který obvykle převládá v půdách, dále se vyskytuje i v tetravalentním mocenství (U^{IV}). Podrobné informace o uranu lze najít v přehledných pracích Kabata-Pendias (2001), Craft et al. (2004) a Hooda (2010).

3.2 Houby a uran

Rolí hub v geochemii uranu se zabývala celá řada prací (Volesky et Holan 1995, Rufyikiri et al. 2002, Bishnoi et Garima 2005, Chen et al. 2005, Gadd 2007, Fomina et al. 2007, Fomina et al. 2008, Gadd et Fomina 2011 a Liang et al. 2015), ze kterých vyplývá, že houby mohou zasahovat do geochemického cyklu uranu podobně jako v případě jiných kovů (Gadd 2010, Gadd et al. 2012). Jak bylo zmíněno v kapitole 1.4, exkrece organických kyselin je důležitou schopností hub, ovlivňuje geochemii okolí hyf v prostředí a v případě uranu vede k jeho rozpouštění a formování uranových komplexů. Uran může být houbovou biomasou efektivně sorbován anebo mohou být na povrchu hyf vytvářeny krystalické agregáty anorganických sloučenin s uranem.

O schopnosti sorbovat uranu spájivými houbami informoval již Volesky et Holan (1995). Akhtar et al. (2007) pozorovali vysokou sorpční kapacitu uranu u mycelia druhu *Trichoderma harzianum* v akvakultuře; tato kapacita byla silně závislá na pH a při optimálních podmínkách dosáhla 612 mg U/ g. Dále byla pozorována vysoká sorpční kapacita i u druhů outkovka pestrá – *Trametes versicolor* a *Phanerochaete chrysosporium*. Biosoropce hub řady kovů včetně uranu je silně závislá na parametrech jako je pH, druh kovu, fyzikálně-chemické ošetření biomasy před pokusem atd. (Bishnoi et Garima 2005).

Saprotrofní houby mohou rozpouštět uran z horniny, kde je přítomen obvykle ve formě oxidů. Jak uvádí Gadd et Fomina (2011) mikrobiální rozpouštění uranu se týká malých rozpustných organo-uranových složek obsahující jiné kovy, zejména železo a hliník. Fomina et al. (2007) přinesli důkazy, že saprotrofní, erikoidní a ektomykorhizní houby vykazují vysokou toleranci a schopnost rozpouštět oxidy uranu (UO_3, U_3O_8) a akumulace uranu v myceliu přesáhla 80 mg/g sušiny. Houby produkovaly nízkomolekulární karboxylové kyseliny (např. kyselinu šťavelovou), které se podílely na rozpouštění uranových minerálů. Testované houby dále produkovaly kyseliny glukonovou, mravenčí a jantarovou, které zlepšovaly účinnost šťavelanů. Akumulovaný uran byl biomineralizován a vytvořil krustu minerálů ze skupiny meta-autunitu na povrch hyf (Fomina et al. 2008). Tvorbu uranových minerálů pozorovali i Liang et al. (2015) u hub z rodu *Aspergillus*. Stejně tak mykorhizní houby voskovička vřesovcová – *Hymenoscyphus ericae* a kořenovec načervenalý – *Rhizopogon rubescens* rostoucí v přítomnosti ochuzeného uranu formovaly na myceliu krustu tvořenou sekundárními minerály uranu (Fomina et al. 2008).

Z pokusů *in vitro* je patrné, že důsledkem běžné činnosti hub může komplexace, biotransformace a (i)mobilizace elementárního uranu i jeho oxidů. Jakou měrou se tyto procesy uplatňují v přírodním prostředí, však není doposud jasné.

3.3 Obsah uranu v plodnicích hub z čistých lokalit

Obsahem uranu v plodnicích hub z čistých lokalit se zabývalo několik prací, ve kterých byly ke stanovení uranu použity odlišné metody: hmotnostní spektrometrie s indukčně vázaným plazmatem (ICP–MS), rentgenová fluorescenční spektrometrie (XRF) či epitermální neutronová aktivační analýza (ENAA) (Falandysz et al. 2001, Stijve et al. 2001, Johanson et al. 2004, Řanda et al. 2005 a Campos et al. 2009). Většina těchto prací ukázala, že uran je prvek, který se nachází v houbách v nízkých koncentracích a obvykle nepřesahuje

nižší stovky ppb[4]. Výjimku tvoří práce Campos et al. (2009), kteří ke stanovení uranu ve 12 druzích hub použili XRF, a jejich výsledky byly výrazně vyšší než výsledky ostatních autorů: pohybovaly se v jednotkách ppm. Srovnat naměřené obsahy uranu lze pouze u jednoho druhu houby a to u lišky obecné – *Cantharellus cibarius*, kterou analyzovali i Řanda et al. (2005). Campos et al. (2009) naměřili 2,3 ± 0,44 ppm a Řanda et al. (2005) 0,0072 ppm. Tento rozdíl by mohl být způsoben řadou faktorů, např. vlivem prostředí, ale Campos et al. (2009) uváděli i vysoké hodnoty koncentrací uranu u hub, které rostou na dřevě a u kterých je všeobecně známo, že obsahují nižší koncentrace kovů (Tyler 1982).

Proto jsme se v naší práci (Borovička et al. 2011, Příloha 1) zabývali analýzou obsahu uranu v plodnicích hub z čistých lokalit – kromě uranu jsme se dále zaměřili i na koncentrace stříbra, olova, thoria a prvků vzácných zemin (REE). Cílili jsme na druhy, které publikovali Campos et al. (2009) ve své studii. Podařilo se nalézt 9 shodných druhů hub (celkem 18 vzorků) z různých lokalit. U všech 18 vzorků byly hodnoty uranu, stanovené pomocí ICP-MS, výrazně nižší než u studie Campos et al. (2009) a odpovídaly hodnotám známým z ostatních studií. Naměřené hodnoty uranu se pohybovaly v jednotkách až nižších desítkách ppb (maximální hodnota 26,3 ppb u pečárky ovčí – *Agaricus arvensis*).

Campos et al. (2009) nepoužili ve své práci standardní referenční materiály, takže (na rozdíl od nás) nedoložili správnost použité analytické metody. Vysvětlit zvýšené koncentrace, které uvádějí Campos et al. (2009), nelze ani předpokladem, že houby byly sbírány na lokalitě s přirozeně vysokým obsahem uranu v substrátu, jelikož další naše práce (Kubrová et al. 2014, Příloha 4) ukázala, že ani obsahy uranu v houbách z lokality postižené těžbou uranových rud nedosahují těchto hodnot (viz níže). Lze se tedy domnívat, že se jedná spíše o analytický problém: nesprávné použití či interpretaci XRF metody.

3.4 Obsah uranu v plodnicích hub z kontaminových lokalit

Data o obsahu uranu v plodnicích hub z kontaminovaných lokalit prakticky chybějí. Baumann et al. (2013) analyzovali pomocí ICP-MS obsah uranu v devíti druzích hub na uranové haldě v oblasti, kde se v minulosti získával uran loužením. Naměřené hodnoty se pohybovaly ve stovkách ppb s nejvyšší naměřenou hodnotou 3470 ppb pro čechratku podvinutou. Vzhledem k minimu informací o obsahu uranu v plodnicích hub

[4] ppb – parts per billion (10^{-9}) – v geovědách doposud běžně používaná jednotka, kterou lze technicky správně vyjádřit jako $\mu g \cdot kg^{-1}$

z kontaminovaných lokalit a snahou o možné vysvětlení vysokých koncentrací naměřených v práci Campos et al. (2009) jsme se rozhodli zaměřit na tuto problematiku, které jsme se věnovali v naší práci (Kubrová et al. 2014, Příloha 4):

Jako vhodnou lokalitu pro sběr vzorků jsme vybrali okolí Bytízu na Příbramsku, kde v minulosti probíhala těžba uranu a dle práce Suchara et Sucharová (2004) se zde vyskytují zvýšené koncentrace uranu v meších a humusovém horizontu. Vzorky hub byly sbírány v lesních porostech a také na přímo na haldách hlušiny. Kromě obsahu uranu jsme analyzovali metodou ICP-MS i obsahy olova, stříbra a thoria. Podle výše zmíněných prací obsah uranu z čistých lokalit nepřekračuje obvykle hodnotu 30 ppb. Obsahy uranu, které jsme naměřili v plodnicích hub, jsou výrazně zvýšené s nejvyšší hodnotou 2611 ppb pro saprotrofní chřapáč jamkatý – *Helvella lacunosa*; některé recentní publikace ale naznačují, že se jedná o ektomykorhizní druh (Nguyen et al. 2013). Houby byly v naší studii rozděleny dle způsobu výživy na saprotrofní a ektomykorhizní. Obsahy uranu v saprotrofních houbách se pohybovaly v rozpětí 5,28 – 2611 ppb s mediánem 82,4 ppb. Obsahy uranu v ektomykorhizních houbách se pohybovaly v rozpětí 3,27 – 1978 ppb s mediánem 63,0 ppb. Je zde patrné, že saprotrofní druhy hub obsahují nepatrně více uranu než druhy ektomykorhizní, což je běžný jev pozorovaný u řady dalších prvků (Falandysz et Borovička 2013).

Nejvyšší koncentrace uranu byly zaznamenány ze vzorků odebraných přímo z haldy a týkalo se to jak saprotrofních, tak ektomykorhizních druhů hub. Mezi těmito vzorky byly i ty, ve kterých byly stanoveny nejvyšší koncentrace uranu: již výše zmíněný chřapáč jamkatý a ektomykorhizní vláknice potměchuťová – *Inocybe dulcamara* s 1978 ppb uranu.

Naše výsledky odpovídají datům publikovaných v práci Baumann et al. (2013) s drobnou výjimkou. Baumann et al. (2013) uvádějí rozpětí naměřených hodnot 30 – 3470 ppb, což odpovídá našim výsledkům, ale nejvyšší hodnotu uvádí v čechratce podvinuté, ve které jsme naměřili naopak nejnižší obsah uranu (3,27 ppb). Tato vysoká hodnota je doprovázena i podezřele vysokým obsahem železa (10020 ppm), což by mohlo ukazovat na analytický problém či kontaminaci vzorku půdními částicemi (Borovička et Řanda 2007, Brzostowski et al. 2011a).

Koncentrace thoria v plodnicích hub jsou velmi nízké s nejvyšší hodnotou 78,6 ppb pro strmělku kosťovitou – *Clitocybe costata*; naměřené hodnoty odpovídají již dříve publikovaným (Borovička et al. 2011, Příloha 1). Obsahy olova jsou v porovnání

s předchozími prvky výrazně vyšší a nejvyšší hodnota 72,0 ppm byla naměřena v pýchavce horské – *Lycoperdon foetidum*, tyto hodnoty odpovídají již dříve publikovaným (Kalač 2010). Houby jsou známé svojí schopností akumulovat nebo dokonce hyperakumulovat stříbro (Borovička et al. 2007, Borovička et al. 2010, Osobová et al. 2011). Nejvyšší naměřená hodnota tohoto prvku byla 53,9 ppm v pečárce ovčí.

Získané obsahy prvků v plodnicích hub je velmi zajímavé porovnat s obsahy prvků v půdě. Analýzou 23 vzorků organického horizontu bylo zjištěno, že obsahy uranu v půdě lze skutečně považovat za zvýšené. Naměřené rozpětí bylo 6,08 - 74,5 ppm, medián hodnot byl 13,7 ppm, rozpětí koncentrace uranu na haldě bylo 11 – 35 ppm. Kabata-Pendias (2001) uvádí celosvětový průměr obsahu uranu v půdách 0,79-11 ppm. Lokalita Bytíz je dále zatížena dlouhodobě spadem z blízké kovohutě a je znečištěna olovem i stříbrem (Ettler et al. 2004, Komárek et al. 2007). Naměřené rozpětí hodnot olova ve vzorcích organického horizontu je 66,8 – 1251 ppm (medián 615 ppm) a lze je tedy dle Kabata-Pendias (2001) bezpochyby považovat za zvýšené. Naměřené rozpětí hodnot stříbra ve vzorcích organického horizontu je 0,11 – 12,3 ppm (medián 0,77 ppm), což lze také považovat za zvýšené hodnoty (Kabata-Pendias 2001). Naměřené rozpětí hodnot thoria ve vzorcích organického horizontu je 2,52-9,87 ppm (medián 5,57 ppm), což jsou normální koncentrace, jaké lze očekávat na granitickém podloží.

V půdním profilu se nejvyšší koncentrace uranu, olova a stříbra nacházely v organickém horizontu a s hloubkou klesaly (Kubrová et al. 2014, Příloha 4, tabulka 3). Nabohacení prvků v organickém horizontu lze vysvětlit sorpcí kovů z atmosférické depozice na organickou hmotu. Thorium bylo jediným prvkem, jehož obsahy s hloubkou rostly. Sekvenční extrakce BCR ukázala významné rozdíly v chování jednotlivých prvků. V případě uranu byla zjištěna dominantní vazba na oxidovatelnou frakci (tedy na organickou hmotu a sulfidy) a ve všech půdních horizontech byla zastoupena frakce vysoce mobilního uranu. Olovo bylo velice mobilní a dominantně se vázalo na redukovatelnou frakci. Thorium bylo silně vázáno a přítomno především v reziduální frakci. Vyměnitelná frakce stříbra byla téměř zanedbatelná a nejvíce stříbra bylo spojeno s redukovatelnou frakcí.

Pokud vezmeme v úvahu celkové koncentrace zkoumaných prvků a jejich mobilitu, můžeme za nejvíce mobilní prvek považovat olovo, následované uranem, thoriem a nakonec stříbrem. Stříbro je tedy prvkem s nejnižší koncentrací a zároveň je nejméně mobilním prvkem ze všech. Přesto však bylo nalezeno v relativně vysokých koncentracích v plodnicích

hub. Tato zjištění naznačují, že houby výrazně zasahují do geochemického cyklu stříbra v půdě a že příjem tohoto kovu houbami je závislý především na jejich biologické aktivitě a nikoli primárně na koncentraci či mobilitě prvku v půdě.

4. STOPOVÉ PRVKY V EKTOMYKORHIZÁCH

4.1 Stopové prvky v ektomykorhizách

Pro rostliny žijící v ektomykorhizní symbióze na antropogenně kontaminovaných lokalitách byla opakovaně prokázána vyšší odolnost vůči zvýšeným koncentracím těžkých kovů v půdách (Dixon 1988, Leyval et al. 1997, Godbold et al. 1998, Schützendübel et Polle 2002, Urban 2011, Colpaert et al. 2011, Ma et al. 2014). Dále bylo prokázáno, že ekotypy ektomykorhizních hub z kontaminovaných lokalit vykazují vyšší toleranci k těžkým kovům než je tomu u izolátů s pozaďových lokalit (Adriaensen et al. 2006, Colpaert et al. 2011).

Řada studií se zabývala obsahy prvků v plodnicích hub (Kalač 2009, 2010, Falandysz et Borovička 2013) či v rhizomorfách a myceliu (Wallander et al. 2002, Wallander et al. 2003, Vinichuk 2013), ale jen malá pozornost byla věnována jejich obsahům v ektomykorhizách. Zřejmě první práci na toto téma publikovali Berthelsen et al. (1995), kteří se zaměřili na koncentrace kadmia, mědi, olova a zinku v 36 vzorcích ektomykorhiz. V té době nebylo běžné použití molekulárně genetických metod, a proto byly ektomykorhizy rozděleny dle morfotypu a nebyly identifikovány na úroveň druhu. Tato studie ukázala, že ačkoliv byl obsah mědi v půdách nízký, ektomykorhizy akumulovaly v průměru 338 ppm mědi s maximální hodnotou 790 ppm. Koncentrace ostatních prvků byla v ektomykorhizách nižší než v půdách a nejvyšší rozdíl byl zjištěn u olova.

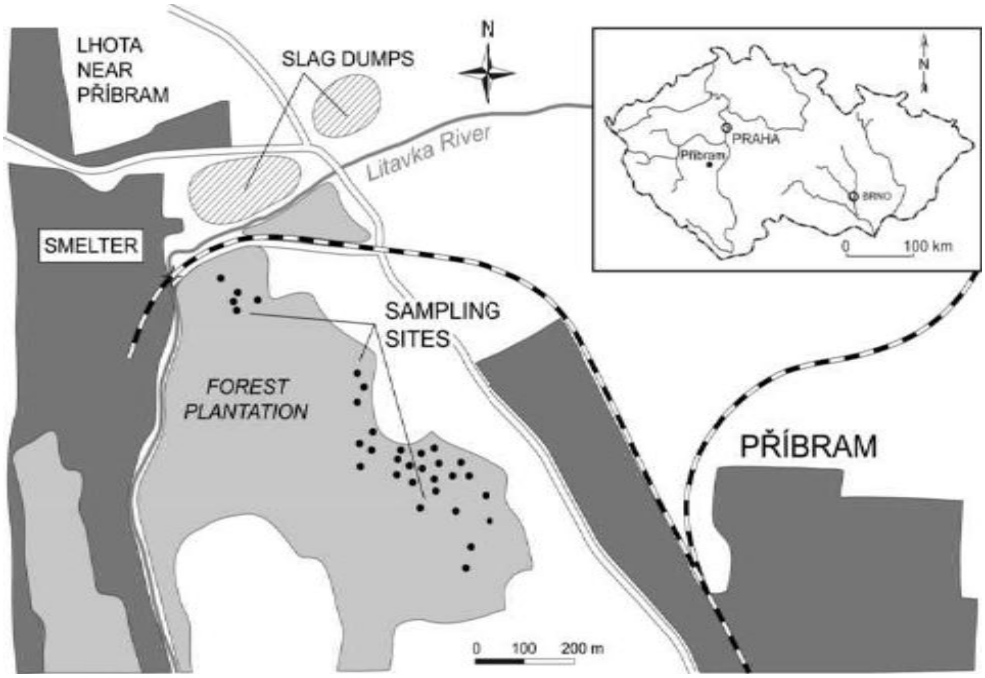
Krupa et Kozdrój (2004) se zaměřili na obsah kadmia a olova v ektomykorhizách, plodnicích hub a jednotlivých částech symbiotické dřeviny (výhonky a kořeny). Ektomykorhizy měly přibližně pětkrát vyšší koncentrace kovů než půdy. Tento výsledek vedl k závěru, že mykorhizní houby v hyfovém plášti ektomykorhiz vážou těžké kovy a budují tak efektivní bariéru proti pronikání kovů do rostlinného partnera (Krupa et Kozdrój 2004). V další publikované práci autoři zjistili, že nejvyšší koncentrace kovů (Cd, Zn a Pb) z testovaných druhů hub měla ektomykorhiza muchomůrky červené (Krupa et Kozdrój (2007).

Kottke et al. (1998) ve své práci publikovali výsledky pro obsah 8 prvků (Al, Ca, Fe, K, Mg, Mn, P, Zn) v 17 druzích ektomykorhizních hub. Nejefektivněji akumuloval v mykorhizách stopové prvky hřib hnědý.

Borovička et al. (2010) se ve své práci zaměřili na obsahy zlata v ektomykorhizách; nejvyšší koncentrace (24,7 ppb) byla nalezena v hřibu hnědém a v porovnání s jemnými kořeny smrků obsahovaly ektomykorhizy 4–10 krát více zlata.

4.2 Stopové prvky v ektomykorhizách ze Lhoty u Příbramě

V naší práci (Cejpková et al. 2016, Příloha 5) jsme se zaměřili na obsahy prvků v ektomykorhizách a jemných kořenech z lokality Lhota u Příbramě (Obr. 3, Příloha 5). V blízkosti této lokality se nachází kovohuť, která je v provozu více než 200 let a v půdě lze nalézt extrémně zvýšené koncentrace celé řady prvků (Ag, As, Cd, Cu, Pb, Sb a Zn; Ettler et al. 2004, 2007, Komárek et al. 2007).



Obr. 3. Místa sběru vzorků ektomykorhiz, Lhota u Příbramě (Cejpková et al. 2016, Příloha 5)

K analýze Cl, Cu a V v ektomykorhizách jsme využili metodu krátkodobé neutronové aktivace (INAA) podle Řanda et al. (2005) a pro analýzu ostatních kovů jsme využili variantu dlouhodobé epitermální neutronové aktivace. Půdní vzorky byly analyzovány pomocí INAA dle Řanda et Kučera (2004).

Někteří zástupci rodu muchomůrka jsou známy svojí schopností akumulovat nebo dokonce hyperakumulovat stříbro ve svých plodnicích (Borovička et al. 2007, 2010). Přestože je stříbro málo mobilní v půdním profilu (Kubrová et al. 2014, Příloha 4), je akumulováno

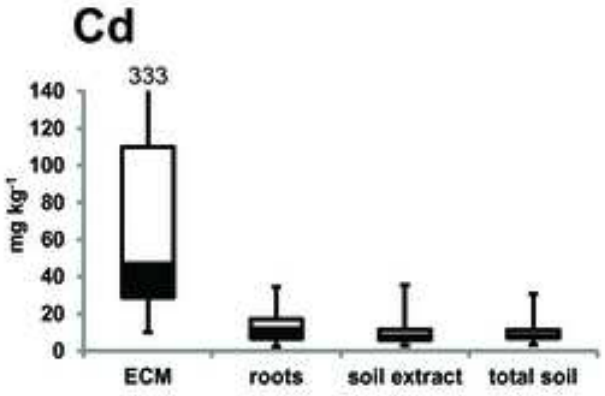
ektomykorhizami ve vysokých koncentracích (47.7-385 ppm). Nejvyšší koncentrace stříbra byly nalezeny v ektomykorhizách hřibu hnědého (Obr. 4), což je zajímavé vzhledem k faktu, že v plodnicích tohoto druhu z této lokality se stříbra nalézá mnohem méně (v průměru 14,2 ppm) a patří tak mezi „slabší“ akumulátory (Borovička et al. 2010).

Obr. 4. Plodnice (vlevo) a ektomykorhizy (vpravo) hřibu hnědého. Foto: Jaroslava Cejpková.

Arsen je houbami efektivně akumulován (Stijve et al. 1990, Slekovec and Irgolic 1996) a metylované sloučeniny As se nacházejí v plodnicích hub (Nearing et al. 2014). Naměřené koncentrace arsenu v ektomykorhizách byly nižší než v půdách, ale vyšší než v jemných kořenech smrků. Koncentrace arsenu byly velmi proměnlivé, např. u hřibu hnědého 3,98-376 ppm).

Dalším prvkem, který je akumulován hlavně saprotrofními houbami, především pečárkami – *Agaricus* ze sekce *Arvenses*, je kadmium (Cocchi et al. 2006). Koncentrace kadmia v plodnicích ektomykorhizních hub jsou nižší než u hub saptrotrofních a obvykle nepřekračují 1 ppm (Kalač et Svoboda 2000, Malinowska et al. 2006). Kalač et al. (1991) publikovali hodnoty pro plodnice hub ze Lhoty u Příbramě a nejvyšší koncentrace byly nalezeny u muchomůrky červené (28,6 ppm). Koncentrace kadmia v ektomykorhizách byly vyšší než v jemných kořenech a také velmi variabilní (10-333 ppm s mediánem 47,0 ppm, Obr. 5). Vzhledem ke koncentracím v půdě bylo kadmium druhým nejvíce akumulovaným

prvkem v ektomykorhizách. Hodnoty nad 100 ppm byly zaznamenány u 6 druhů, včetně hřibu hnědého, což odpovídá výsledkům publikace Krupa et Kozdrój (2004). Naopak velmi nízké koncentrace kadmia v ektomykorhizách byly publikovány v práci Berthelsen et al. (1995), kteří na kontaminované lokalitě naměřili průměr 4,6 ppm.



Obr. 5. Koncentrace kadmia v ektomykorhizách (ECM), kořenech, půdním extraktu a jeho celkový obsah v půdě (Cejpková et al. 2016, Příloha 5).

Meď je akumulována ektomykorhizními houbami v plodnicích, koncentrace však obvykle nepřekročí 50 ppm (Malinowska et al. 2006, Vetter 2005); vyšší hodnoty jsou udávány např. pro čechratku podvinutou (Brzostowski et al. 2011a-b). Koncentrace mědi zjištěné v ektomykorhizách z lokality Lhota byly jen o něco málo vyšší než v jemných kořenech a nejvyšší hodnota byla 162 ppm. Nejvyšší koncentrace byly obecně nalezeny v ektomykorhizách hřibu hnědého (v průměru 120 ppm). Naše výsledky jsou tedy výrazně nižší, než byly publikovány Berthelsen et al. (1995), kteří uvádějí rozpětí 176-790 ppm!

V ektomykorhizách i jemných kořenech byly naměřeny nižší koncentrace antimonu než v půdě, a proto můžeme hovořit o bioxkluzi. Koncentrace v ektomykorhizách se pohybovaly v jednotkách až nižších desítkách ppm, nejvyšší byla naměřena v plesňáku zemním (117 ppm); o interakcích hub a antimonu nejsou k dispozici prakticky žádné údaje (Pierart et al. 2015).

Dalším prvkem, u kterého můžeme hovořit o bioexkluzi v ektomykorhizách, je vanad, ovšem až na jednu výjimku. U ektomykorhiz muchomůrky červené (Obr. 6) byly v porovnání s ostatními druhy nalezeny vyšší koncentrace vanadu (26,4 a 61,2 ppm, oproti průměrné

koncentraci 2,74 ppm). Muchomůrka červená je známým akumulátorem vanadu v plodnicích (viz kapitola 1.5.1), vysoké koncentrace v ektomykorhizách tedy naznačují biologickou aktivitu mycelia (příjem, biotransformaci a případný transport do plodnic).

Obr. 6. Plodnice (vlevo) a ektomykorhizy (vpravo) muchomůrky červené. Foto: Jaroslava Cejpková.

Naměřené koncentrace zinku v ektomykorhizách jsou v rozpětí 197-1280 ppm a tyto hodnoty jsou totožné s výsledky publikované v práci Berthelsen et al. (1995): 105-1 090 ppm. Zároveň se jedná o nejvyšší zaznamenané koncentrace ze zkoumaných kovů. Hodnoty přesahující 1000 ppm byly nalezeny u čechratky podvinuté a plesňáka zemního.

Houby jsou známé produkcí chlormetanu (Anke et Weber 2006) a různých organických chlorovaných sloučenin (Drehmel et Chilton 2002). Zdá se, že je zde druhová závislost a vysoké koncentrace jsou charakteristické např. pro rod muchomůrka (Stijve 1984, Petrini et al. 2009). Naopak nízké koncentrace jsou nacházeny např. v čechratce podvinuté (Řanda et al. 2005). Medián hodnot chloru v ektomykorhizách je 2 700 ppm, což je výrazně víc než u jemných kořenů (962 ppm). Nejvyšší koncentrace chloru byly nalezeny

v ektomykorhize hřibu hnědého (5 614 ppm). Obsahy dalších analyzovaných prvků (Co, Cs, Se, Th, U aj.) v ektomykorhizách byly výrazně nižší a často pod detekčním limitem INAA.

Jak ukázala naše práce (Borovička et al. 2011, Příloha 1) uran a thorium nejsou akumulovány houbami a koncentrace v plodnicích nepřesahují 0,55 ppm U, respektive 0,65 ppm Th. V pozdější práci (Kubrová et al. 2014, Příloha 4) jsme publikovali koncentrace v ekomykorhizách a jemných kořenech z čisté a kontaminované lokality. Ektomykorhizy a jemné kořeny obsahovaly téměř stejné koncentrace uranu. Ovšem zatímco maximální hodnota z čisté lokality nepřekročila 1 ppm U v ektomykorhize, obsahy z kontaminované lokality se pohybovaly v nižších jednotkách ppm (maximum 6,95 ppm).

Selen je prvek, který je akumulován určitými druhy ektomykorhizních hub (např. hřib smrkový) a běžná koncentrace v plodnicích se pohybuje mezi 0,15-1,50 ppm (Borovička et Řanda 2007). V ektomykorhizách je selen obvykle pod limitem detekce INAA, obvykle tedy pod 2 ppm.

Mezi další prvky, které jsou akumulovány houbami, patří cesium a rubidium (Horyna et Řanda 1988, Vinichuk et al. 2010). Přestože hodnoty cesia byly vyšší v ektomykorhizách než jemných kořenech, nepřesáhly 1,50 ppm. Naměřené koncentrace rubidia byly vyšší v ektomykorhizách než v jemných kořenech a dosáhly 20,2 ppm.

Jak ukázali ve své studii Kottke et al. (1998), ektomykorhizy hřibu hnědého mají vysoký potenciál k ukládání řady prvků, jako jsou Fe, K, N, P a Zn. To potvrdily i naše výsledky, protože ektomykorhizy hřibu hnědého se v porovnání s ostatními detekovanými druhy ukázaly být velmi efektivními akumulátory Ag, Cd, Cl a Cu.

4.3 Kvantifikace mycelia hřibu hnědého a muchomůrky červené v ektomykorhizách

V práci Cejpková et al. (2016, Příloha 5) jsme využili metodu qRT-PCR ke kvantifikaci houbové biomasy v ektomykorhize. Tato metoda byla již v minulosti úspěšně využita ke kvantifikaci mycelia saprotrofních i ektomykorhizních hub v řadě studií (Parladé et al. 2007, Hortal et al. 2008, De la Varga et al. 2011, Borovička et al. 2014). Kvantifikace proběhla u hřibu hnědého a muchomůrky červené, v obou případech za pomoci dvou odlišných párů specifických primerů, které byly pro tento účel navrženy. Zjistili jsme značnou variaci koncentrace houbové biomasy v ektomykorhize, ale medián hodnot pro oba druhy hub byl blízko 5 % houbové biomasy v ektomykorhize. Naše výsledky jsou zřetelně nižší, než

v práci Antibus et Sinsabaugh (1993). Ti detekovali rozsah 18-34 % houbové biomasy v ektomykorhize. Zeppa et al. (2000) publikovali hodnoty ještě o něco vyšší: pro mladou ektomykorhizu to bylo 51,5 % a pro starou ektomykorhizu 35,1 %. Příčina odlišnosti výsledků zůstává nejasná. Obě z výše citovaných prací se nezabývaly ektomykorhizami na smrku ztepilém (jako naše práce). Lze očekávat, že i v rámci sezóny se množství houbové biomasy může měnit a může být ovlivněno řadou environmentálních faktorů. Přesto, že naše výsledky mají velkou variaci (hřib hnědý 0,50-36,8 % a m. červená 0,81-21,0 %), nedosahují tak vysokých hodnot jako publikovali Zeppa et al. (2000). Nižší koncentrace mycelia v mykorhizách na lokalitě Lhota by mohla být způsobena toxickým prostředím tamních půd s obsahem olova v nižších jednotkách procent (Ettler et al. 2004). V neposlední řadě je také třeba uvést, že obě uvedené práce publikovaly hodnoty založené na použití ergosterolu jakožto markeru pro houbovou biomasu a nikoliv založené na qRT-PCR. Žádné jiné výsledky, které bychom mohli použít pro srovnání, jsme však v literatuře nenalezli.

Pozorování, že ektomykorhizy obsahují vyšší koncentrace kovů než jemné kořeny (Krupa et Kozdrój 2004, Kubrová et al. 2014, Cejpková et al. 2016), vede k myšlence, že je kov akumulován v houbové biomase. Na základě znalosti obsahu kovů v jemných kořenech, ektomykorhizách a při znalosti hmotnostního poměru biomasy rostliny a houby v ektomykorhize je možné odhadnout koncentrace kovu v houbové biomase ektomykorhizy. Jak je patrné z tabulky 3 (Cejpková et al. 2016, Příloha 5), vypočítaná průměrná koncentrace Zn v houbové biomase v ektomykorhize hřibu hnědého je 2668 ppm, což odpovídá publikované hodnotě (2600 +/- 50 ppm) pro houbový plášť rhizomorf klouzka obecného (Turnau et al. 2001). Další prvky, které lze srovnat s touto studií je chlor, měď a kadmium. Námi vypočtené koncentrace pro tyto prvky v ektomykorhizách jsou výrazně vyšší (Cejpková et al. 2016, Tabulka 3, Příloha 5), což ale může souviset s odlišným druhem houby, hostitelskou dřevinou a podmínkami na lokalitě. Jak již bylo zmíněno výše, ektomykorhizy hřibu hnědého mají vysoký potenciál k ukládání právě zmíněných prvků. Metoda micro-PIXE, kterou použili Turnau et al. (2001), by mohla být vhodným nástrojem pro budoucí ověření námi zjištěných koncentrací prvků v hyfovém plášti ektomykorhiz.

5. DISTRIBUCE AKTIVITY RADIOCESIA V HŘIBU HNĚDÉM

5.1 Distribuce aktivity radiocesia v hřibu hnědém

V průběhu roku 2011 se v českých médiích[5] objevily klamavé zprávy (tzv. hoaxy), že pokožka klobouku hřibu hnědého – *Boletus badius* (nyní *Imleria badia*) je oproti jiným částem plodnice vysoce radioaktivní, a měla by se tedy před konzumací oloupat. Přestože se tyto zprávy neopíraly o žádné vědecké studie, brzy se rozšířily, především díky masivnímu šíření po internetu. Schopnost hub akumulovat radiocesium je v houbách zhruba 100krát vyšší než v zelených rostlinách (Stijve 1994). Radioaktivitou hub na našem území se zabývala celá řada prací (Řanda et al. 1987, 1988a,b, 1989; Horyna et Řanda 1988, Klán et al. 1988, Kalač 2001, 2008). Z nich jednoznačně vyplývá, že kumulace radiocesia vykazuje několik závislostí. Jedná se především o druhovu závislost: byly nalezeny rody, jejichž zástupci patří mezi nízké akumulátory radiocesia (muchomůrka – *Amanita*, holubinka – *Russula*, čirůvka – *Lepista* a většina dřevních hub). Mezi nejsilnější akumulátory patří především ektomykorhizní rod lakovka – *Laccaria*, dále čechratka podvinutá, hřib hnědý a klouzek sličný. Stejné druhy hub, které vyrostly v oblasti s nízkým radioaktivním spadem, měly nižší obsahy radiocesia než ty, které vyrostly v místech s vyšším spadem (např. jihovýchodní část Středočeského kraje, Jeseníky, Šumava apod.).

Bylo vypočteno, že konzumace 1 kg sušených hub za rok s aktivitou 10 kBq/kg sušiny znamená pro organismus dávku 0,2-0,3 mSv, což je 20-30 % dávky z přirozeného pozadí, a nelze tedy předpokládat somatické ani genetické změny (Borovička et al. 2012, Příloha 2). Běžná konzumace hub tedy nepředstavovala závažné zdravotní riziko. Navíc bylo zjištěno, že více než 80 % aktivity radiocesia přechází varem do výluhu (Kalač 2008).

Dostupné hodnoty aktivity radiocesia uvedené v literatuře se obvykle vztahují k celé plodnici, jindy však ke klobouku či třeni (Malinowska et al. 2006). Radiocesium není v rámci plodnice homogenně distribuováno – vyzrálé plodnice obsahují vyšší hodnoty v lupenech a klobouku, oproti tomu mladší plodnice mají vyšší obsahy ve třeni (Guillén et Baeza 2014).

[5] http://www.hoax.cz/hoax/radioaktivni-houby/ [cit. 1. 10. 2015]

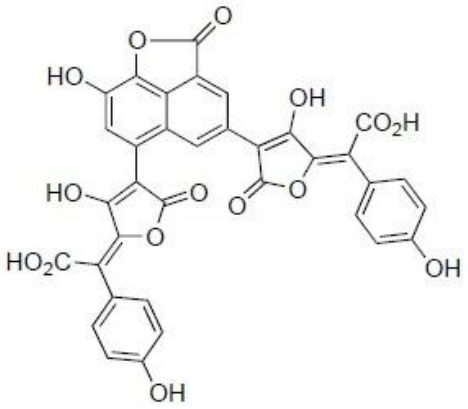
http://hobby.idnes.cz/jiri-baier-myty-o-houbach-0xw-/houby.aspx?c=A120808_163946_houby_mce [cit. 1. 10. 2015]

http://prehravac.rozhlas.cz/audio/2459460 [cit. 1. 10. 2015]

I přes zjištění publikovaná na konci 80. let 20. století jsme se rozhodli stanovit aktivitu radiocesia v různých částech plodnice hřibu hnědého a případně tak potvrdit anebo vyvrátit tvrzení kolující v mediích a na sociálních sítích (Borovička et al. 2012, Příloha 2). Proto jsme v roce 2012 v blízkosti obce Krasoňovice (okres Kutná Hora), kde byl v roce 1986 zaznamenán vysoký radioaktivní spad (Řanda 1989), nasbírali 10 dospělých plodnic h. hnědého. Plodnice byly standardně očištěny a rozděleny na třeně, rourky (hymenofor), dužninu klobouku a pokožku klobouku.

Výsledky měření ukázaly, že nejvyšší aktivita radiocesia je v dužnině klobouku (1 498 Bq/kg sušiny) a nejnižší aktivita byla naměřena ve třeni (788 Bq/kg sušiny). Aktivita pokožky klobouku byla 1 061 Bq/kg sušiny. Zjištěná aktivita je tedy zhruba 10x nižší oproti konci 80. let 20. století (Řanda et al. 1988a). Jak bylo zmíněno v kapitole 1.5.3, činí hygienický limit 6 000 Bq/kg sušiny, a je tedy patrné, že limitní hodnota nebyla překročena. V pokožce klobouku nebyla zjištěna zvýšená radioaktivita oproti ostatním částem plodnice, a její loupání nemá tedy žádný význam.

V pokožce klobouku h. hnědého jsou sice přítomná barviva badion A a norbadion A (Obr. 7), která mají schopnost komplexovat cesium (Garaudeé et al. 2002, Desage- El Murr et al. 2003) – v tomto smyslu by hypotéza o hromadění radiocesia v pokožce klobouku dávala smysl. Ovšem ionty radiocesia v plodnici „soutěží“ o vazebná místa v pigmentu s ostatními homologními ionty (stabilní Cs, K, Rb), která jsou v porovnání s cesiem v nadbytku, takže ve výsledku k hromadění nedochází (Borovička et al. 2012, Příloha 2).



Obr. 7. Struktura norbaidonu A (Desage El-Murr et al. 2003).

6. ZÁVĚR

Dnes již není pochyb, že houby hrají významnou roli v biogeochemických cyklech celé řady chemických prvků, že přispívají ke zvětrávacím procesům v půdách a že jsou významnými symbionty zelených rostlin. I přesto, že je tomu více než 100 let, co byly plodnice hub poprvé chemicky analyzovány, jejich role v těchto procesech nebyly doposud uspokojivě vysvětleny. Výzkumy ukazují, že houby dokáží ve svých plodnicích akumulovat řadu prvků, v některých případech dokonce hyperakumulovat, a to i v prostředí, ve kterém se tyto prvky nacházejí jen ve stopovém množství. Zatímco u rostlin byla u hyperakumulovaných kovů prokázána jejich protektivní úloha (odrazují býložravce), u hub je biologická role hyperakumulace kovů doposud neznámá.

Koncentrace některých kovů v houbách jsou doposud málo prozkoumány. Proto jsem se ve své první práci zaměřila především na obsahy Ag, Pb, Th, U a REE v plodnicích hub. Výsledky ukázaly, že plodnice hub nasbírané na kontaminované lokalitě Bytíz, obsahovaly 4 krát vyšší koncentrace uranu než plodnice z pozaďových lokalit. I přesto houby z Bytízu obsahovaly velice nízké koncentrace uranu, které obvykle nepřekročily 1 ppm. Výsledky sekvenční extrakce BCR ukázaly významné rozdíly v chování kovů v půdním profilu. Stříbro (kromě celkově nízkých obsahů) se ukázalo být velmi málo mobilní v půdním profilu a naopak velice mobilní byl uran. I přes tuto skutečnost se uran v plodnicích hub vyskytoval ve velmi nízkých koncentracích (medián 0,09 ppm) a stříbro bylo naopak houbami silně akumulováno (medián 6,10 ppm). Lze se tedy domnívat, že chemické chování prvku ani jeho koncentrace v půdě nehrají při akumulaci zásadní roli a hlavní je biologická činnost houby.

Pomocí aplikace molekulárně genetických metod bylo prokázáno, že mycelium pečárky Bernardovy se nachází i relativně hluboko v půdním profilu a že se neomezuje pouze na svrchní půdní vrstvu s nejvyšším obsahem organických látek. Tato saprotrofní houba zřejmě může čerpat kovy i z větších hloubek než se doposud předpokládalo, čemuž odpovídá i izotopické složení olova zjištěné v jejích plodnicích.

Podařilo se nám stanovit celou řadu prvků v ektomykorhizách a výsledky pro řadu z nich (Ag, As, Cl, Co, Rb, Se, Sb, Th, U a V) nebyly doposud nikdy publikovány. Naše výsledky naznačují, že akumulace prvků v ektomykorhizách se řídí podobnými pravidly, jako akumulace v plodnicích hub, tedy že záleží na prvku a druhu houby. Ukázalo se, že houby,

které akumulují určitý prvek ve svých plodnicích, jej můžou, ale také nemusí akumulovat v ektomykorhizách. Muchomůrka červená je známým akumulátorem vanadu ve svých plodnicích a naše výsledky naznačují, že vanad akumuluje i ve svých ektomykorhizách. Naopak hřib hnědý, který je slabým akumulátorem stříbra, silně akumuloval stříbro ve svých ektomykorhizách – a to i navzdory jeho nízké mobilitě v půdním profilu. Dále je patrné, že některé prvky jsou více akumulovány v ektomykorhizách než v jemných kořenech (např. chlór, kadmium či stříbro), ale jsou i prvky, u kterých tomu tak není (např. antimon).

I přesto, že se podařilo získat celou řadu nových informací o interakcích hub a stopových prvků, zbývá řada nezodpovězených otázek. Např. není jasné, zda se prvky akumulují v houbové části ektomykorhizy, anebo vstupují do rostlinné části ekromykorhizního kořene. K rozřešení tohoto problému by šlo využít metodu mikroPIXE, která by umožnila prvky v ektomykorhizách přesně lokalizovat.

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Příloha 1

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Uranium, thorium and rare earth elements in macrofungi: what are the genuine concentrations?

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Abstract Concentrations of uranium, thorium and rare earth elements (REE) in 36 species of ectomycorrhizal (26 samples) and saprobic (25 samples) macrofungi from unpolluted sites with differing bedrock geochemistry were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Analytical results are supported by use of certified reference materials (BCR-670, BCR-667, NIST-1575a) and the reliability of the determination of uranium was verified by epithermal neutron activation analysis (ENAA). It appears that data recently published on these elements are erroneous, in part because of use of an inappropriate analytical method; and in part because of apparent contamination by soil particles resulting in elevated levels of thorium and REE. Macrofungi from unpolluted areas, in general, did not accumulate high levels of the investigated metals. Concentrations of uranium and thorium were generally below 30 and 125 μg kg^{-1} (dry weight), respectively. Concentrations of REE in macrofungi did not exceed 360 μg kg^{-1} (dry weight) and their distribution more or less followed the trend observed in post-Archean shales and loess.

Keywords ICP-MS · ENAA · REE · Fungi · Bioaccumulation · Metals

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Introduction

In recent years, interest in the biogeochemical roles of fungi in the environment has increased rapidly (Fomina et al. 2010; Gadd 2007, 2010; Rosling et al. 2009). Part of this research involves studying the ability of macrofungi to accumulate trace elements in fruit-bodies. So far, the main interest has focused on toxic heavy metals and metalloids (Cd, Hg, Pb, As), various essential elements (Se, Zn, Cu, Fe, etc.), and noble metals (Ag, Au) (Kalač and Svoboda 2000; Kalač 2010). Available data on some elements are rather scant or even equivocal.

Specifically, ambiguous data have been reported for U, Th and Nd. Whereas several authors (e.g., Bakken and Olsen 1990; Falandysz et al. 2001; Stijve et al. 2001a; Johanson et al. 2004; Řanda et al. 2005)

reported rather low concentrations of these metals in macrofungal fruit-bodies (at μg kg^{-1} level), Campos et al. (2009) published much higher values, generally at the mg kg^{-1} level. Since recent papers highlighted the possible role of fungi in the environmental biogeochemistry of U (Fomina et al. 2007, 2008), there is an obvious need for more knowledge of macrofungal ability to accumulate U.

The aim of this study is to report reliable results for U, Th and rare earth elements (REE) in a representative set of macrofungi from unpolluted sites. Emphasis is placed on the quality of chemical analysis by comparison with certified reference materials and the use of two independent and highly sensitive analytical methods: inductively coupled plasma mass spectrometry (ICP-MS) and non-destructive epithermal neutron activation analysis (ENAA).

Materials and methods

A representative set of ectomycorrhizal (ECM) and saprobic (SAP) macrofungi was selected from various pristine localities on differing geological bedrock in the Czech Republic. Samples were collected, cleaned and dried as described previously (Borovička et al. 2010). In order to compare our results with those of Campos et al. (2009), we have included most of the species analyzed in their study.

For ICP-MS analysis, 250–350 mg portions of dried homogenized fungal sample were digested with Teflon-distilled HNO_3 (J.T. Baker) in a 60 ml PFA vessel (Savillex) on a hot plate at 250°C for a minimum 16 h. After digestion, samples were transferred to a volumetric flask, diluted to 50 ml by Millipore Milli-Q Element water and stored in a refrigerator in polyethylene bottles (Kartell) until analysis. Just before analysis, the solution was filtered over a 0.45 μm Millipore syringe filter. In the case of the reference material BCR-670 (Duck Weed), a test portion of 120–150 mg was processed by a similar procedure; siliceous material naturally occurring in plant biomass was dissolved by adding 2 ml HF (Merck, Suprapur) in an open vessel on the hot plate, evaporating to near dryness and re-dissolving in concentrated HNO_3. Trace elements were analyzed by magnetic sector-based ICP-MS using the instrument Element 2 (Thermo Scientific). Standard analytical conditions of the instrument were utilized to analyze the solutions. All elements were measured in low resolution mode. The calibration curves were prepared using a blank and multi-element stock reference standard solutions (Analytika Ltd., Czech Republic, and EPOND, Switzerland).

For ENAA, 110–200 mg pellets with 16 mm diameter were prepared using a hydraulic press (with a head made of hardened steel) and sealed into polyethylene capsules. Standards (0.09 and 1 μg U) were prepared by pipetting of diluted calibration solution on Whatman chromatographic paper with the same diameter as the samples. Neutron irradiation was carried out in the LVR-15 reactor of the Nuclear Research Institute, Řež, at flux rates of 8×10^{13} and 3×10^{13} n cm^{-2} s^{-1} for thermal and fast neutrons, respectively, using a pneumatic system with 3 s transport time. Irradiation was performed in a Cd cylindrical box (diameter 25 mm, height 10 mm, wall thickness 1 mm); just before irradiation, the Cd box with the sample was cooled in liquid nitrogen. After 30 s irradiation and 9 min decaying, counting (15 min) was undertaken. Gamma-ray spectra of the irradiated samples and standards were measured by a low energy germanium planar detector Canberra GL0515R, FWHM resolution 550 eV for the 122 keV photons of ^{57}Co. The detector was shielded by 5 cm of Pb with a 15 mm thick Fe layer inside in order to suppress X-ray $K_{\alpha 1}$ of Pb (74.969 keV). This characteristic X-ray is induced by gamma-ray and bremsstrahlung produced by negatronic emmiters of other radionuclides originated during neutron irradiation. Uranium was determined using the radioisotope ^{239}U (74.7 keV, $t_{1/2} = 23.45$ min), the product of the neutron capture reaction on ^{238}U. Epithermal activation is advantageous due to a high resonance integral of the target isotope ^{238}U; for details see Řanda et al. (2005).

After 1 month of decaying, irradiated samples (activity negligible) were removed from the polyethylene capsules, processed in the same way as described above, and subjected to ICP-MS analysis.

Results and discussion

Certified reference materials

In order to verify the quality of the analysis, we used the standard reference material BCR-670 with

certified values for U, Th and REE and indicative value for Pb. For checking the quality of Ag and Pb determination, internal fungal reference material M-122 (*Boletus reticulatus*) and standard reference material NIST-1575a (Pine needles) were used.

By ICP-MS, samples were analyzed in a set of 3 individual measurements; the reference materials BCR-670 and M-122 were always included. The results, shown as arithmetical mean and standard deviation (in parentheses) are presented in Table 1. In comparison with certified values (uncertainty in brackets), we have attained excellent agreement for U, Th, Pr, Dy, Ho and Pb (indicative value); in addition, results for La were good. Our results were slightly lower for Ce and Gd and slightly higher for Nd, Sm, Tb, Er, Tm, Yb and Lu. However, all obtained results generally fall within the certified concentration ranges. The only exception was Eu: the results for BCR-670 are excessive because of interferences from barium oxide. However, Ba content of macrofungi is low and its concentrations in the analyzed solutions rarely exceeded 3 μg l^{-1}, whereas in BCR-670, Ba concentration was about 120 μg l^{-1}. Since our results for Eu in macrofungi match well with those previously published (see below), we have included them in our study despite the fact that their reliability was not confirmed by the analysis of the certified reference material. The reference material NIST-1575a with certified value of 0.17 ± 0.01 mg kg^{-1} Pb and the internal reference material M-122 with concentrations 7.29 ± 0.43 mg kg^{-1} of Ag and 0.55 ± 0.04 mg kg^{-1} Pb were repeatedly analyzed successfully.

In order to obtain confirmatory analytical results, we used ENAA for the determination of uranium. The result for BCR-670 obtained by this method (110 μg kg^{-1}, analyzed in duplicate) is somewhat higher than the certified concentration range (Tables 1, 2). This discrepancy might be explained by interference from the X-ray $K_{\alpha 1}$ of Pb (74.969 keV), which, unfortunately, cannot be suppressed totally in the shield box.

In comparison with macrofungal samples, BCR-670 contains higher concentrations of some activated

Table 1 Results from ICP-MS and ENAA, reference and indicative values for the reference material BCR-670 (μg kg^{-1} dry weight)

ICP-MS	Result	Certified
U	83.4 (4.1)	82 [8]
Th	149 (11)	159 [18]
La	481 (26)	487 [20]
Ce	958 (38)	990 [40]
Pr	119 (3)	121 [6]
Nd	500 (21)	473 [15]
Sm	108 (5)	94 [7]
Eu	55.9 (3.6)	23.2 [2.5]
Gd	79.9 (23)	98 [8]
Tb	16.4 (0.3)	14 [1.1]
Dy	83.1 (1.1)	79 [7]
Ho	16.6 (0.9)	15.8 [1.8]
Er	48.6 (1.6)	44 [2.8]
Tm	7.0 (1.3)	5.7 [0.7]
Yb	44.4 (3.8)	40 [4]
Lu	7.3 (1.3)	6.3 [0.5]
ICP-MS	Result	Indicative
Pb	1985 (26)	2060 [120]
ENAA	Result	Certified
U	110 ± 9	82 [8]

Results from ICP-MS are given as arithmetical mean and standard deviation (in parentheses) calculated from 3 individual sets of measurements. Reference and indicative values are given with uncertainty in brackets. The result from ENAA is given as arithmetic mean of 2 analyzed samples with approximate concentration error (statistical uncertainty)

Table 2 Comparison between ENAA and ICP-MS

Sample	ENAA	ICP-MS
BCR-670	108 ± 9	74.3
BCR-667	2468 ± 62	–
Bolbitius vitellinus	11.0 ± 3	9.77
Tricholoma populinum	13.3 ± 2	16.9
Macrolepiota rhacodes	16.5 ± 3	10.5
Macrolepiota rhacodes	20.9 ± 3	17.2
Suillus luteus	134 ± 3	138

Results for uranium (μg kg^{-1} dry weight) on certified reference materials and macrofungal samples obtained firstly by non-destructive ENAA, then ICP-MS (the same sample of biomass was analyzed)

The results from ENAA are given with approximate concentration error (statistical uncertainty). The sample of *Suillus luteus* (ECM species) with a relatively high concentration was collected on an abandoned uranium dump in Bytíz (Příbram mining district, Czech Republic) for the purpose of analytical comparison

elements (Al 10×, Na 30×, and Mn 60×, approximately), resulting in higher activity (dead time circa 20× higher) and, in consequence, higher influence of X-ray $K_{\alpha 1}$ of Pb; such influence might be significant, especially at ultra-trace level. On the other hand, when analyzing the geological reference material BCR-667 (Estuarine sediment) at modified conditions (after 18-min decaying), the obtained result of 2.47 ± 0.06 mg kg^{-1} fell tightly within the certified concentration range (2.26 ± 0.15 mg kg^{-1}) (Table 2). Nevertheless, the apparent consensus between both independent methods ICP-MS and ENAA confirms the quality of our analysis.

Uranium and thorium

Accumulation of metals in macrofungal fruit-bodies is a well-known but poorly understood process. Some fungal species may accumulate (or even "hyperaccumulate") high levels of a particular metal, despite its concentration in soil substrate being very low. Such a phenomenon, whose biological importance has not been explained yet, has been repeatedly reported for Ag (Borovička et al. 2007, 2010). Apparently, U and Th are not accumulated in macrofungal fruit-bodies. Our results for U and Th concentrations (Tables 3, 4) are generally below 30

Table 3 Trace elements in ectomycorrhizal macrofungi (dry weight) obtained by ICP-MS

Bedrock	Species	U (μg kg^{-1})	Th (μg kg^{-1})	Ag (mg kg^{-1})	Pb (mg kg^{-1})
GN	*Amanita citrina*	1.08	1.35	0.21	1.16
QS	*Amanita muscaria*	4.17	3.34	1.77	0.12
QS	*Boletus badius*	1.42	3.54	1.36	0.41
CA	*Boletus edulis*	5.31	7.97	7.67	0.55
GR	*Boletus edulis*	6.92	1.57	4.31	0.24
GR	*Boletus edulis*	2.70	0.67	5.34	0.22
GN	*Cantharellus cibarius**	4.64	13.1	2.59	0.82
PS	*Cantharellus cibarius**	6.45	31.4	0.40	1.45
LM	*Hebeloma crustuliniforme*	2.75	0.22	0.18	0.03
CS	*Hebeloma sinapizans**	6.17	1.66	0.24	0.15
OS	*Hebeloma sinapizans**	7.37	15.4	0.10	0.69
LM	*Hebeloma sinapizans**	1.53	<dl	0.51	0.11
CS	*Laccaria amethystina*	2.37	5.17	0.70	0.46
PS	*Laccaria* sp.	5.69	6.09	0.18	1.00
QS	*Leccinum aurantiacum*	6.07	16.4	29.5	0.72
QS	*Paxillus involutus*	1.53	1.81	1.77	0.18
AM	*Ramaria eumorpha*	<dl	1.90	0.81	0.03
LM	*Russula exalbicans*	3.16	30.5	3.65	0.13
LM	*Russula exalbicans*	1.73	7.29	8.46	0.12
GN	*Strobilomyces strobilaceus*	10.7	30.3	5.19	0.19
CS	*Suillus collinitus*	1.57	6.61	0.15	0.20
CS	*Suillus collinitus*	0.59	2.62	0.21	0.22
QS	*Suillus luteus*	0.11	3.66	0.42	1.30
QS	*Suillus luteus*	1.55	3.65	0.66	1.89
GN	*Tricholoma populinum*	17.1	25.8	2.33	0.06
SN	*Tricholoma sulphureum*	<dl	1.36	0.30	0.20

Species analyzed by Campos et al. (2009) are indicated by an *asterisk*

Type of geological bedrock: *AM* amphibolite, *CA* Carboniferous sandstones, *CS* Cretaceous sediments (excluding sandstones), *GN* gneiss, *GR* granitic rocks, *LM* Paleozoic limestones, *OS* Ordovician sediments, *PS* Proterozoic sediments, *QS* Quaternary sediments, *SN* Cretaceous sandstones

Table 4 Trace elements in saprobic macrofungi (dry weight) obtained by ICP-MS

Bedrock	Species	U (μg kg^{-1})	Th (μg kg^{-1})	Ag (mg kg^{-1})	Pb (mg kg^{-1})
QS	*Agaricus arvensis*	26.3	5.69	15.8	13.1
AS	*Agaricus campestris**	14.7	11.8	66.6	1.09
GN	*Bolbitius vitellinus*	13.4	122	0.24	0.25
SN	*Calvatia excipuliformis*	3.77	2.83	1.79	2.49
SN	*Calvatia excipuliformis*	0.10	1.37	2.15	1.02
PS	*Clitocybe costata*	3.85	0.75	7.95	1.01
DM	*Clitocybe geotropa**	5.04	31.6	2.44	0.65
QS	*Gymnopilus spectabilis**	4.10	0.73	0.99	0.12
QS	*Gymnopilus spectabilis**	4.18	0.93	27.0	0.04
GN	*Helvella lacunosa*	1.33	6.03	0.49	0.05
PS	*Hypholoma fasciculare**	3.28	8.58	1.88	0.19
QS	*Hypholoma fasciculare**	<dl	<dl	1.30	0.21
SN	*Lepista nuda*	0.27	2.69	1.10	0.74
AS	*Leucoagaricus leucothites*	1.42	8.29	48.9	0.40
GR	*Macrolepiota procera**	1.89	2.75	1.95	10.9
PS	*Macrolepiota procera**	11.6	83.1	1.35	5.07
GR	*Macrolepiota rhacodes*	13.4	3.15	5.33	2.52
QS	*Mycena zephirus*	0.10	1.15	0.15	0.10
CS	*Omphalotus olearius**	0.08	1.51	1.38	0.05
CS	*Pleurotus pulmonarius*	3.25	11.4	7.55	0.13
GN	*Pluteus cervinus*	1.10	1.76	0.22	0.09
CS	*Tricholomopsis rutilans**	1.81	1.05	0.62	0.65
QS	*Tricholomopsis rutilans**	0.53	2.95	8.34	0.27
GN	*Tricholomopsis rutilans**	<dl	1.56	5.07	0.42
GR	*Tricholomopsis rutilans**	3.95	0.37	6.14	0.11

Species analyzed by Campos et al. (2009) are indicated by an *asterisk*

Type of geological bedrock: *AS* anthropogenic soil, *CS* Cretaceous sediments (excluding sandstones), *DM* dolomitic marbles, *GN* gneiss, *GR* granitic rocks, *PS* Proterozoic sediments, *QS* Quaternary sediments, *SN* Cretaceous sandstones

and 125 μg kg^{-1}, respectively. No significant difference between concentrations in ECM and SAP fungi was found using the Student's *t* test of significance. The highest level of U (26 μg kg^{-1}) was found in *Agaricus arvensis*; in the case of Th, the concentration range is wider, with the highest value of 122 μg kg^{-1} for *Bolbitius vitellinus*.

The results generally agree with data reported by Bakken and Olsen (1990), Falandysz et al. (2001), Stijve et al. (2001a), Johanson et al. (2004), and Řanda et al. (2005). However, there are slight differences: Bakken and Olsen (1990) found somewhat lower values for Th and Johanson et al. (2004) also reported low data for Th, but a wider concentration range for U. Such differences might be explained by problems in chemical analysis but, more likely, by a low number of analyzed samples in both studies and possibly also by environmental factors. For this reason, we do not discuss the few data for Th published by Latiff et al. (1996).

Campos et al. (2009) reported U and Th concentrations in macrofungi in the range of 0.80–4.13 and 1.43–3.63 mg kg^{-1}, respectively. However, these high concentrations cannot be explained by a "specific ability of tested species" to accumulate these elements. We have shown that the same fungal species as analyzed by Campos et al. (2009), collected from various sites in the Czech Republic (with differing bedrock geochemistry), exhibit much lower concentrations. In the case of U, our data are, moreover, supported by two independent analytical methods. The explanation of such discrepancy cannot

be related to some specific environmental factors such as the bedrock geochemistry, since all specimens analyzed by Campos et al. (2009) originated from sites above quartzite. None of our samples from similar environments exhibited such high U/Th concentrations. Curiously enough, the highest concentrations of U/Th reported by Campos et al. (2009) were found in wood-rotting fungi *Hypholoma fasciculare* and *Gymnopilus spectabilis*—despite the concentrations of U/Th in wood being much lower than those in soils. These results, three orders of magnitude higher than those of ours, were obtained using X-ray fluorescence spectrometry (XRF). Whereas the authors correctly claim that this analytical method "is one of the simplest, most accurate and most economic for the determination of the chemical composition of many types of mineral and organic substrates", this is not the case for very low levels (e.g. mg kg^{-1}) that they reported. It would have been useful if they had provided results for standard reference materials and the spectral lines used for the chemical analysis (for consideration of potential spectrometric interferences). Our study indicates that the only realistic results reported by Campos et al. (2009) are those for Pb. Furthermore, XRF is not suitable for determination of U and Th at ultra-trace or even trace levels.

With regard to the general ability of macrofungi to take up U and Th we have compared the data with elements known to be highly accumulated (Ag) and discriminated (Pb) (Tables 3, 4). Concentrations of both elements are much higher than those of U and Th. Correlation analysis (tested by the Pearson product moment correlation coefficient) revealed a significant correlation ($P < 0.01$) between U and Th in ECM fungi ($r = 0.61$) and between U and Pb in SAP fungi ($r = 0.58$).

Fomina et al. (2007) have demonstrated that fungi exhibit high uranium oxide tolerance and possess the ability to solubilize UO_3 and U_3O_8 and to accumulate U within the mycelium to over 80 g kg^{-1} dry weight biomass. The hyphae were found to be encrusted with uranium precipitates associated with phosphorus and some fungal species caused the biomineralization of uranyl phosphate minerals of the meta-autunite group. A similar ability of fungi has been demonstrated also in metallic depleted uranium (Fomina et al. 2008).

As suggested by Fomina et al. (2007, 2008), the fact that fungi are able to solubilize uranium solids indicates their possible role in biogeochemical cycling of U in the environment. This role, indeed, should be considered at U-polluted sites. On the other hand, the role of macrofungi in the geochemistry of U in pristine environments seems to be limited, at least when compared to other elements like Ag; Ag concentrations in soils are usually lower than those of U, but, despite this fact, Ag is absorbed by hyphae, transported and accumulated in fruit-bodies very effectively, since metallothioneins have been confirmed to play a significant role in Ag sequestration process (Osobová et al. 2011). A similar significant role of fungi could be considered, for example, to that of As, which is also highly accumulated by many macrofungi and biotransformed into methylated organic compounds (Šlejkovec et al. 1997).

Concentrations of U in unpolluted soils depend on the type of geological bedrock; concentrations in common rocks usually fall within the range of 0.3–6 mg kg^{-1} (Kabata-Pendias 2001). The very low U concentrations in fruit-bodies do not indicate a specific and significant contribution of macrofungi to the biogeochemical cycling of U. Interestingly, Johanson et al. (2004) reported concentrations of 0.04–10.3 mg kg^{-1} U in fungal mycelia collected in the field; U concentrations in mycelia and fruit-bodies correlated well with those measured in soils. With regard to the U content in fruit-bodies, the concentration ratio (concentration in fruit-bodies divided by concentration in mycelia) was 0.01. This would indicate that U enters fungal hyphae but that it is neither effectively accumulated, nor transported to the fruit-bodies. In this context, it should be noted that the extremely high U concentrations in fungal mycelia reported by Fomina et al. (2007, 2008) do not represent U content *sensu stricto* but include also the "mycogenic" U precipitates outside the fungal hyphae.

Several authors have reported activities of U and Th isotopes in macrofungi (Mietelski et al. 2002; Wichterey and Sawallisch 2002; Baeza and Guillén 2006; Baeza et al. 2006; Turhan et al. 2007). In general, activities of ^{234}U, ^{238}U, ^{228}Th, ^{230}Th and ^{232}Th in macrofungi from background areas were below 10 Bq kg^{-1} (dry weight). Slightly elevated activities were found at U-mining areas in Germany (Wichterey and Sawallisch 2002); according to this study, the contribution of ^{238}U to the effective dose via ingestion of wild-growing mushrooms is negligible.

Rare earth elements

Concentrations of REE in ECM and SAP fungi are presented in Table 5. REE distribution in two selected species, *Tricholoma populinum* (ECM, C-05) and *Macrolepiota procera* (SAP, M-19), is presented in Fig. 1 and shows a typical negative Eu-anomaly. Concentrations were normalized according to Boynton (1984). The REE pattern more or less follows the trend observed in post-Archean shales and loess (Rudnick and Gao 2003).

Our data generaly agree well with those few published in the literature (Aruguete et al. 1998; Falandysz et al. 2001; Stijve et al. 2001a). Some results reported by Stijve et al. (2001b, 2002) are somewhat higher, in all probability due to contamination by soil particles (see below). Despite the fact that we were not able to directly confirm the quality of our Eu determinations, the levels we found match perfectly those previously reported (Falandysz et al. 2001; Stijve et al. 2001a) and, with a typical negative Eu-anomaly, do not disturb the trend commonly found in post-Archean shales and loess (Fig. 1). Similarly to U and Th, it appears that values for Nd (2.80–7.10 mg kg^{-1}) reported by Campos et al. (2009) appear to be excessive and erroneous.

Sample contamination by thorium and REE

Possible contamination of samples by inorganic soil particles should be taken into account when analyzing and evaluating Th and REE concentrations in macrofungi. Stijve et al. (2001a) found elevated REE levels in *Gyrophragmium dunalii*, a secotioid fungus (currently classified as *Agaricus aridicola*) where REE were highly enriched in comparison with *Agaricus* spp. having typically clean fruit-bodies, with no adhering soil particles. In *Allopsalliota* (formerly *Agaricus*) *geesterani*, which fruit-body also develops underground and is difficult to clean, REE concentrations were also elevated. Generally, macrofungi with fruit-bodies developing underground or growing in sand dunes are difficult to clean and, therefore, results for Th and REE might be excessive; this could be the case of REE in *Podaxis pistillaris* (Stijve et al. 2001b, 2002), Th and La in *Termitomyces* sp. (Latiff et al. 1996), and Th in *Scleroderma verrucosum* (Horovitz et al. 1974).

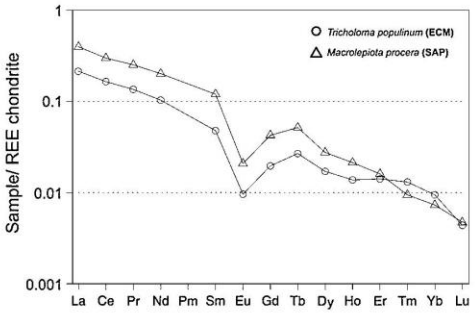

Fig. 1 Chondrite-normalized distribution of rare earth elements (REE) in two selected species: ectomycorrhizal *Tricholoma populinum* (collected on gneissic bedrock) and saprobic *Macrolepiota procera* (collected on Proterozoic sediments)

Analysis of insufficiently cleaned material (or dried samples from herbaria, which are very difficult to clean) also leads to higher Th and REE concentrations—this is seen, e.g., in the case of *Albatrellus pes-caprae* Fl1073 and all species in Table 3 in Stijve et al. (2001b, 2002). We have observed a similar phenomenon in *Pisolithus arhizus*, where Th and REE concentrations were strikingly different in a routinely cleaned sample and a sample cleaned with a

Table 5 Rare earth elements in ectomycorrhizal (ECM) and saprobic (SAP) macrofungi; a statistical summary showing median and maximum value (μg kg^{-1} dry weight); data not shown

	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
ECM														
Median	23.1	42.1	5.56	19.9	4.06	0.68	2.35	0.59	2.16	0.42	1.28	0.17	1.26	0.13
Maximum	206	276	42.8	103	22.2	4.78	13.7	3.36	21.4	4.58	14.5	2.04	13.8	1.93
SAP														
Median	13.4	21.8	2.53	10.9	2.46	0.68	1.37	0.27	1.24	0.21	0.79	<dl	0.87	0.10
Maximum	207	357	40.2	139	37.3	12.7	30.2	9.00	57.9	12.2	33.5	4.37	28.8	4.08

special care (data not shown). On the other hand, as correctly pointed out by Stijve et al. (2004), such contamination would not influence concentrations of heavy metals like Cd or Ag, which are usually much enriched in fungal biomass. For a detailed discussion on this subject and further analyses see Stijve et al. (2004).

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2006. Actually, most of the finds were made in the Bohemian part of the country before 1960; no more recent occurrence there can be confirmed. However, quite numerous finds of this species have been reported from 9 other locations since 2007, mostly in anthropogenic habitats in town parks in Bohemia (7) and in Moravia (1), the only exception being one find in an agricultural landscape. *Amanita vittadinii* has been observed in some of those places for several consecutive years. It is not clear whether this is just an accidental phenomenon or a new growth trend but the present concept of protection of this species will obviously have to be reviewed in future.

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K radioaktivitě hřibu hnědého

Radioaktivita je přirozeným jevem: radioaktivní izotopy draslíku, uranu, thoria (a některé produkty jejich radioaktivní přeměny) stojí za přirozenou radioaktivitou v životním prostředí kolem nás i v nás. Již desítky let nás však na každém kroku doprovází také radioaktivita, za kterou stojí člověk – toto celosvětové „zamoření" bylo způsobeno především testy jaderných zbraní v atmosféře. Na území Evropy však byla nejpodstatnější událostí havárie reaktoru elektrárny v Černobylu 26. dubna 1986.

Nejvýznamnější radionuklidem, který v Černobylu unikl do atmosféry a posléze se rozšířil do celé Evropy, je cesium ^{137}Cs (radiocesium), což je jeden z produktů štěpení uranu. Radiocesium se přeměňuje přes krátkodobý metastabilní izotop baria ^{137m}Ba na stabilní izotop baria ^{137}Ba. Poločas této přeměny, kterou doprovází emise záření beta a gama, je 30,17 let. To znamená, že bude trvat více než 300 let, než jeho aktivita v prostředí klesne na zanedbatelné množství. Pro úplnost poznamenáváme, že dalším uniklým radioaktivním izotopem cesia bylo ^{134}Cs, to však dnes vzhledem k 15× kratšímu poločasu rozpadu nemá v životním prostředí praktický význam (a podobné je to i s některými dalšími štěpnými produkty).

Pro posouzení škodlivosti konkrétního radionuklidu pro lidský organismus však nestačí znát jen typ záření, které emituje, a poločas jeho přeměny. Důležité jsou i jeho chemické vlastnosti. Cesium má podobné vlastnosti jako draslík, je to jeho chemický homolog, a podobně jako on je z těla vylučováno relativně rychle. U dospělého člověka je asi 10 % přijatého cesia vylučováno s biologickým poločasem 2 dny (biologický poločas: doba, za kterou je z těla vyloučena polovina látky), zbývajících 90 % má pak biologický poločas 110 dní; u dětí a dospívajících je vylučování cesia z těla dokonce o něco rychlejší (Anonym

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2005). V případě hub by mohlo být navíc pozitivní, že obsahují vysoké koncentrace draslíku, který cesium z těla může „vytěsňovat". Všechna tato fakta znamenají, že nebezpečnost ^{137}Cs je pro lidský organismus relativně nízká. Podstatně vyšší nebezpečí by pro člověka mohl znamenat např. radionuklid ^{90}Sr, který také unikl z Černobylu, protože stroncium je homologem vápníku a hromadí s v kostech – to ovšem houby neakumulují, a tak v tomto případě nepředstavuje problém (Mietelski et al. 1993, 1994).

Radioaktivní spad z Černobylu postihl území naší republiky velmi nerovnoměrně (Kukal a Reichmann 2000) a nejvíce byly postiženy ty oblasti, kde byly v době přechodu radioaktivního mraku vodní srážky (Řanda a kol. 1989). Radioaktivní cesium z tohoto spadu postupně migruje půdou směrem do hloubky a váže se na půdní součásti, především jílové minerály. V půdě se však dostává i do kontaktu s houbami, jejichž mycelium má schopnost ho absorbovat, transportovat a akumulovat v plodnicích.

Schopnost hub akumulovat radiocesium je známa už více než čtyřicet let – jeho koncentrace v houbách jsou obecně až 100× vyšší než v zelených rostlinách (Stijve 1994). O problematice radioaktivity hub bylo na stránkách Mykologického sborníku v minulosti několikrát informováno (Řanda a kol. 1988; 1989). V současnosti jsou o tomto fenoménu k dispozici podrobné informace díky přehledným článkům v odborné i populární literatuře (Reisinger 1994; Kalač 2001, 2008, 2012; Borovička 2007).

Pro houbaře je dobré vědět, že houby v plodnicích skutečně akumulují radiocesium a že radioaktivita plodnic může být mnohem vyšší, než jaká je v půdním substrátu. Netýká se to všech hub, ale především mykorhizních druhů, zejména některých pavučinců, lakovek, čechratky podvinuté a hřibu žlučníku. Mezi jedlými druhy je jedním z nejvýznamnějších akumulátorů hřib hnědý. Naopak nízké schopnosti akumulovat radiocesium mají saprotrofní houby jako jsou pečárky a bedly.

Tato schopnost hub akumulovat radiocesium není náhodná – houby totiž akumulují neradioaktivní (stabilní) cesium (izotop ^{133}Cs), které je přirozenou součástí hornin a půd, a to radioaktivní se prostě „sveze" spolu s ním. Přestože hodnoty radioaktivity některých hub dosahovaly na přelomu 80. a 90. let 20. století na našem území relativně vysokých hodnot, riziko pro konzumenty hub nebylo závažné. Typické hodnoty aktivity ^{137}Cs v sušině jedlých hub byly na konci 80. let kolem 10 000 $Bq.kg^{-1}$. Konzumace 10 kg čerstvých hub ročně (což přibližně odpovídá konzumaci asi 1 kg sušených hub) tedy zapříčiňovala efektivní dávku 0,2-0,3 mSv (milisievertu), což odpovídá 20-30 % dávky, kterou obdržíme z přirozeného pozadí. V takovýchto případech nelze předpokládat žádné somatické ani genetické změny (Řanda a kol. 1988, Klán a kol. 1987). Pro srovnání lze uvést, že současný roční limit dávky (expozice) je stanoven na

1 mSv pro veřejnost a 50 mSv pro pracovníky, kteří jsou ve styku s radioaktivitou. V současnosti, 27 let po havárii, kdy se radiocesium v půdě „rozředilo" a částečně vymřelo, radioaktivita v houbách podstatně klesla a není žádný důvod k obavám při jejich konzumaci.

Neznamená to ovšem, že bychom radioaktivitu v houbách stále nedetekovali. A neznamená to ani, že by v médiích, na internetu a mezi veřejností čas od času nekolovaly zavádějící nebo poplašné zprávy – v roce 2011 se objevila jedna v souvislosti s havárii elektrárny v japonské Fukušimě, na stránkách Mykologického sborníku o ní referoval Borovička (2011). Druhá taková zpráva se objevila na více místech v médiích a týkala se radioaktivity hřibu hnědého. Podle ní se radiocesium v hřibu hnědém – *Boletus badius* představuje zdravotní riziko a koncentruje v pokožce klobouku, takže je třeba ji před zpracováním pro kuchyni loupat. Bohužel ji šíří – či snad je dokonce jejím autorem – známý popularizátor houbaření Jiří Baier (např. Český rozhlas 6, pořad Zaostřeno na občana, 13.10.2011, nebo na serveru idnes[c]). Protože v literatuře, kterou máme k dispozici, nejsou uváděné hodnoty aktivity ^{137}Cs pro pokožku klobouku, ale jen např. pro klobouk a třeň (např. Malinowska a kol. 2006), rozhodli jsme se pravdivost této informace ověřit.

Metodika

Jako místo sběru vzorků jsme zvolili oblast v okolí Zbraslavic (okres Kutná Hora), kde byl v roce 1986 zaznamenán vysoký radioaktivní spad a aktivita radiocesia v hřibu hnědém dosahovala hodnot až 38 100 Bq.kg^{-1} v sušině (Řanda 1989). Lokalitu jsme zvolili náhodně – šlo o vzrostlý smíšený les (smrk, jedle, bříza) v blízkosti Krasoňovic. Plocha sběru měla rozměry přibližně 100×50 m (souřadnice GPS středu plochy jsou N 49.806017, E 15.151881). Dne 22. října 2012 jsme na ploše sebrali 10 dospělých plodnic hřibu hnědého, které nebyly poškozené slimáky, a měly tedy přirozené proporce, pokud jde o zastoupení jednotlivých částí plodnice.

Houby byly standardně očištěny jako pro kuchyňskou úpravu (hmotnost po očištění byla přibližně 300 g) a rozděleny na třeně, hymenofor (rourky), dužninu klobouku a pokožku klobouku. Hřib hnědý nemá loupavou pokožku, jako je tomu např. u některých klouzků, a tak byla pokožka seříznuta nožem v tenké vrstvě, jak by to učinil běžný houbař. Jednotlivé části plodnic byly pokrájeny, smíchány jako jeden samostatný vzorek a usušeny v sušičce při 50C. Vzorky byly poté rozemlety v mlýnku na jemný prášek a na hydraulickém lisu slisovány v tablety o hmotnosti přibližně 2 g (tímto způsobem jsme získali dostatečně reprezentativní vzorek).

[c] http://hobby.idnes.cz/jiri-baier-myty-o-houbach-0xw-/houby.aspx?c=A120808_163946_houby_mce

	hmotnostní podíl složky v plodnici (%)	sušina (g)	měrná aktivita složky v Bq.kg^{-1}	aktivita složky v 1 kg sušiny plodnice v Bq
třeň	25,6	7,40	788	202
dužnina klobouku	20,8	5,99	1 498	311
pokožka klobouku	8,47	2,44	1 061	90
hymenofor (rourky)	45,1	13,0	1 078	486
	sušina 10 plodnic:	28,83	aktivita 1 kg sušiny:	1 089

Tab. 1: Aktivita ^{137}Cs v jednotlivých složkách směsného vzorku 10 plodnic hřibu hnědého – *Boletus badius.*

Stejným způsobem byla také slisována tableta ze škrobu (Riedel-de Han, Německo), na kterou byl posléze nakápnut 1 ml standardního roztoku o známé aktivitě ^{137}Cs; tato tableta byla použita jako srovnávací standard s aktivitou radiocesia 2 142 Bq. Jednotlivé vzorky byly měřeny na detektoru záření gama (HPGe detektor Canberra s relativní účinností 78 %) po dobu 28 hodin, standard byl měřen 10 minut. Na lokalitě byly na několika místech odebrány také vzorky půdního substrátu (Ah horizont), ze kterých byl připraven směsný vzorek. Půda byla usušena při pokojové teplotě, přesítována na frakci pod 2 mm a rozemleta v achátovém mlýnu. Tableta o hmotnosti přibližně 1,4 g byla měřena stejným způsobem jako vzorky hub, čas měření činil 89 hodin. Aktivita radiocesia byla stanovena na základě γ linky 661,7 keV, stanovené hodnoty jsou zatíženy nejistotou přibližně 5 %.

Výsledky a diskuse

Aktivita ^{137}Cs jednotlivých částí směsného vzorku hřibu hnědého od Krasoňovic je uvedena v Tabulce 1; je vyjádřena v becquerelech (1 Bq = 1 přeměna za sekundu) na kilogram suché hmotnosti. V tabulce jsou dále prezentovány hmotnosti sušiny a podíl jednotlivých částí plodnic na celkové hmotnosti. Nejvyšší hodnota aktivity radiocesia byla zjištěna v dužnině klobouku, jen o něco nižší (ale prakticky stejné) hodnoty byly naměřeny v pokožce klobouku a hymenoforu (rourkách). Ve třeni byla aktivita nejnižší, téměř poloviční oproti dužnině klobouku. Hmotnostně nejvyšší zastoupení v plodnici hřibu hnědého má hymenofor, nejnižší pokožka klobouku.

Zjištěné hodnoty aktivity radiocesia jsou relativně nízké, přibližně 10× nižší oproti aktivitám typických jedlých hub z konce 80. let 20. století a prakticky odpovídají hodnotám, které byly z hřibu hnědého na našem území známy před rokem 1985 (Řanda a kol. 1988). Obvyklý hygienický limit pro potraviny na kilogram čerstvé hmotnosti je pro dospělé 600 Bq, pro děti 350 Bq (Kalač 2008). Po přepočtu na sušinu, která u hub představuje kolem 10 % čerstvé hmotnosti, se tedy dostáváme na aktivitu 6 000 Bq.kg^{-1}, respektive 3 500 Bq.kg^{-1}, přičemž je zřejmé, že tyto hodnoty nebyly překročeny. K tomu je vhodné poznamenat, že Evropské společenství v roce 1987 stanovilo jako nejvyšší přípustnou hodnotu

pro houby aktivitu 12 500 Bq.kg^{-1} sušiny, Mezinárodní agentura pro atomovou energii doporučila hodnotu poněkud nižší, 10 000 Bq.kg^{-1} sušiny (Kalač 2008).

Zjištěná aktivita radiocesia ve směsném vzorku půdního Ah horizontu byla 244 Bq.kg^{-1}. Hodnoty zjištěné v biomase hřibu hnědého jsou tedy přibližně 5× vyšší než v půdním substrátu, což svědčí pro akumulační schopnosti tohoto druhu houby. Malinowska a kol. (2006) nalezli v hřibu hnědém z různých částí Polska aktivitu radiocesia v rozmezí 330-6 670 Bq.kg^{-1}. Pokud jde o srovnání aktivity ^{137}Cs v klobouku a ve třeni, aktivita v klobouku byla obvykle o něco vyšší (maximálně 2×) než ve třeni.

Pokožka hřibu hnědého obsahuje hnědé barvivo norbadion A, jehož molekuly jsou schopné efektivně komplexovat alkalické kovy, mezi které patří i izotopy cesia (Aumann a kol. 1989). V tomto ohledu má tedy zpráva o hromadění radiocesia v pokožce klobouku zdánlivě smysl. Ionty radocesia se však vyskytují v celé plodnici a o vazebná místa v pigmentu „soutěží" s ionty draslíku, rubidia a stabilního cesia, které jsou oproti radiocesiu v obrovském nadbytku: hřib hnědý obsahuje na kilogram sušiny přibližně 20 000 mg draslíku, 80 mg rubidia a 5 mg cesia (Borovička 2012, nepublikováno). Ve výsledku tedy k hromadění radiocesia v pokožce nedochází: i když hodnota její měrné aktivity není zanedbatelná (1061 Bq.kg^{-1}), její příspěvek k celkové aktivitě plodnice činí pouze 90 Bq, čili 8,3 %.

Závěr

V pokožce klobouku hřibu hnědého nebyla oproti ostatním částem plodnice zjištěna zvýšená radioaktivita. Zprávy o akumulaci radiocesia v pokožce klobouku, které se v letech 2011-2012 objevily v českých médiích, lze tedy považovat za nepravdivé a loupání pokožky klobouku hřibu hnědého nemá z hlediska prevence žádný smysl. Hodnoty radioaktivity v hřibu hnědém zjištěné na lokalitě u Krasoňovic jsou nízké a pro konzumenty nepředstavují riziko: varování před údajným nebezpečím radiocesia v hřibu hnědém se nezakládají na pravdě; dramaticky vyšší koncentrace na jiných lokalitách lze totiž prakticky s jistotou vyloučit.

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Jan Borovička, Jaroslava Kubrová, Zdeněk Řanda: On radioactivity of Boletus badius

The capacity of mushrooms to absorb radiocaesium has been known for more than 40 years. Some news popped up in the Czech Republic lately indicating that the presence of radiocaesium (^{137}Cs) in the Bay Bolete (*Boletus badius*) poses a health threat to humans. According to such news it is recommended as a precaution to peel off the cap cuticle, where the concentration of radiocaesium is alleged to be highest, so that this species can be consumed safely. In the study referred to in this article its authors analyzed a composite sample of 10 carpophores of *Boletus badius* (300 g of fresh weight) taken from Chernobyl fallout contaminated site in a locality near Zbraslavice (Krasoňovice village, Central Bohemia). The mushrooms were cut into the sections of stipe, cap, body flesh and hymenophore, and cap cuticle was also separated. The values of radiocaesium activity in the different parts did not exceed the recommended sanitary limitations laid down in the respective standards. The highest value (1 498 Bq.kg^{-1} of dry weight) was measured in cap flesh, the lowest in stipe. Hence, the peeling of cap "skin" of the Bay Bolete as a health prevention measure could not be proved as substantiated. The accumulations of radiocaesium in this fungal species do not represent any health risk to consumers.

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Příloha 3

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Lead isotopic signatures of saprotrophic macrofungi of various origins: Tracing for lead sources and possible applications in geomycology

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ABSTRACT

Four saprotrophic species of macrofungi (*Leucoagaricus leucothites, Agaricus bernardii, Agaricus campestris* and *Agaricus xanthodermus*) were collected from 4 sites in the Czech Republic and analyzed for Pb content and Pb isotopic composition. Lead concentrations were relatively high in *L. leucothites* (up to 130 mg kg^{-1}) collected in site heavily polluted by a lead smelter, but much lower (0.2–6.5 mg kg^{-1}) in samples of the *Agaricus* species collected from urban, rural and pristine areas, respectively. The $^{206}Pb/^{207}Pb$ isotopic ratio in fruit bodies had a wide range of variation, and except for the smelter-polluted site in Příbram, did not reflect that in the organomineral topsoil horizons at particular sites. In the urban area of Prague, a detailed study of Pb uptake was conducted. The $^{206}Pb/^{207}Pb$ isotopic ratio in 19 samples of *A. bernardii* varied in a surprisingly wide range, from 1.124 to 1.175. In 5 specimens, the majority of "accumulated" Pb was undoubtedly transported from the topsoil layers (0–5 cm) characterized by low $^{206}Pb/^{207}Pb$ isotopic ratios, corresponding with gasoline-derived Pb from traffic emissions. In most samples, however, lead must have been transported from lower depths. Since the mycelium of *A. bernardii* was not restricted to the topsoil but could be detected both visually and using specific PCR even in a depth of 30 cm, such uptake appears to be possible. At suitable sites, Pb isotopes might represent an interesting tool for tracing the fungal uptake and transport of Pb in soils.

1. Introduction

Lead is a non-essential and toxic metal whose biogeochemical cycle has been affected by man to a great degree. It enters the environment mainly during production (mining and smelting), use and recycling of Pb-containing products and combustion of fossil fuels, namely coal and formerly also leaded gasoline (Komárek et al., 2008). Lead is a toxic metal and its elevated concentrations in the environment are of great ecological significance because this element is known to affect biological activity of soils and human health (Filippelli et al., 2012; Marzadori et al., 1996; Pasqualetti et al., 2012).

Lead isotopes have been introduced as "fingerprints" of environmental pollution and isotopic analyses of environmental samples are considered an efficient tool for tracing the sources of local and global Pb pollution (Bellis et al., 2005; Bindler et al., 2004; Conkova and Kubiznakova, 2008; Mihaljevič et al., 2006a). Each source of Pb can have distinct or sometimes overlapping isotopic ratio ranges. The isotopic composition of Pb in soils reflects mixing of these sources and source apportionment can be quantified in cases where major sources of Pb are characterized and have specific ratios (Komárek et al., 2008).

Nevertheless, discussion on possible applications and use of Pb isotopes for estimating of human environmental impact still goes on (Klaminder et al., 2011; Le Roux et al., 2008; Reimann et al., 2008a,b,c; Shotyk, 2008; Sucharová et al., 2011). Despite a large interest in applications of Pb isotopes in environmental studies, very little work has been done on Pb isotopic composition of macrofungi (mushrooms). To our knowledge, the only authors who reported on stable Pb isotope composition of macrofungi were Komárek et al. (2007) who investigated samples from a smelter-polluted site in the Czech Republic.

Macrofungi are efficient accumulators of various metals and metalloids (Falandysz and Borovička, 2013) and concentrations of accumulated elements such as Ag or Cd in their fruit bodies are

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commonly much higher than those in the underlying soil substrates (Falandysz and Danisiewicz, 1995; Svoboda and Chrastný, 2008). In several macrofungal species, hyperaccumulation of Ag has been reported (Borovička et al., 2007) with concentrations in fruit bodies from pristine sites exceeding even 1000 mg kg^{-1}. Mechanisms of metals uptake, transport to fungal fruit bodies and their origin in soils are poorly known (Osobová et al., 2011). However, high Ag levels in macrofungi suggest a massive colonization of a large soil volume since Ag is typically low in unpolluted soils, with total concentrations mostly below 0.1 mg kg^{-1} (Evans and Barabash, 2010) and EDTA extractable fraction below 10% of the total content (Borovička et al., 2010a).

Macrofungi do not accumulate all heavy metals however. Despite the fact that soil fungi are able to transform the mineral pyromorphite into Pb-oxalate and metallic Pb into pyromorphite when grown on these substrates *in vitro* (Rhee et al., 2012), concentrations of Pb and similar heavy metals like U and Th are typically very low in macrofungi from both pristine and polluted areas, much lower than concentrations of these elements in underlying soils (Borovička et al., 2011; Komárek et al., 2007). Low ability of macrofungi to take up Pb and the highly variable Pb isotopic composition of pollution sources in Europe suggest possible applications of Pb isotopes as tracers of fungal uptake of Pb and possibly also other metals in soils (Clarholm and Skyllberg, 2013; Smits and Hoffland, 2009).

The analysis of stable isotopes (N, C) has become a useful tool for investigations of fungal ecology (Agerer et al., 2012; Griffith, 2004; Hobbie and Agerer, 2010; Hobbie and Högberg, 2012; Hobbie et al., 2012a,b; Hobbie et al., 2001). On the other hand, isotopes of heavier stable elements like Sr have only rarely been applied (Blum et al., 2002; Chen et al., 2012; Wallander, 2000; Wallander et al., 2006). The aim of this paper was to investigate Pb isotopic composition of saprotrophic macrofungi collected from areas influenced by differing sources of Pb pollution, and a detailed study of Pb uptake was conducted at one selected site.

2. Materials and methods

2.1. Sampling

We decided to focus on saprotrophic fungi which are known to accumulate more Pb than the ectomycorrhizal ones (García et al., 1998) and feed on decomposition of organic matter which is mostly accumulated in surface soil horizons. Furthermore, element accumulation in saprotrophic macrofungi is not affected by various factors attributable to ectomycorrhizal fungi like exploration types of ectomycorrhizae, host plant species, specific ability of particular species to leach minerals and others (Agerer, 2001; Finlay, 2008; Landeweert et al., 2001; Rosling et al., 2003).

The macrofungal samples (4 species of the Agaricaceae family: *Leucoagaricus leucothites*, *Agaricus bernardii*, *Agaricus campestris*, and *Agaricus xanthodermus*) were harvested from 4 areas in the Czech Republic in the fall of 2007; the *Agaricus* collections are deposited in the herbarium at the Mycological Department, National Museum, Prague (PRM 857488, PRM 858095, and PRM 858110, respectively). Samples of *L. leucothites* were found along grassy roadsides in 5 close places near the town Příbram, Central Bohemia [Urbic Technosol (Siltic) above sedimentary bedrock], an area that is heavily polluted by Pb smelting operations (Ettler et al., 2004). Samples of *A. bernardii* were collected on more or less bare soil under shrubs in the capital city of Prague, from a single plot (10 × 3 m) in the immediate vicinity of a road with heavy traffic [Urbic Technosol (Siltic) above sedimentary bedrock]. Samples of *A. campestris* were collected from a meadow (10 × 10 m) in a rural area near the village of Mokrsko, Central Bohemia [Stagnic Cambisol (Dystric) on granodiorite overlain by Quaternary sediments]. Samples of *A. xanthodermus* were collected in a semi-natural broad-leaved forest (*Fagus*, *Quercus*) in one place (20 × 20 m) in the vicinity of the village of Hostim, Central Bohemia [Haplic Cambisol (Ferric) on Devonian limestones]. GPS coordinates of the sampling sites: Příbram (N49 42.148, E13 59.788); Mokrsko (N49 44.729, E14 20.004); Hostim (N49 57.322, E14 08.447); Prague (N50 4.705, E14 25.777).

Three surface soil samples (~5 cm depth), corresponding to the Au horizon (Příbram, Prague) and the Ah horizon (Mokrsko, Hostim) were collected throughout the sampling sites. They were roughly homogenized, dried to constant weight at room temperature and put through a sieve with 2 mm mesh. Furthermore, at the site of *A. bernardii* in Prague, a soil profile of 30 cm depth was sampled (1 cm steps). These soil samples were gently homogenized in plastic bags and lyophilized; there was no need to sieve them. A representative part of every sample was separated and kept frozen at −20 °C for DNA extraction.

2.2. Sample processing and ICP–QMS analyses

Macrofungal fruit bodies were processed as described elsewhere (Borovička et al., 2010b). The digestion of individual samples (weights in the range of 200–300 mg) was carried out in PTFE Savillex® beakers (Minnetonka, USA) in 10 ml of concentrated HNO_3 at 150 °C overnight. The resulting solution was subsequently evaporated and dissolved in 2% (v/v) HNO_3, transferred to a 25 ml volumetric flask and stored in 30-ml Nalgene® HDPE bottles until analysis. For solution preparation and dilution, analytical grade HNO_3 (Merck, Germany) additionally purified by sub-boiling distillation, and deionized water obtained from a Millipore® Academic purifying system (Millipore, USA) were used. The procedural blank was below 0.05 μg l^{-1}.

In soil samples from the profile in Prague, organic carbon (C_{ox}) was determined by wet combustion with a mixture of potassium dichromate and sulfuric acid; pH values were measured in distilled water and 1 M KCl with a SenTix21 electrode using the soil:solution ratio of 1:2.5.

In order to determine the complex leachable fraction of Pb in soils, 0.05 M EDTA (Fluka) standardized extraction was carried out as described in Quevauviller (1998). Shortly after the extraction, 5 ml aliquots of each sample were dissolved in a microwave oven using about 5 ml of conc. HNO_3. The sample was then diluted to 50 ml with deionized water and stored in the dark at 4 °C until analysis. The soil samples from the soil profile from Prague were processed in a similar way, but 0.11 M CH_3COOH (Merck) extraction was carried out, corresponding to the first step of the BCR sequential extraction (Rauret et al., 1999); nitric acid extraction (1 M HNO_3) was processed in the same way.

The Pb contents and isotopic composition of the mineralized macrofungi and soil extracts were determined by inductively coupled plasma quadrupole based mass spectrometry (ICP–QMS, X Series 2, Thermo Scientific). Correction for mass bias in determination of the isotope ratios was performed by SRM 981 measurement (Common lead, NIST, USA). The measurement conditions are described elsewhere (e.g. Ettler et al., 2004). Control of the quality of the analytical data was performed by measuring the mineralizates of the reference materials SRM 1515 (Apple leaves, NIST, USA) and SRM 1575 (Pine needles, NIST, USA). The differences in the measured and certified values did not exceed 3% RSD. The standard errors for measurement of the $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ ratios were <0.3% RSD and <0.4% RSD, respectively. The accuracy of the measurements was tested on reference materials BCR 2 and AGV 2 certified by the US Geological Survey (USGS, 1998). Correlation between observed data was tested using Spearman's rank correlation coefficient (rho) using the software by Wessa (2012).

2.3. Designing of specific primer and molecular analyses

To detect mycelium of *A. bernardii* in soil, a specific forward primer AgBer3 (5′-ATG TCA TTT ATT ACA CTC TAT G-3′), was designed as follows. Four ITS rDNA (ITS1-5.8S-ITS2) sequences of *A. bernardii* (GenBank: AY484678, AJ418774, AF432880 and a sequence from field collected fruit body of this species) and two sequences of *Agaricus bitorquis* as a closely related outgroup species (GenBank: AM110150, AJ884649), known to occur nearby the investigated site in Prague, were aligned using the biological sequence alignment editor BioEdit (Hall, 1999). A sequence motif differing in these two species was found in the ITS1 region. This motif is identical in all 4 mentioned *A. bernardii* sequences and has been used as a target locus of the specific primer. The sequence of the locus recognized by the primer differs from that of *A. bitorquis* in 3 of the 4 nucleotides at 3′ end and in 1 nucleotide near 5′ end. The melting point of hybridized primer, homo-dimer stability and melting point of the secondary structure were checked using DinaMelt tools (http://dinamelt.bioinfo.rpi.edu).

In soil samples, nuclear DNA was extracted from approx. 250 mg of homogenized lyophilized soil using the UltraClean soil DNA isolation kit (MoBio). For qualitative determination of *A. bernardii* in particular soil layers, semi-nested PCR targeted to the ITS rDNA region was carried out using the primer pairs ITS1F-ITS4 and AgBer3-ITS4, with the same PCR conditions as used by Borovička et al. (2010b). In *A. bernardii*, nuclear DNA was extracted from a small piece of biomass separated from the herbarium specimen from the investigated site (PRM 857488) using the UltraClean Fecal DNA Isolation Kit (MoBio). Selected amplicons were purified by isopropanol precipitation and sequenced at Macrogen Inc., Korea. The obtained sequences were edited using BioEdit.

For quantification of the mycelial biomass of *A. bernardii* in the soil profile, we used real-time polymerase chain reaction (RT-PCR) with the specific primer AgBer3 (forward) and ITS2 (reverse, White et al., 1990) in Hot FirePol®EvaGreen® qPCR Supermix (Solis BioDyne, Tartu, Estonia) prepared according to the manufacturer's instructions. Each 20 µl reaction contained approx. 10 ng of template DNA. The cycling conditions in the StepOnePlus instrument (Applied Biosystems) were initial denaturation for 12 min at 95 °C, followed by 40 cycles of 15 s denaturation at 95 °C, annealing for 20 s at 55 °C, and synthesis for 20 s at 72 °C. The results (based on 3 replicates) were expressed in terms of the number of rRNA gene copies per 1 g soil and corrected for DNA extraction efficiency. In order to determine the extraction efficiency, 2×10^{10} gene copies of an internal standard (linearized plasmid carrying fragment of cassava mosaic virus, GenBank accession AJ427910) were added into each sample before extraction and quantified in the DNA extract as described in Thonar et al. (2012).

3. Results and discussion

In order to characterize the complex leachable fraction of Pb in topsoils at the investigated sites, we have performed EDTA extraction which was demonstrated to provide representative results for soil horizons rich in organic matter (Komárek et al., 2006). Lead isotopic ratios measured in topsoil extracts from particular sites were strikingly different (Fig. 1A). The differences should be attributed to the contrasting prevailing anthropogenic Pb sources: leaded gasoline in Prague ($^{206}Pb/^{207}Pb \sim 1.11–1.13$; Novák et al., 2003) and Pb lead smelter-derived air pollution control residues in Příbram ($^{206}Pb/^{207}Pb \sim 1.167–1.177$; Ettler et al., 2004). In Mokrsko and Hostim, mixing of various anthropogenic and background sources can be expected, with coal combustion as the major artificial source ($^{206}Pb/^{207}Pb \sim 1.18–1.19$; Mihaljevič et al., 2009), which is often difficult to discern from the background values (Komárek et al., 2008).

The highest Pb concentrations were found in caps of *L. leucothites* from the heavily Pb-polluted area near Příbram (Table 1); macrofungi collected from this location are known contain extraordinarily high Pb contents (Kalač et al., 1991; Komárek et al., 2007). In contrast to this, the lowest Pb concentrations were found in samples from the rural area of Mokrsko and the semi-natural forest near Hostim (Table 1). Despite the samples of *A. bernardii* being collected in a long-term polluted synantropic habitat, lead concentrations in caps were relatively low (Table 2).

Lead isotopic composition detected in saprotrophic macrofungi (caps) does not precisely reflect that found in topsoil extracts at particular sites (Fig. 1B), with values from distinct sites overlapping to some extent. At all sites, the $^{206}Pb/^{207}Pb$ isotopic ratios in fungal samples were somewhat higher than those in the soil extracts. Low $^{206}Pb/^{207}Pb$ ratios in some specimens of *A. bernardii* from Prague give a clear evidence of heavy influence of traffic-related emissions; such a low ratios were not observable neither in topsoils, nor biota on the scale of the Czech Republic (Sucharová et al., 2011). However, $^{206}Pb/^{207}Pb$ ratios in *L. leucothites* from a heavily contaminated site were very close to those in *A. campestris* from the rural area at Mokrsko. Possible use of Pb isotopes for tracing the origin of macrofungi or even a distinction between clean and polluted sites seems therefore unfeasible; compare with Marzano et al. (2001), who effectively used artificial radioactive isotopes ^{137}Cs and ^{134}Cs derived from the Chernobyl accident for tracing geographical origin of *Boletus* spp. in Italy. On the other hand, the relatively high $^{206}Pb/^{207}Pb$ ratios in several samples of *A. xanthodermus* from Hostim ($\sim$1.21) indicate significant contribution of natural, rock-derived Pb ($\sim$1.205 and higher; Komárek et al., 2008).

The range of $^{206}Pb/^{207}Pb$ ratios in caps of *A. bernardii* from Prague was surprisingly wide (1.124–1.175) and did not fully correspond to the isotopic fingerprint found in topsoil extracts (1.114–1.130). This indicates a significant contribution of a Pb source not derived from leaded gasoline, presumably situated deeper in the soil. In order to extend the dataset, we have analyzed additional samples of *A. bernardii* available from 2007; in total, we have obtained results for 19 fruit bodies, with caps and stipes analyzed separately (Table 2). Lead concentrations in caps of *A. bernardii* were mostly higher than those in stipes. In caps and stipes, there was a significant positive correlation between both Pb concentrations (rho = 0.785, $\alpha = 0.01$) and $^{206}Pb/^{207}Pb$ ratios (rho = 0.593, $\alpha = 0.01$). Furthermore, the total Pb content in fruit bodies was positively correlated with total fruit body biomass (rho = 0.682, $\alpha = 0.01$).

In vascular plants, foliar uptake of aerial Pb must be considered (Hovmand et al., 2009; Kabata-Pendias, 2011) and species-dependent Pb isotopic variations have been reported for *Betula*

Table 1
Lead concentrations (mg kg^{-1}, dry weight) in fungal samples (caps) from Příbram (smelter-polluted site), Mokrsko (rural area) and Hostim (semi-natural forest).

Příbram *L. leucothites* Pb (mg kg^{-1})	Mokrsko *A. campestris* Pb (mg kg^{-1})	Hostim *A. xanthodermus* Pb (mg kg^{-1})
82.1	0.20	0.77
85.7	0.36	1.74
23.3	0.24	1.18
130	0.34	1.62
67.8	0.23	1.84
29.5	0.26	1.50
28.2	0.61	1.05
13.1	0.27	1.16
–	0.70	1.37
–	0.34	1.37

Table 2
Lead concentrations (mg kg^{-1}, dry weight) and additional data on samples of *Agaricus bernardii* from Prague.

Sample	cap Pb (mg kg^{-1})	stipe Pb (mg kg^{-1})	fruit body Pb (mg kg^{-1})	cap g (d.w.)	stipe g (d.w.)	fruit body g (d.w.)	fruit body mg Pb	cap $^{206}Pb/^{207}Pb$	stipe $^{206}Pb/^{207}Pb$
APB 01	2.00	0.74	1.71	10.88	3.17	14.05	24.1	1.1679	1.1484
APB 02	1.39	0.80	1.23	4.34	1.64	5.98	7.34	1.1649	1.1492
APB 03	1.13	0.76	1.04	3.81	1.10	4.91	5.13	1.1623	1.1467
APB 04	0.89	0.74	0.86	3.06	0.95	4.01	3.44	1.1440	1.1564
APB 05	0.63	0.55	0.61	2.99	0.73	3.72	2.28	1.1548	1.1641
APB 06	6.81	4.96	6.47	4.73	1.08	5.81	37.6	1.1245	1.1305
APB 07	0.89	0.85	0.88	2.84	1.13	3.97	3.49	1.1468	1.1549
APB 08	1.23	1.00	1.15	3.66	1.80	5.46	6.28	1.1650	1.1635
APB 09	1.37	0.93	1.27	2.64	0.76	3.40	4.32	1.1752	1.1654
APB 10	1.30	1.10	1.25	3.72	1.41	5.13	6.40	1.1506	1.1604
APB 11	1.02	1.11	1.03	3.34	0.81	4.15	4.29	1.1704	1.1609
APB 12	4.90	2.80	4.39	3.32	1.05	4.37	19.2	1.1313	1.1387
APB 13	1.92	3.21	2.01	1.93	0.14	2.07	4.16	1.1635	1.1683
APB 14	1.25	0.78	1.11	4.95	2.15	7.10	7.85	1.1669	1.1557
APB 17	2.25	1.60	2.07	3.01	1.18	4.19	8.65	1.1731	1.1515
ABP 20	1.70	1.27	1.62	2.05	0.50	2.55	4.12	1.1557	1.1649
ABP 21	5.01	3.03	4.65	2.65	0.59	3.24	15.1	1.1422	1.1374
ABP 22	3.49	2.25	3.22	4.11	1.13	5.24	16.9	1.1314	1.1366
ABP 23	6.24	4.40	6.01	7.56	1.04	8.60	51.7	1.1241	1.1318

d.w.: dry weight.

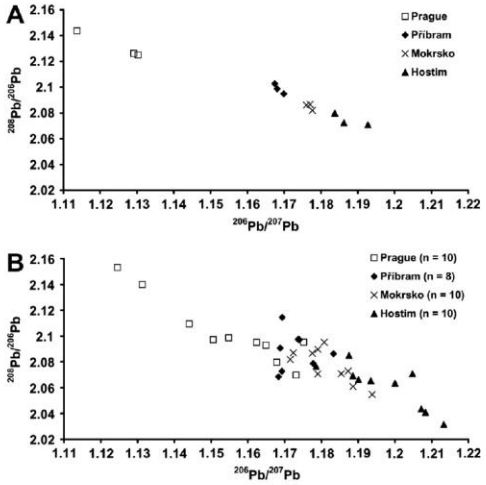

Fig. 1. (A) Lead isotopic composition of EDTA soil extracts at the investigated sites. (B) Lead isotopic composition of macrofungal samples (caps only) from the investigated sites.

and *Sorbus* leaves (Reimann et al., 2008a). In contrast to this, fungal fruit bodies are short-lived and their possible contamination by airborne particulate matter typical for industrial environments ($^{206}Pb/^{207}Pb$ = 1.17–1.19; Mihaljevič et al., 2006b) is therefore negligible. Because the isotopic composition of Pb is not affected to a measurable extent by physical or chemical fractionation processes (Bollhöfer and Rosman, 2001), the isotopic fingerprints of fruit bodies reflect just soil-derived Pb transported by mycelium. This might therefore be a tool for tracing Pb origin in underlying soils.

Since *A. bernardii* apparently does not actively accumulate Pb, its passive uptake by mycelium could be presumed from the most mobile "exchangeable/acid-extractable fraction" (0.11 M acetic acid; Rauret et al., 1999), as suggested by Komárek et al. (2007) who, however, investigated fungi from soils with total Pb concentrations up to 40,000 mg kg^{-1}. But in sites with lower Pb concentrations, the active chemical action of fungal hyphae via enzymes (Baldrian, 2008) and organic acids (Gadd, 2007) possibly leads to solubilisation of specifically bound Pb fractions. When studying the availability of the ^{210}Pb isotope, dilute-acid (1 M HCl) soluble fraction was considered as available by Guillén et al. (2009); dilute-acid fraction (0.5 M HNO_3) practically corresponds to the 0.05 M EDTA extracts (Komárek et al., 2006).

In order to find out which isotopic fraction of Pb is exactly taken up by mycelia of *A. bernardii*, we have tried to grow its isolate *in vitro* on media highly enriched with gamma-sterilized (40 kGy) soil samples (fraction <0.25 mm) from Prague with distinct $^{206}Pb/^{207}Pb$ ratios in extractable fractions. Unfortunately, *A. bernardii* produces very fine and sparse mycelia that could not have been perfectly separated from the media. Since any contamination would seriously skew the genuine isotopic ratios, the experiment failed. But despite the lack of the data on experimentally observed Pb uptake by mycelium, we may compare both possibilities, considering the exchangeable and dilute-acid fractions as "end members" of what can be taken up by the fungus. Vertical distributions of Pb isotopic ratios in 0.11 M acetid acid and 1 M nitric acid soil extracts are shown in Fig. 2A; extractable amounts of Pb are indicated in Fig. 2B.

The $^{206}Pb/^{207}Pb$ ratio increases with depth and acetid acid soluble fractions are characterized by generally lower values when compared to nitric acid extracts (compare with Teutsch et al., 2001). However, the isotopic shift is negligible in topsoil and, on the average, the difference between both extractants resulted in the $^{206}Pb/^{207}Pb$ ratio of only about 0.008 higher for nitric acid extracts (calculated from the depth of 5–30 cm). The gasoline-derived Pb ($^{206}Pb/^{207}Pb$~1.11–1.13) is dominant in the surface horizon only (0–4 cm depth) and a distinct increase of the $^{206}Pb/^{207}Pb$ ratio values is seen below.

Acetic acid extractable Pb decreases more or less continuously from 2 mg kg^{-1} in the surface layer to 0.5 mg kg^{-1} in the deepest layer; an anomaly (6.7 mg kg^{-1}) has been detected at 19 cm depth. In contrast to this, lead extracted by nitric acid reached 122 mg kg^{-1} in the topsoil horizon and decreased with depth to 29.6 mg kg^{-1}; the concentration anomaly detected in the acetic acid extract in 19 cm depth is followed (120 mg kg^{-1}).

Lead isotopic fingerprints of fruit bodies reflect mixing of all "accumulated" Pb. It can be concluded that the prevailing origin of Pb in 5 fruit bodies (APB 6, 12, 21–23) with low $^{206}Pb/^{207}Pb$ ratios is unambiguously in the topsoil layers (0–5 cm). In these layers, the highest mobility of Pb was observed. Accordingly, the

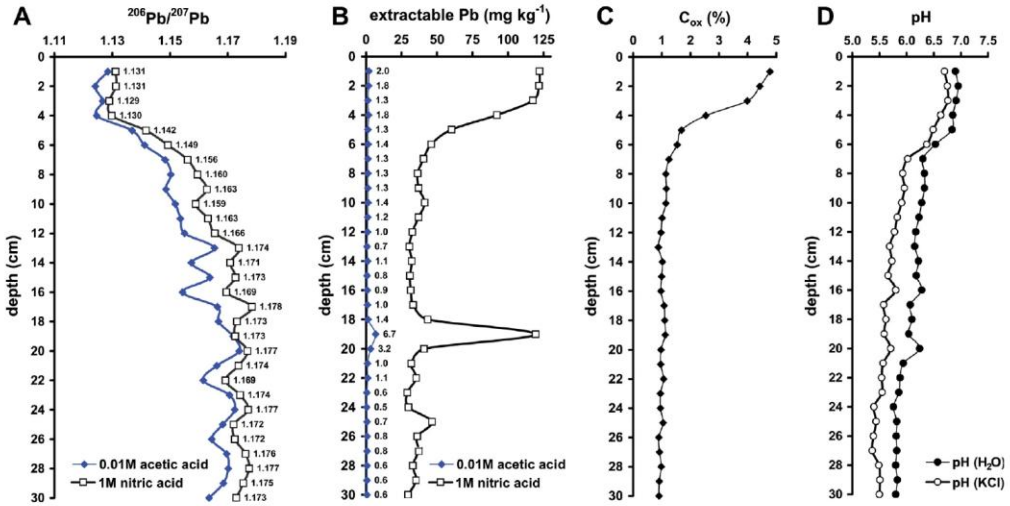

Fig. 2. (A) Lead isotopic ratios in 0.01 M acetic acid and 1 M nitric acid extracts from individual layers of the Au horizon in the soil profile in Prague (0–30 cm depth). (B) Acetic acid and nitric acid extractable Pb (mg kg^{-1}) in the soil profile. (C) Distribution of organic carbon (C_{ox}, in mass%) in the soil profile. (D) Distribution of pH values (measured in distilled water and 1 M KCl) in the soil profile.

highest concentrations of Pb were determined in *A. bernardii* specimens with the lowest $^{206}Pb/^{207}Pb$ ratios (Fig. 3, Table 2).

In most samples, however, the $^{206}Pb/^{207}Pb$ ratios were higher, indicating deeper Pb origin. The highest value found (1.175) would indicate a minimum depth of circa 13–17 cm or 19–20 cm, with respect to the ratios corresponding to nitric acid or acetic acid extractable fractions, respectively. If proportions and mixing of contrasting Pb sources (with respect to $^{206}Pb/^{207}Pb$ ratios and Pb mobility) from the whole soil profile are considered, the role of even deeper horizons should be taken into account. It is however, impossible to determine how the Pb uptake, transport and mixing took place in individual fruit bodies.

It is generally believed that saprotrophic fungi colonize organic rich topsoil horizons but there is little data available on vertical distribution of their mycelia in soils (Robinson et al., 2009). As saprotrophic fungi obtain nutrients from dead organic matter, they are restricted to the upper horizons in forest soils (Luis et al., 2005) and in contrast, ectomycorrhizal species are dominant in bulk soil and stone compartments investigated in various forest soils (Christ et al., 2011). However, in the "classical" study on fairy rings, Shantz and Piemeisel (1917) reported the case of the soil under the stimulated zone of a grassland fairy ring (agricultural soil), which is permeated with mycelial filaments of an *Agaricus* species from a depth of about 8 cm to a depth of over 30 cm.

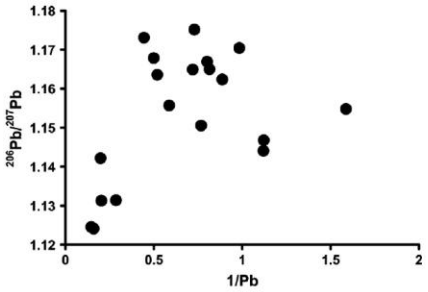

Fig. 3. Lead isotopic composition vs. Pb concentration in 19 caps of *Agaricus bernardii* from Prague.

In the investigated Au horizon in Prague (for C_{ox} contents and pH values see Fig. 2C and D), dense whitish mycelial filaments were also noted in the soil profile under the fruit bodies even at the depth of 30 cm. A specific briny smell, typical for *A. bernardii* (Parra, 2008), was observable in the soil samples, indicating the mycelium belonged to this species. Molecular methods currently enable direct detection of particular organism in environmental samples by use of specific primers (Gryndler et al., 2011, 2010; Kerrigan, 2007). *A. bernardii*-specific PCR has confirmed the occurrence of this species throughout the profile (Fig. 4); the identity of three amplicons was confirmed by DNA sequencing. Despite the mycelial biomass of *A. bernardii* being most abundant at the depth of approx. 4–6 cm, a significant increase was observed on the bottom (Fig. 4) and *A. bernardii* would have certainly been detected even below. The relatively rich occurrence of *A. bernardii* in the whole investigated soil profile confirms that Pb transport from the rather deep soil layers (as indicated by the $^{206}Pb/^{207}Pb$ ratios) is possible with regard to soil colonization by the fungus.

A similar phenomenon can be observed in *A. campestris* from Mokrsko and *A. xanthodermus* from Hostim, where the $^{206}Pb/^{207}Pb$ ratios in only a few samples reflected the topsoil horizon (compare Fig. 1A and B) and other values were higher, in *A. xanthodermus* even corresponding to the natural, bedrock-derived Pb.

Especially in soils with low Pb contents and contrasting vertical distribution of the $^{206}Pb/^{207}Pb$ ratios in individual horizons, lead isotopic fingerprints of both saprotrophic and ectomycorrhizal fungi (fruit bodies, possibly also ectomycorrhizae) might indicate the origin of the "accumulated" Pb: the $^{206}Pb/^{207}Pb$ isotopic ratio, typically increasing from organic horizons to parent rock, might point to contact and activity of fungal mycelia in mineral soil. Combination of this approach with the ^{210}Pb isotope might be particularly interesting, since Kirchner and Daillant (1998) and Guillén et al. (2009) proved that ^{210}Pb is taken up from soil as a Pb isotope

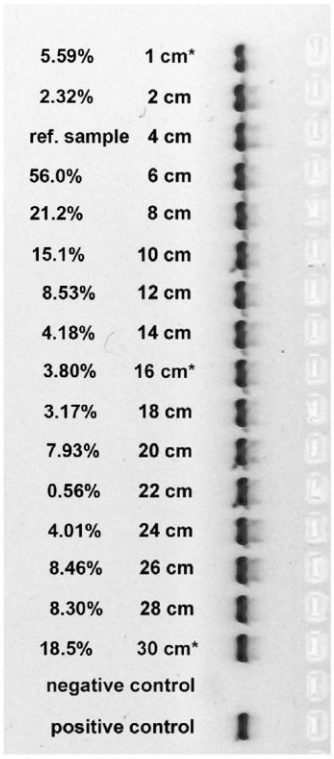

Fig. 4. DNA gel electrophoresis indicating positive detection of *Agaricus bernardii* in selected soil layers by PCR with specific primer. Identity of three PCR-amplicons (indicated by asterisks) was confirmed by DNA sequencing. In the left, relative abundance (%) of mycelium of *A. bernardii* (measured by RT-PCR as concentrations of rRNA gene copies) in the soil profile is indicated. The maximum concentration of the rRNA gene copies (3.9×10^7 per g soil), corresponding to the highest amount of *A. bernardii* soil biomass, was detected in the sample indicated as reference (ref. sample); at 4 cm depth. The percentage values for individual samples in the profile are related to this maximum.

and not its parent isotope ^{226}Ra. Unfortunately, the simultaneous use of the ^{137}Cs/^{134}Cs ratio as demonstrated by Rühm et al. (1997) is not possible as the ^{134}Cs isotope ($t_{1/2}$ = 2.0652 y) from the Chernobyl fallout is practically decayed.

4. Conclusions

Lead isotopic composition of the investigated saprotrophic macrofungi from smelter-polluted, urban, agricultural, and pristine areas in the Czech Republic varies in a rather wide range and, with the exception of the heavily smelter-polluted site in Příbram, does not reflect that in the organomineral topsoil horizons. Therefore, use of saprotrophic macrofungi in studies monitoring artificial Pb pollution of soils seems not to be possible.

Mycelium of *A. bernardii* was not restricted to topsoil layers at the locality in Prague but could be detected both visually and using specific PCR even at a depth of 30 cm. The ^{206}Pb/^{207}Pb isotopic ratio measured in the fruit bodies of this species sampled in one place varied in a wide range (1.124–1.175). In 5 specimens, the majority of "accumulated" Pb was undoubtedly transported from the topsoil layers (0–5 cm) characterized by low ^{206}Pb/^{207}Pb isotopic ratios, corresponding with gasoline-derived Pb from traffic emissions. In most samples, however, lead must have been transported from below. Despite the fact that the ^{206}Pb/^{207}Pb isotopic ratio does not indicate direct origin of Pb in the soil profile, it can by hypothesized that Pb could have been transported from the depth of about 13–17 cm at least, probably deeper. The relatively high ^{206}Pb/^{207}Pb isotopic ratios found in fruit bodies of *A. campestris* and *A. xanthodermus* suggest that also these two species likely accumulate Pb from low in the soil profile.

The uptake, transport and accumulation of elements in fungal mycelia, ectomycorrhizae and fruit bodies are poorly understood phenomena of considerable environmental significance. At suitable sites, lead isotopes might represent a useful tool for tracing the fungal uptake of Pb (and indirectly also other metals) which might contribute to better understanding of the role of macrofungi in biogeochemical cycling of elements in soils.

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On the possible role of macrofungi in the biogeochemical fate of uranium in polluted forest soils

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HIGHLIGHTS

- Uranium is not accumulated in ectomycorrhizal and saprotrophic macrofungi.
- Uranium is not accumulated in ectomycorrhizal roots of *Picea abies*.
- Accumulation of metals in macrofungi does not depend on their fractionation in soils.

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ABSTRACT

Interactions of macrofungi with U, Th, Pb and Ag were investigated in the former ore mining district of Příbram, Czech Republic. Samples of saprotrophic (34 samples, 24 species) and ectomycorrhizal (38 samples, 26 species) macrofungi were collected from a U-polluted Norway spruce plantation and tailings and analyzed for metal content. In contrast to Ag, which was highly accumulated in fruit-bodies, concentrations of U generally did not exceed 3 mg/kg which indicates a very low uptake rate and efficient exclusion of U from macrofungi. In ectomycorrhizal tips (mostly determined to species level by DNA sequencing), U contents were practically identical with those of the non-mycorrhizal fine spruce roots. These findings suggest a very limited role of macrofungi in uptake and biotransformation of U in polluted forest soils. Furthermore, accumulation of U, Th, Pb and Ag in macrofungal fruit-bodies apparently does not depend on total content and chemical fractionation of these metals in soils (tested by the BCR sequential extraction in this study).

1. Introduction

Uranium (U) is a radioactive toxic heavy metal [1] with typical background concentrations in rocks and soils of only a few mg/kg [2]. Environmental contamination by U may be caused by various processes including mining and processing operations, coal combustion, testing and use of weapons with depleted uranium (DU). Artificially elevated concentrations of U in topsoils usually reach tens or a few hundred mg/kg [3], but natural accumulation in soils can on rare occasions reach 4000 mg/kg [4]. Under the conditions found in the natural environment, the two important oxidation states are U(IV) and U(VI); the chemistry of U(VI) is dominant in most soils [3]. Furthermore, organic compounds influence the mobility and chemistry of U in the environment [5,6].

Geochemical behavior of many heavy metals in the environment is, at least to some extent, affected by microbial [7,8] and fungal activity [9–11] and U is no exception. *In vitro* experiments have shown that both free living (saprotrophic) and symbiotic (mycorrhizal) fungi exhibit a high U oxide tolerance and possess the ability to solubilize UO_3 and U_3O_8 and accumulate U within the mycelium to over 80 mg/g (dry weight); some fungi even cause the biomineralization of well-crystallized uranyl phosphate

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minerals [12]. Furthermore, fungi are capable of dissolving DU and transforming the released mobile U complexes into minerals of the meta-autunite group [13].

Apparently, the fungal activities, summarized by Gadd and Fomina [14], may have relevance to contaminated terrestrial habitats. The dependence of almost all land plants on symbiotic fungi, and the fact that symbiotic fungi are capable of U transformations, may make fungal activity of importance in the biogeochemical fate of U in polluted environments and possibly also in phytoremediation or phytostabilization strategies. Arbuscular mycorrhizal (AM) fungi have been demonstrated to enhance U concentrations in roots of host plants and reduce root-to-shoot translocation of U [15]. However, most of the available knowledge on interactions between fungi and U is based on *in vitro* experiments.

Both saprotrophic (SAP) and symbiotic macrofungi are known as effective accumulators or even hyperaccumulators of toxic heavy metals, noble metals, metalloids and radionuclides [16,17]. Despite the astonishing ability of macrofungi to accumulate some metals, concentrations of U in fruit-bodies from pristine environments are very low, with bioaccumulation factors well below 1 [18]; however, there is a lack of data on interactions between macrofungi and U in polluted environments.

Similarly to AM fungi, ectomycorrhizal (EM) fungi may contribute to the retention of U in soils via its accumulation in ectomycorrhizae and extraradical mycelia. Furthermore, accumulation of U in mycelia and fruit-bodies of fungal saprotrophs may contribute to retention and redistribution of U in organic soil horizons. We have therefore conducted a field study where fungal fruit-bodies and ectomycorrhizae (EM tips) were used as indicators to assess the possible role of macrofungi in biogeochemical cycling of U in polluted forest soils. To compare the accumulation ability, Th and Pb were analyzed as elements known to be excluded in macrofungi [18,19] and Ag as an effectively accumulated element [20]. Furthermore, the accumulation ability of macrofungi was investigated in relation to the chemical fractionation (mobility, bioavailability) of the investigated metals in soil.

2. Experimental

2.1. Selected sites

The Bohemian Massif in the Czech Republic is well-known for various U deposits. For the purpose of this study, we chose the former U mining district of Příbram in Central Bohemia (Fig. 1a) which is a region with perigranitic monometallic veins [21]. In this area, biogeochemical monitoring has revealed a very distinct localized anomaly of U concentrations in mosses and humus, apparently caused by long-term mining and processing operations [22]. We selected a spruce forest plantation (mixed with birch, pine, poplar and lime trees on its margins) on granitic bedrock near the village of Bytíz, near the abandoned shaft 11a (closed in 1991) and tailings. Macrofungi, EM tips, fine spruce roots and soils were sampled throughout the selected part of the forest (Fig. 1b). In addition, some macrofungi and soils were collected directly at the tailings (Fig. 1c). For the purpose of comparison, EM tips and fine spruce roots were also collected in forest plantations at two sites with background U concentrations in soils in the Czech Republic: Kladská village (West Bohemia) and Chmelná village near Nová Cerekev (the Highland) above granitic and gneissic bedrocks, respectively (Fig. 1a).

2.2. Macrofungi, EM tips and fine roots

Samples of macrofungi were collected, identified and processed as described elsewhere [18]. EM tips and non-mycorrhizal fine roots of Norway spruce (*Picea abies*) were sampled from the Oe horizon, transported to the laboratory, stored in a fridge at 4 °C and processed within 24 h. Briefly, the roots were washed in a stream of tap water. Then, under a stereo-microscope EM tips and fine roots were separated and cleaned thoroughly using tweezers and dissecting needles in distilled water and dried on filter paper. In order to identify the fungal species forming the EM tips we carried out DNA sequencing. DNA was extracted from a miniscule piece of frozen EM tip using the NucleoSpin Plant II DNA isolation kit (Macherey-Nagel, Germany). To amplify fungal DNA, we performed Polymerase Chain Reaction (PCR) with the primer pair ITS1F-ITS4 as described elsewhere [23]. In the case of negative results (low PCR product yield, sequencing failure), the more specific primer pair ITS1F-ITS4B [24] or semi-nested PCR were used [25]. The obtained fragments were purified by isopropanol precipitation and sequenced (Macrogen Inc., Korea). All sequences were identified to species/genus/family/order level by querying the GenBank database, using the nucleotide–nucleotide (blastn) BLAST search option, available through the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov) and the UNITE online database ([26]; UDB accession numbers). Quality sequences were accessioned into the EMBL-Bank database.

2.3. Soils

The selection of a representative soil profile pit was on the basis of a thorough preliminary soil survey involving the examination of 16 profiles in the area of circa 9000 m^2 in the spruce forest plantation where fungal samples were collected (N49 40.708, E14 04.246). Morphological description and horizon designation followed the scheme of Jahn et al. [27]. Individual soil profiles were classified according to the World Reference Base for Soil Resources [28]. Soil samples from the soil profile (individual soil horizons) were analyzed at the Research Institute for Soil and Water Conservation (Czech Republic, Prague).

Analytical procedures for soil characterization followed standard methods. The pH values were measured in distilled water and 1 M KCl with a SenTix21 electrode using the soil:solution ratio of 1:2.5 for all horizons. The following analyses were done for particular horizons: (i) particle size distribution using the pipette method; (ii) cation exchange capacity and base saturation using the method of Mehlich; (iii) organic carbon (C_{ox}) by wet combustion with a mixture of potassium dichromate and sulphuric acid; (iv) total nitrogen (N_t) using the Kjeldahl method [29].

In order to assess the extent of metal pollution, 23 samples of Oa horizon were collected throughout the forest plantation and 6 rather heterogeneous organomineral topsoils were also sampled at the tailings in places where macrofungi were collected (Fig. 1b and c). Soils were air-dried and sieved through a 2 mm stainless steel mesh. A representative part of each sample was milled in an agate mill and used for chemical analyses.

2.4. Chemical analyses

Concentrations of U, Th, Pb and Ag in macrofungi were determined using the *Element2* (Thermo Scientific, Germany) sector field mass spectrometer with inductively coupled plasma (ICP-SF-MS) as described previously [18]. Concentrations of U in EM tips and fine roots from the polluted site were determined by instrumental neutron activation analysis (INAA) using the radioisotope ^{239}U (74.7 keV, $t_{1/2}$ = 23.45 min) according to Řanda et al. [30]. In EM tips and fine roots from pristine sites with lower U concentrations, a more sensitive variant with epithermal neutrons and the radioisotope ^{239}Np (106.13 keV, $t_{1/2}$ = 2.36 d) was used.

Total concentrations of U, Th and Ag in soils (sample weight ~250 mg) were determined non-destructively by INAA using the radioisotopes ^{239}Np for determination of U, ^{233}Pa (311.9 keV,

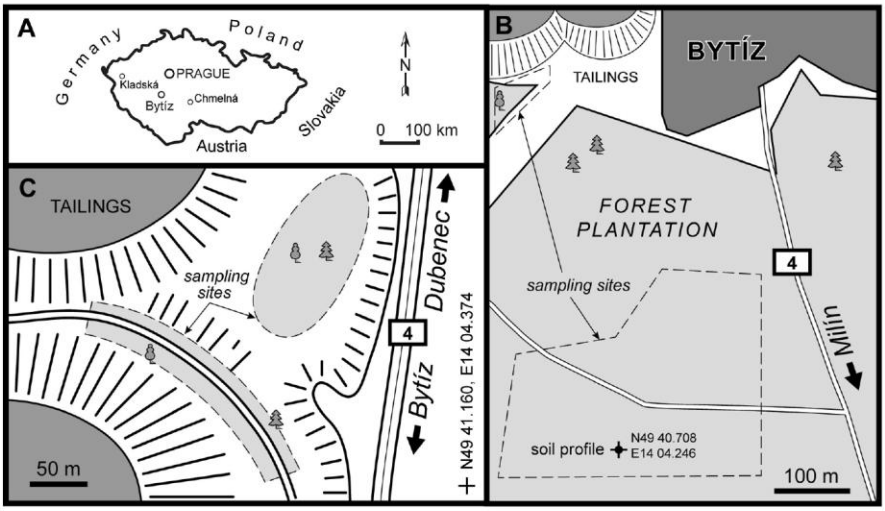

Fig. 1. (A) Sampling sites in the Czech Republic. (B) Sampling area in forest plantation at Bytíz (Příbram district). (C) Sampling sites within the tailings.

$t_{1/2}$ = 27.4 d) for determination of Th and ^{110m}Ag (657.75 keV, $t_{1/2}$ = 249.88 d) for determination of Ag. Lead concentrations in soils, which cannot be determined by INAA, were analyzed by instrumental photon activation analysis (IPAA) using the radioisotope ^{203}Pb (279.19 keV, $t_{1/2}$ = 51.873 h) according to Řanda et al. [31]. The quality of analytical procedures was repeatedly verified using certified reference materials processed similarly to our biological and soil samples: NIST 1566b (Oyster Tissue), BCR 670 (Duck Weed), NIST 2711 (Montana Soil) and BCR 667 (Estuarine Sediment).

In order to determine fractionation of the investigated metals in individual soil horizons, BCR sequential extraction (fraction < 2 mm, unmilled) was carried out according to Rauret et al. [32] in 3 analytical replicates. Concentrations of metals in soil extracts were analyzed by ICP-SF-MS (samples from steps 2 and 3 after dilution). The results are presented as mean ± standard deviation in the (I) exchangeable, (II) reducible, (III) oxidizable, and (IV) residual fractions. The BCR fractions, however, are more correctly operationally defined as the metal extracted by (I) 0.11 M acetic acid, (II) 0.5 M hydroxylammonium chloride, (III) hydrogen peroxide + 1.0 M ammonium acetate, and (IV) sum of fractions I–III subtracted from the total metal content. The quality of the BCR sequential extraction procedure was verified by the use of the certified reference material BCR 483 (Sewage Sludge Amended Soil) in 3 analytical replicates. The Pb values obtained for the steps I–III (0.32 ± 0.02 mg/kg, 360 ± 20.5 mg/kg, and 57.5 ± 5.12 mg/kg, respectively) fell within the indicative concentration ranges: (I): 0.756 ± 0.7 mg/kg, (II): 379 ± 21 mg/kg, (III): 66.5 ± 22 mg/kg [33].

3. Results and discussion

3.1. Soil

The investigated forest plantation in Bytíz is covered by Cambic Leptosols. The studied soil profile (Fig. 2) is developed on granite and is relatively shallow (60 cm deep). As documented in Table 1, the soil is extremely acidic, with pH values decreasing up the soil profile. Cation exchange capacity is higher in the upper part of the soil profile and decreases with depth. The values of base saturation

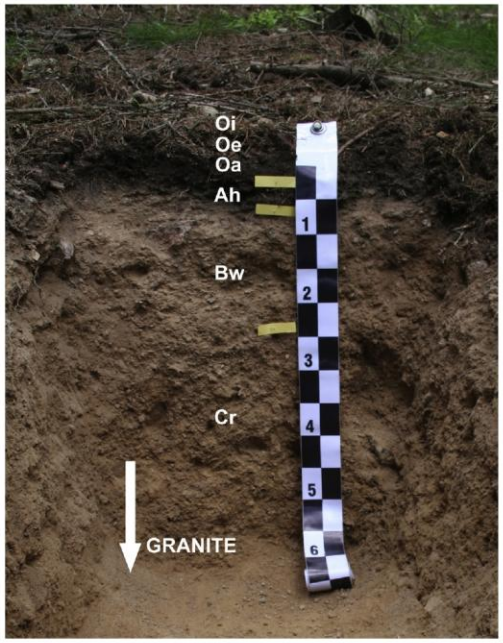

Fig. 2. Soil profile of Cambic Leptosol at Bytíz.

correspond to the character of pedogenesis. The contents of C_{ox} and N_t are the highest in the upper part of the profile and the C/N ratio values showed some enrichment of soil organic matter by N_t in the Oi, Oe, Oa, and Ah horizons. As expected on the granite bedrock, the analysis of the particle size showed the predominance of sand fraction.

Table 1
Chemical properties, soil organic matter and particle size distribution of the soil profile in forest plantation at Bytíz.

Depth (cm)	Horizon	pH_{H_2O}	pH_{KCl}	BS (%)	CEC (mmol$^+$/100 g)	C_{ox} (%)	N_t (%)	C/N	Clay (%)	Silt (%)	Sand (%)	Texture class
4-3	**Oi**	4.80	4.14	8	64.4	40.2	1.04	38.6	25.3	36.8	37.9	Loam silt
3-2	**Oe**	4.89	4.27	34	88.6	32.1	1.52	21.1	21.8	61.8	16.4	Loam
2-0	**Oa**	4.44	3.71	62	80.7	20.3	1.43	14.2	18.2	65.4	16.4	Silt loam
0-5	**Ah**	3.67	2.86	46	28.7	4.62	0.31	14.9	13.1	36.2	50.7	Loam
5-22	**Bw**	3.54	2.98	34	8.21	0.38	0.05	7.60	10.1	37.6	52.3	Sandy loam
22-60	**Cr**	3.91	2.89	15	7.58	0.12	0.05	2.40	6.50	35.5	58.0	Sandy loam

BS: base saturation. CEC: cation exchange capacity. C_{ox}: organic carbon. N_t: total nitrogen.

Table 2
Statistical summary of the total metal concentrations (related to dry matter) in soil samples from forest plantation (Oa horizon, 23 samples) and tailings (organomineral topsoil, 6 samples) at Bytíz. See also Supplementary Table s1.

Forest	Concentration (mg/kg)			
	Minimum	Median	Mean	Maximum
U	6.08	13.7	19.5	74.5
Th	2.52	5.57	5.90	9.87
Pb	66.8	615	565	1251
Ag	0.11	0.77	1.30	12.3
Tailings	Concentration (mg/kg)			
	Minimum	Median	Mean	Maximum
U	11.5	20.4	21.2	34.8
Th	6.21	9.54	10.2	15.2
Pb	135	880	957	2114
Ag	0.21	0.29	0.56	1.37

Statistical uncertainty of INAA/IPAA determinations of U, Th, Pb and Ag was mostly below 1%, 1%, 3% and 7%, respectively.

3.2. Distribution and mobility of metals in soils

The investigated forest plantation can be considered U-polluted, with concentrations of U in the range of 6–75 mg/kg in the Oa horizon; relatively high U levels (11–35 mg/kg) were also determined in the substrates from the tailings (Table 2, Supplementary Table S1). The U pollution, however, did not influence the mineral horizons Bw and Cr, where U concentrations correspond with natural levels [2,3]. Furthermore, the area is polluted by Pb, which is the result of long-term smelting operations in the vicinity [34]. Silver is another pollutant derived from these operations [35]. Its concentrations in O horizons are elevated, but lower than those reported from the close vicinity of the smelter [20]; the median of 0.77 mg/kg in the Oa horizon is quite close to values that would be expected in the background [36]. Concentrations of Th are similar to those in unpolluted environments [2].

In the soil profile (Table 3), the highest concentrations of U, Pb and Ag were encountered in the O horizons (Oa > Oe > Oi) and decreased rapidly with depth (Ah > Bw > Cr). This enrichment in O horizons can be explained by sorption of metals to organic matter (O and Ah horizons), resulting in a slow flux of these pollutants to the underlying Bw and Cr horizons. The only element with the highest concentrations in the mineral horizons Bw, Cr and parent rock is Th but this should represent natural distribution of this metal in unpolluted Cambic Leptosols developed on granites. Similarly, the highest concentrations of metals in organic layers were reported from polluted areas by other authors [35,37].

Table 3
Total metal concentrations (related to dry matter) in the soil profile (Cambic Leptosol) in forest plantation at Bytíz.

Horizon	Concentration (mg/kg)			
	U	Th	Pb	Ag
Oi	0.43	0.18	28.5	0.17
Oe	14.2	3.11	382	0.45
Oa	23.2	5.78	789	0.99
Ah	7.16	12.2	388	0.52
Bw	4.09	15.3	38.4	0.06
Cr	5.41	21.8	42.5	0.07
Granite	6.59	21.4	<25.0	<0.09

Statistical uncertainty of INAA/IPAA determinations of U, Th, Pb and Ag was mostly below 1%, 1%, 5% and 7%, respectively.

Cambic Leptosols are oxidomorphic soils. The low pH values in the soil profile would suggest that U is present as U(VI) with UO^{2+} as a major ion [3]. However, in organic-rich horizons, interactions of U with organic matter are expected and organic compounds (e.g., the humic acid) greatly influence the mobility and chemistry of U [5,6]. Little is known about the geochemical behavior and fractionation of U in polluted soils. Several authors have investigated the biogeochemical fate of DU in polluted environments [6,38–40] but none of these authors focused on forest soils. Results from sequential extraction for U in forest soils were published by Virtanen et al. [41] but these authors studied various size fractions of four different mineral soils from an unpolluted boreal area. Despite the investigated soil profiles being hardly comparable with regard to soil classification, the BCR fractionation of U in our soil profile (Table 4) is very similar to that observed by Oliver et al. [42]. In Oe, Oa and Ah horizons, most of the U was associated with the oxidizable fraction and U was highly immobile in the Bw and Cr horizons, with nearly 90% in the residuum.

Some minor fractions of exchangeable Th were noted in the Oe and Oa horizons, but the majority of Th (~90%) throughout the soil profile was generally present as tightly bound in the residuum, which is in good agreement with Virtanen et al. [41]. Pb was the most mobile element, especially in O and Ah horizons. It was primarily associated with the reducible fraction, followed by the oxidizable, residual and exchangeable fractions, respectively. Similar results have been published from various polluted environments in the Czech Republic, but in some studies somewhat higher percentages for the oxidizable fraction were reported [35,43,44]. Most Ag was associated with the reducible fraction, followed by somewhat lower percentages for oxidizable and residual fractions, respectively. The exchangeable Ag content was found to be negligible. Very few data have been published on Ag fractionation

Table 4
BCR fractionation of soil U, Th, Pb and Ag in individual soil horizons from the soil profile at Bytíz.

(I) Exchangeable fraction[a]									(II) Reducible fraction[a]								
	U		Th		Pb		Ag			U		Th		Pb		Ag	
[b]	μg/kg	%	μg/kg	%	μg/kg	%	μg/kg	%	[b]	μg/kg	%	μg/kg	%	mg/kg	%	μg/kg	%
Oe	260 ± 8	2	125 ± 4	4	1078 ± 23	<1	3.47 ± 0.3	<1	**Oe**	939 ± 19	7	125 ± 5	4	292 ± 6	76	225 ± 32	50
Oa	211 ± 5	1	70.1 ± 6	1	1654 ± 60	<1	2.75 ± 0.5	<1	**Oa**	2222 ± 11	10	115 ± 5	2	610 ± 115	77	365 ± 6	37
Ah	149 ± 1	2	39.8 ± 1	<1	3409 ± 157	<1	1.59 ± 0.2	<1	**Ah**	1027 ± 23	14	56.7 ± 2	<1	267 ± 27	69	173 ± 17	34
Bw	55.0 ± 2	1	13.2 ± 0.2	<1	302 ± 41	<1	0.37 ± 0.1	<1	**Bw**	261 ± 19	6	55.6 ± 13	<1	13.0 ± 2	34	29.2 ± 4	42
Cr	48.0 ± 3	1	6.15 ± 0.4	<1	233 ± 4	<1	0.37 ± 0.1	<1	**Cr**	283 ± 10	5	60.4 ± 9	<1	10.8 ± 2	25	34.4 ± 4	57

(III) Oxidizable fraction[a]									(IV) Residual fraction[a]								
	U		Th		Pb		Ag			U		Th		Pb		Ag	
[b]	μg/kg	%	μg/kg	%	mg/kg	%	μg/kg	%	[b]	μg/kg	%	μg/kg	%	mg/kg	%	μg/kg	%
Oe	8737 ± 172	62	195 ± 23	6	72.0 ± 1	19	171 ± 24	38	**Oe**	4264	30	2666	86	16.9	4	51.5	11
Oa	14907 ± 954	64	169 ± 144	3	99.2 ± 5	13	240 ± 42	24	**Oa**	5860	25	5426	94	78.1	10	383	39
Ah	1848 ± 92	26	539 ± 7	4	16.7 ± 2	4	84.0 ± 6	16	**Ah**	4136	58	11565	95	101	26	257	50
Bw	194 ± 15	5	974 ± 241	6	1.07 ± 0.1	3	2.40 ± 2	3	**Bw**	3580	88	14257	93	24.0	63	28.0	54
Cr	342 ± 7	6	1578 ± 183	7	1.04 ± 0.02	2	<1.70	1	**Cr**	4737	88	20155	92	30.4	72	35.2	40

[a] "%" is the percentage of total recovered metal in that fraction.
[b] Soil horizon.

and behavior in soils [45–47] and our results possibly represent the first data reported for the BCR sequential extraction scheme. When considering the total concentrations and mobility of the investigated metals (Tables 1–3), Pb should be considered the most bioavailable element followed by U, Th and Ag, respectively.

At the sites near Kladská and Chmelná, where EM tips were collected, the U concentrations in soils correspond to background levels [2,3] and both sites can therefore be considered unpolluted (Supplementary Tables S2 and S3).

3.3. Macrofungi

According to previously published data [18], concentrations of U in macrofungi from unpolluted environments are generally below 30 μg/kg; Johanson et al. [48] published similar results with an extreme value of 216 μg/kg found in *Cortinarius odorifer*. In the polluted area of Bytíz, U concentrations were distinctly elevated (Tables 5 and 6) but did not exceed 3 mg/kg, with the highest levels in SAP fungi (*Helvella lacunosa*: 2.61 mg/kg). In EM fungi, the highest contents of U were found in *Inocybe dulcamara* (1.00 and 1.98 mg/kg). This is particularly interesting since the highest content of U we have ever detected in a fungus was also in *I. dulcamara* (14.2 mg/kg) collected at tailings in Rožná, another U mine in the Czech Republic (unpublished).

In both SAP and EM macrofungi, the highest concentrations were detected in samples collected directly at the tailings where the fungi grow in association with solitary trees or utilize dead organic matter near these trees [49–51]. Only a very few results on U in macrofungi from U mine tailings were published by Wichterey and Sawallisch [52], who reported a maximum activity of 25.9 Bq/kg (wet weight) for the radioisotope ^{238}U in specimens growing on soils with median ^{238}U activity of circa 300 Bq/kg. Such activities would, however, correspond with unpolluted environments (circa 210 μg ^{238}U/kg for macrofungi and 2.42 mg ^{238}U/kg for soils, both related to dry matter). Recently, values for nine macrofungal species collected at a test site within a U-contaminated area in Germany were reported [53]. Similarly to our results, the U concentrations varied over a rather large range of 0.03–1.2 mg/kg. However, the highest value of 3.47 mg/kg U published for *Paxillus involutus* was followed by a spuriously high Fe content of 10,020 mg/kg which might be caused by an analytical problem or contamination by soil particles (cf. Borovička and Řanda [54] and Brzostowski et al. [55,56]).

It must be stressed, however, that comparisons of U concentrations in macrofungi from various sites must be taken with care. Despite the fact that Gast et al. [57] did not observe any influence of soil pH and organic matter content on accumulation of Cd, Cu, Pb and Zn in macrofungi, environmental conditions (pH, Eh, organic matter) greatly influence the chemical speciation of U which may affect its uptake by macrofungi.

With a maximum value of 78.6 μg/kg found in *Clitocybe costata*, Th concentrations in macrofungi were very low, indicating an effective exclusion of this element as also observed by previous studies [18,48,58]. Concentrations of Pb in macrofungi were much higher, with the highest content of 72.0 mg/kg found in *Lycoperdon foetidum*; among macrofungi, the genus *Lycoperdon* is known to possess the greatest ability to take up Pb [59,60]. While the ability of macrofungi to take up U, Th and Pb has been shown to be very limited, concentrations of Ag in our samples (median values of 7.32 mg/kg and 1.07 mg/kg in SAP and EM fungi, respectively) are very high, despite the fact that Ag was found to be (i) the element with the lowest total concentrations in soils, and (ii) the most immobile element.

These data and the fact that macrofungi efficiently accumulate or even hyperaccumulate Ag in ionic form [20,61,62], allow us to hypothesize, that macrofungi significantly contribute to the biogeochemical cycling of Ag in soils. This capability would also be of ecological importance as the rotting Ag-rich fruit-bodies significantly alter the spatial taxonomic and quantitative homogeneity of the soil microbiota [63].

The mechanisms and influence of soil geochemistry on metal uptake and transport by macrofungi are poorly understood. As pointed out by Komárek et al. [35], the vast majority of geomycological field studies lack data concerning the chemical fractionation of metals in soils: as different metal phases are associated with different soil fractions from which they might be taken up by macrofungi, the sole knowledge of total metal contents could be insufficient. However, according to Hooda [64], fractionation schemes seldom serve any purpose for assessing metal bioavailability (for plants), largely because plant uptake generally correlates only with that extracted in the first step of any sequential extraction procedure, which often includes their most labile fraction.

Our data would suggest that the fungal uptake of the investigated metals will largely depend upon the biological activity of the

Table 5
Metal concentrations (related to dry matter) determined in saprotrophic macrofungi collected in the vicinity of Bytíz.

Species	Sample	Concentration (μg/kg)				Species	Sample	Concentration (μg/kg)			
		U	Th	Pb	Ag			U	Th	Pb	Ag
Agaricus arvensis	JK 02	78.2	7.14	5164	28888	*Leucoagaricus leucothites*	JK 193	696	12.6	1964	19266
Agaricus arvensis	JK 07	56.4	10.2	12449	53942	*Lycoperdon foetidum*	JK 186	138	16.7	72009	7773
Agaricus leucotrichus	JK 48	26.3	5.69	13129	15510	*Lycoperdon perlatum*	JK 213	68.1	2.92	32108	8346
Agaricus silvaticus	JK 188	308	12.0	35476	27598	*Lycoperdon perlatum*	JK 217	134	4.92	36425	16372
Armillaria ostoyae	JK 221	19.9	6.17	227	7518	*Lycoperdon perlatum*	JK 218	87.9	4.65	26665	6535
Bolbitius vitellinus	JK 66	319	14.2	3155	1276	*Macrolepiota procera*	JK 179	54.5	2.98	8237	8556
Calocera viscosa	JK 180	106	17.7	946	59.6	*Macrolepiota procera*	JK 181	59.0	5.32	21266	5257
Calvatia excipuliformis	JK 03	320	4.04	7926	7239	*Macrolepiota procera*	JK 182	66.7	4.94	28584	8356
Calvatia excipuliformis	JK 214	134	5.35	45467	8090	*Macrolepiota procera*	JK 211	86.7	2.81	14377	3589
Chlorophyllum rhacodes	JK 215	66.0	5.34	14531	25804	*Mycena pura*	JK 198	25.6	5.70	195	815
*Clitocybe costata**	JK 63	438	35.0	8904	6054	*Mycena zephirus*	JK 14	76.8	25.7	3864	1554
*Clitocybe costata**	JK 219	400	78.6	3128	5567	*Mycena zephirus*	JK 208	9.11	4.55	651	318
*Clitocybe costata**	JK 238	7.13	7.88	1868	4657	*Psathyrella spadiceogrisea*	JK 69	52.3	26.7	481	1758
Clitocybe fragans	JK 26	87.4	38.5	3186	142	*Rhodocollybia butyracea*	JK 220	93.3	6.33	3185	7495
Clitocybe nebularis	JK 212	57.1	4.30	9628	7398	**Statistical summary**		**Concentration (μg/kg)**			
*Helvella lacunosa**	JK 67	2611	38.0	6837	13600			**U**	**Th**	**Pb**	**Ag**
Hypholoma fasciculare	JK 207	5.28	3.46	452	250	**Minimum**		5.28	2.81	195	59.6
Lepiota magnispora	JK 192	357	6.84	5394	4599	**Median**		82.4	5.93	7381	7318
Lepista flaccida	JK 222	255	5.54	1598	6151	**Mean**		216	12.9	13100	9833
Lepista nuda	JK 210	51.4	4.57	15923	14004	**Maximum**		2611	78.6	72009	53942

Relative standard deviation of the ICP-SF-MS measurements of U, Th, Pb and Ag concentrations was approx. 1%. Samples collected from tailings are indicated by asterisk (*).

organism. The distribution and activity of fungal mycelia in the soil profile likely differ among species and can only be studied by use of molecular methods [65–67]. It can be expected that the chemical action of fungi driven by enzymes [68] and organic acids [9] influences various element soil fractions and that the elements can be transported from various soil depths [69,70]. Moreover, the element uptake in macrofungi appears highly species-dependent as reported for various elements and fungal species: Ag in *Amanita* spp. [61], As in *Sarcosphaera coronaria* [71], Fe in *Suillus variegatus* [54], Se in *Scutiger pes-caprae* [72], Sb in *Suillus* spp. [73], V in *Amanita muscaria* [74], and others.

It is generally accepted that prerequisite to the accumulation of metal in the cell is its mobilization from soil, metal uptake and translocation mechanism, and competence to detoxify the (over)accumulated metal species [9–15]. There is evidence that the fungal mycelia are able to mobilize U (including metallic U or U oxides) via the combined acidification and complexation with excreted oxalate, and fix the released extracellular uranyl species by precipitation with excreted phosphate and carboxylate anions that eventually leads to formation of secondary mycogenic minerals [11–14]. Consequently, the mycelia of the SAP/entomopathogenic *Beauveria caledonica* and the EM *Rhizopogon rubescens* cultured *in vitro* in the presence of U oxides accumulated up to 80 and 15 mg/g of U precipitates on their exterior surfaces, respectively [12]. Regarding the intracellular accumulation of U in fungi, uranyl ions are, like in plants [75], thought to be bioavailable in soils [12]. Indeed, the U uptake and translocation rates in the AM *Rhizophagus intraradices* cultured at pH 4 with uranyl ion as the predominant U species were substantially higher than those with the fungus cultured at higher pH of 5.5 or 8.0 at which most U was present as uranyl phosphate and carbonate, respectively [76].

Table 6
Metal concentrations (related to dry matter) determined in ectomycorrhizal macrofungi collected in the vicinity of Bytíz.

Species	Sample	Concentration (μg/kg)				Species	Sample	Concentration (μg/kg)			
		U	Th	Pb	Ag			U	Th	Pb	Ag
Amanita muscaria	JK 04	29.8	3.76	532	1457	*Russula exalbicans*	JK 61	36.0	3.00	1867	612
A. submembranacea	JK 68	94.6	45.4	2653	48157	*Russula exalbicans*	JK 62	46.3	5.61	4187	590
Boletus badius	JK 13	5.88	6.04	518	1030	*Russula exalbicans*	JK 203	248	6.41	84.5	842
Boletus edulis	JK 05	19.3	3.64	4953	1229	*Russula chloroides*	JK 184	60.8	13.2	236	1039
Cantharellus cibarius	JK 187	39.0	12.9	260	1091	*Russula illota*	JK 205	138	7.32	314	93.0
Cantharellus cibarius	JK 190	15.5	6.11	832	3861	*Russula ochroleuca*	JK 209	17.5	6.17	3434	75.2
Cortinarius trivialis	JK 195	128	11.7	222	832	*Russula puellaris*	JK 189	70.8	5.41	1268	308
Elaphomyces sp.	JK 250	30.3	15.3	226	1865	*Russula puellaris*	JK 206	13.9	6.85	7822	257
Gomphidius glutinosus	JK 201	16.5	7.64	401	101	*Russula subfoetens*	JK 204	97.1	9.20	497	214
*Inocybe dulcamara**	JK 64	1978	49.3	4316	5595	*Suillus collinitus*	JK 191	314	23.5	2761	2629
*Inocybe dulcamara**	JK 65	1004	40.7	9987	4226	*Suillus luteus*	JK 71	139	5.54	5379	648
*Inocybe dulcamara**	JK 197	78.5	8.73	174	1302	*Suillus luteus*	JK 73	160	6.80	4359	813
Lactarius controversus	JK 200	47.1	18.8	297	2860	*Suillus luteus*	JK 74	102	9.82	8353	689
Lactarius controversus	JK 202	94.0	22.1	583	2734	*Suillus luteus*	JK 75	151	20.3	10181	403
Lactarius deterrimus	JK 185	65.2	6.52	217	58.5	*Tricholoma populinum*	JK 15	323	22.8	441	1152
Lactarius torminosus	JK 196	137	5.46	302	482	*Xerocomus chrysenteron*	JK 199	13.7	5.43	844	1590
Leccinum rufum	JK 06	33.7	5.55	696	1396	**Statistical summary**		**Concentration (μg/kg)**			
Leccinum rufum	JK 08	58.3	6.36	376	1301			**U**	**Th**	**Pb**	**Ag**
*Leccinum scabrum**	JK 72	78.9	4.04	3516	768	**Minimum**		3.27	3.00	84.5	58.5
Paxillus involutus	JK 216	3.27	3.09	523	2652	**Median**		63.0	7.09	764	1065
Ramaria eumorpha	JK 183	47.1	8.26	1065	1817	**Mean**		157	12.0	2285	2599
Russula aeruginea	JK 194	24.9	8.72	2170	1991	**Maximum**		1978	49.3	10181	48157

Relative standard deviation of the ICP-SF-MS measurements of U, Th, Pb and Ag concentrations was approx. 1%. Samples collected from tailings are indicated by asterisk (*).

Table 7
Uranium concentrations (related to dry matter) in ectomycorrhizal tips and fine spruce roots collected from polluted forest plantation (Oe horizon) at Bytíz (A) and pristine areas in the vicinity of Kladská and Chmelná (B). Closest BLAST matches of the ITS sequences and their EMBL-Bank accession numbers are indicated.

A: Bytíz, uranium polluted forest site

Sample	ECM/root dry weight (mg)	U (ECM tips) (mg/kg)	U (roots) (mg/kg)	Closest BLAST match, similarity	EMBL-Bank
ECM 162/2	3.94/106	2.07	2.88	*Amanita* sp., EF493271, 94%	–
ECM 233/1	1.66/26.9	1.35	2.03	*Amphinema byssoides*, JN943932, 99%	LK932092
ECM 165	2.96/–	2.16	–	*Boletus badius*, HQ207696, 100%	LK932093
ECM 166	8.08/91.4	3.71	1.32	*Boletus badius*, HQ207696, 100%	LK932094
ECM 169	3.95/–	3.04	–	*Boletus badius*, HQ207696, 100%	LK932095
ECM 231/1	0.49/34.2	<0.74	2.45	*Boletus badius*, HQ207696, 100%	LK932096
ECM 231/2	0.65/34.2	1.53	2.45	*Boletus badius*, HQ207696, 100%	LK932097
ECM 231/4	0.60/34.2	3.64	2.45	*Boletus badius*, HQ207696, 100%	LK932098
ECM 235/1	2.17/29.6	2.31	3.45	*Boletus badius*, HQ207696, 100%	LK932099
ECM 161	3.04/84.7	4.33	3.22	*Boletus edulis*, HM579930, 100%	LK932100
ECM 236/1	2.06/23.6	2.33	17.7	*Clavulina* sp., JF519096, 100%	LK932101
ECM 236/2	2.95/23.6	1.43	17.7	*Clavulina* sp., JF519096, 100%	LK932102
ECM 236/3	1.79/23.6	3.09	17.7	*Clavulina* sp., JF519096, 95%	LK932103
ECM 163/2	3.24/110	1.76	1.70	*Russula ochroleuca*, HM189930, 100%	LK932104
ECM 235/2	1.74/29.6	6.95	3.45	*Russula ochroleuca*, HM189930, 100%	LK932105
ECM 164	8.21/97.7	1.88	0.99	*Russula xerampelina*, UDB011175, 100%	LM643862
ECM 162/1	5.85/106	3.29	2.88	*Thelephora terrestris*, HM189966, 100%	LK932106
ECM 232/1	0.63/36.7	3.67	1.71	*Tricholoma vaccinum*, FJ845444, 99%	LK932107
ECM 231/3	0.40/34.2	<0.74	2.45	Not identified	–
ECM 232/2	1.03/36.7	0.68	1.71	Not identified	–
ECM 234/1	1.22/26.8	1.29	2.04	Not identified	–
ECM 163/1	3.97/110	2.87	1.70	Not identified	–

B: Chmelná (CH) and Kladská (KL), unpolluted forest sites

Sample	ECM/root dry weight (mg)	U (ECM tips) (mg/kg)	U (roots) (mg/kg)	Closest BLAST match, similarity	EMBL-Bank
192/1 (CH)	0.32/43.9	<0.58	0.03	*Clavulinaceae*, AJ534710, 100%	LK932108
192/2 (CH)	0.51/43.9	<0.16	0.03	*Clavulinaceae*, AJ534710, 100%	LK932109
190/7 (CH)	1.02/33.5	0.09	0.08	*Craterellus tubeaformis*, AY195572, 99%	LK932110
248/1 (KL)	5.98/29.4	0.17	0.26	*Cortinarius gentilis*, EU266692, 100%	LK932111
250/1 (KL)	1.82/24.4	0.18	0.41	*Cortinarius croceus*, UDB001144, 99%	LK932112
246/3 (KL)	1.28/19.9	<0.07	<0.02	*Lactarius tabidus*, HM189833, 100%	LK932113
217/3 (CH)	1.99/11.0	<0.05	0.13	*Pucciniales*, FJ554109, 99%	LK932114
249/1 (KL)	2.30/31.5	0.11	0.07	*Pucciniales*, FJ554109, 99%	LK932115
219/4 (CH)	1.45/21.6	0.14	0.06	*Russula emetica*, JQ888196, 99%	LK932116
246/2 (KL)	3.57/19.9	0.05	0.02	*Russula paludosa*, UDB017996, 100%	LK932117
247/1 (KL)	8.25/34.9	0.05	0.01	*Russula paludosa*, AJ971402, 99%	LK932118
247/2 (KL)	4.07/34.9	<0.04	0.01	*Russula paludosa*, JQ888199, 100%	LK932119
247/3 (KL)	3.27/34.9	0.05	0.01	*Russula paludosa*, JQ888199, 100%	LK932120
246/4 (KL)	1.05/19.9	<0.07	0.02	*Tomentellopsis submollis*, AM086445, 100%	LK932121
191/1 (CH)	0.85/21.2	<0.19	0.08	*Tylopilus felleus*, HM190016, 100%	LK932122

Statistical uncertainty of INAA determination of U in EM tips and root samples was mostly below 7% and 3%, respectively. UDB accession numbers refer to the UNITE database [26].

In spite of the fact that U chemistry in soils would be expected to be complex, the pH conditions and the occurrence of U in the exchangeable BCR fraction throughout the soil profile observed at Bytíz would suggest that the uranyl ion was available for fungal uptake: however, the information regarding the mechanism of U uptake and intracellular handling in eukaryotic organisms is scarce. It is reasonable to assume that the accumulation of physiologically irrelevant metals will employ mechanisms designed for the acquisition and handling of structurally and chemically similar essential metal species—none of them, however, being a large metal oxycation. To our knowledge, the mechanism of U uptake has been detailed only in animal cells—the cultured kidney cells were shown to take up U by endocytosis, storing intracellular U in the absorbed vesicles [77]. Intriguingly, small, U-containing vesicles emerging in a dose-dependent manner were described also in mycelia of AM fungi [78].

For the metal transport to be efficient, the cells should employ selective high-affinity membrane transporter proteins. The (bio)chemical similarity of Cu^+ and Ag^+ would suggest that the observed exceptional disposition of macrofungi to effectively accumulate Ag will, at least in part, rely upon the proteins of the Cu transporter (CTR) family, which exist in all eukaryotes [79]. The high-affinity Ag^+ uptake executed by human plasma membrane CTR1 has been demonstrated recently [80]. While the information regarding the molecular determinants underlying the translocation of Ag in fungi or other multicellular eukaryotes is missing, there is evidence that the detoxification of both Ag^+ accumulated in mycelia and Ag^+ deposited in fruit-bodies is dominated by complexation with small, cysteine-rich metallothionein peptides [20,62,81].

3.4. Ectomycorrhizae

EM fungi can protect symbiotic trees against heavy metal toxicity by executing a metal barrier function in ectomycorrhizae through mechanisms such as extracellular precipitation or chelation, biosorption, exclusion, and cellular uptake of excess toxic metal species [9,11,82,83]. However, unlike in AM fungi, the role of the EM symbionts in the plant-U interaction has not so far been addressed.

In order to test whether the ectomycorrhizae would serve as the sink for U, spruce EM tips formed by various fungal species and the control non-mycorrhizal fine roots collected from the polluted Bytíz forest plantation (Table 7A) and two distinct pristine areas (Table 7B) were analyzed for their U content. In EM tips from the pristine sites, the U concentrations did not exceed 0.18 mg/kg; in the fine roots, the U concentrations were very similar. In EM tips

from Bytíz, the U concentrations were distinctly elevated, with the highest value of 6.95 mg/kg found in *Russula ochroleuca*. However, when compared to the fine roots, no U accumulation was observed. It thus appears that, unlike with Zn, Cu and Pb [84,85], the EM tips do not tend to accumulate U from polluted soils. No striking differences were observed in the potential of individual fungal species to store U in EM tips (cf. Kottke et al. [86]).

It has been documented that although the AM fungus *R. intraradices* translocates U towards the mycorrhizal roots [76], the metal remains entrapped within the intraradical mycelial structures, rendering U immobile for the uptake into the plant tissues. As a consequence of the U filtering function of *R. intraradices*, the AM roots contained elevated U concentrations, when compared to the non-mycorrhizal ones, and the fungus reduced the translocation of U to the shoots [87–92]. These properties make *R. intraradices* a suitable mycobiont in phytostabilization of U in polluted soils [91]. Low U contents in EM tips from Bytíz suggest that a similar function cannot be attributed to EM fungi, which apparently exclude U, and our findings therefore do not indicate a potential use of EM fungi in U remediation or phytostabilization strategies.

4. Conclusions

Recent studies have indicated that fungi might play important roles in the biogeochemical cycling of U and their possible use in bioremediation strategies has been suggested [14]. However, the data obtained for macrofungi in this field study do not indicate a major role of these organisms in the biogeochemical fate of U in polluted forest soils. Concentrations of U in macrofungi collected from the polluted area near Bytíz in the former Příbram U mining district were low and generally did not exceed 3 mg/kg which indicates a very low uptake rate and efficient exclusion of U. The concentrations of U observed in EM tips and the non-mycorrhizal fine roots were essentially identical. It can therefore be concluded that the environmental role of EM macrofungi in the biogeochemical cycling of U is considerably limited. This is, however, in contrast to what has been observed in AM symbiosis. Furthermore, it was demonstrated that the uptake and accumulation of U, Th, Pb and Ag in macrofungal fruit-bodies does not primarily depend on their total content and chemical fractionation in soils. The relatively high U contents and mobility in soils did not lead to its effective accumulation in macrofungi. In contrast, the highly accumulated Ag was the element with the generally lowest total soil concentrations and mobility.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2014.07.050.

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Příloha 5

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Covering Letter

EDITOR
Editorial Office
Soil Biology & Biochemistry

Jan Borovička
Nuclear Physics Institute & Institute of Geology ASCR
Prague, Czech Republic

Prague, 6 January 2016

Dear Editor,

The work of our team has much focused on interactions between metals and macrofungi in the last decade. For this time, we have finished a study on trace element bioaccumulation in ectomycorrhizae (ECMs) from smelter-polluted area. There are only a very few papers dealing with the subject of element accumulation in ECMs (about 5) and only one multielement study has been published to date; but this was focused on nutrients and essential metals.

Our manuscript presents results of multielement analysis (toxic metals, metalloids, and chlorine) of ECMs identified by DNA sequencing – and this has never been done before. And for the first time, concentration of fungal biomass in ECMs of two macrofugal species was determined by qRT-PCR approach.

We believe the subject of our research fits to the scope of your journal and the findings would be interesting to readers. We have thus decided to send you our manuscript and I am kindly asking you to consider it for review in Soil Biology & Biochemistry.

Thank you very much. On behalf of co-authors,

Jan Borovička
biogeochemist and mycologist
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http://www.researcherid.com/rid/A-1022-2008

Graphical Abstract (for review)

***Highlights (for review)**

- Cadmium, chlorine, and silver are highly accumulated in ectomycorrhizae
- Vanadium is accumulated in ectomycorrhizae of Amanita muscaria
- Mycelial biomass in ectomycorrhizae of B. badius and A. muscaria was quantified
- Ag, Cd, and Zn levels in mycelia possibly reach lower thousands of mg/kg

Bioaccumulation of heavy metals, metalloids, and chlorine in ectomycorrhizae from smelter-polluted area

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Abstract

Ectomycorrhizae (ECMs) on *Picea abies* collected from Oe soil horizon in smelter-polluted area at Lhota near Příbram (Czech Republic) were identified by ITS rDNA sequencing and analyzed for metal(loid) and chlorine contents. Concentrations were compared with those found in non-mycorrhizal fine roots, total soils and soil extracts. Cadmium, chlorine, and silver were the elements markedly accumulated in ECMs, with *Boletus badius* being the most efficient accumulator among the identified 11 species. Vanadium was accumulated only in ECMs of the *Amanita muscaria*. The proportions of *Boletus badius* and *Amanita muscaria* mycelia quantified in wild-grown ECMs using a qRT-PCR approach showed substantial variations, but the median values were close to 5 % (w/w) for both species. The assessment of the element concentrations in ECM hyphae revealed in *B. badius* mean Ag, Cd, Zn and Cl concentrations of 1.68, 1.51, 2.67 and 37.1 g kg^{-1} dry weight, respectively. The present results further straighten the idea of active role of ectomycorrhizal fungi in soil-fungal-plant interactions and indicate high element accumulation capacity of ectomycorrhizal mycelia.

Keywords

Ectomycorrhizal fungi; Ectomycorrhiza; Quantitative real-time PCR; Roots; Soil; Cadmium; Silver

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1. Introduction

Ectomycorrhizal (ECM) fungi significantly interfere in biogeochemical cycles of elements in soils (Gadd, 2007). The major active roles of ECM fungi in ecosystems involve, besides the degradation and cycling of soil organic matter (Clemmensen et al., 2013; Phillips et al., 2014), contribution to weathering processes through excretion of organic acids and subsequent transfer of mobilized nutrients towards the colonized plant roots (Schmalenberger et al., 2015), and trace element translocation, accumulation and biotransformation (Clarholm and Skyllberg, 2013; Falandysz and Borovička, 2013).

Metal/metalloid accumulation ability in fruit-bodies of ECM macrofungi suggests major role of these organisms in sequestration and cycling of the accumulated elements (particularly Ag, As, Cd, Cu, Se, and Zn). Ectomycorrhizae (ECMs) represent mutual organs where the fungal-plant nutrient exchange takes place; studies have also suggested that they benefit the host by forming a protective barrier against heavy metal toxicity (Leyval et al. 1997, Jentschke and Godbold 2000, Krupa and Kozdrój 2004, 2007; Gadd, 2007; Urban, 2011; Colpaert et al., 2011). Extraradical mycelium is hardly separable from soil under the natural conditions. However, analysis of ECMs might represent an interesting tool for inspecting the soil-fungal-plant interactions *in situ*.

Only a very few data have been published on concentrations of trace elements in ECMs, both from pristine and polluted sites. To our knowledge, the largest dataset published so far was a study by Kottke et al. (1998) who focused on nutrients and essential metals P, K, Mg, Ca, Fe, Zn, Al, and Mn. Berthelsen et al. (1995) and Krupa and Kozdrój (2004, 2007) have reported substantially elevated concentrations of Cu, Cd, Pb and Zn in ECMs. In contrast, the concentrations of Au (Borovička et al., 2010a) and U (Kubrová et al., 2014) were found to be low even in ECMs from auriferous and U-polluted areas.

The aim of this study was to inspect concentrations of selected elements in ECMs from smelter-polluted area with the mycobionts identified at species level by DNA sequencing. In order to

provide a semi-quantitative assessment of the element concentrations in fungal hyphae forming the mantle around a fine root tip and the Hartig net (Landeweert et al., 2001), the proportion of mycelial biomass was quantified using a quantitative real-time PCR (qRT-PCR) approach in ECMs of two macrofungal species.

2. Materials and methods

2.1. Investigated area and sample collection

Norway spruce (*Picea abies*) forest plantation above sedimentary bedrock (greywacke) at Lhota near Příbram was selected for this study. Long-term Ag-Cu-Pb-Zn ore mining activities in the region and decades of lead smelting (Sucharová and Suchara, 2003) resulted in elevated levels of Ag, As, Cd, Cu, Pb, Sb, and Zn levels in soils (Ettler et al. 2004, 2007; Borovička et al. 2006, 2010b; Komárek et al. 2007). Samples of ECMs, roots and soils were collected from Oe horizon at 32 randomly selected places distributed throughout the area (Fig. 1); GPS coordinates of sampling points are listed in Supplementary Table S1.

2.2. Sample preparation

ECMs and non-mycorrhizal fine roots were processed as described by Kubrová et al. (2014). Dried samples were heat-sealed into polyethylene (PE) capsules; the sample weight was in the range of 0.5-16 mg for ECMs and 35-62 mg for roots. Soil samples (Oe horizon) were collected directly from the places where ECMs/roots were sampled, dried, and sieved through a 2 mm stainless steel mesh. A representative part of each sample was milled in an agate mill and aliquots of approximately 250 mg were used for determination of total element contents.

84 *2.3. Chemical analyses*

85

86 To analyze the concentrations of Cl, Cu, and V in ECMs, roots, soils, and macrofungal fruit-
87 bodies, short-time neutron activation analysis (INAA) was used according to Řanda et al. (2005).
88 Times of irradiation-decay-counting of 2-10-10 minutes for ECMs, 1-10-10 minutes for roots and
89 fruit-bodies, and 1-13-13 for soils were used as optimum conditions. The measurement of gamma
90 spectra was performed using the high-purity germanium (HPGe) coaxial detector PGT IGC 20 (20%
91 relative efficiency, resolution FWHM 1.75 for the 1332.5 keV photons of ^{60}Co). After 4 weeks of
92 decaying, ECMs and roots were re-irradiated and further analyzed as described below.
93 Concentrations of other elements in ECMs and roots were assessed by using long-time
94 instrumental neutron activation analysis with epithermal neutrons (ENAA). This was performed by
95 2.5-3 h irradiation in a specially designed Al/Cd box as described by Řanda (1976). The measurements
96 of gamma spectra were performed after 3-4 and 18-25 days of decaying using the HPGe coaxial
97 detector Canberra GC7020 (78% relative efficiency, resolution FWHM 1.87 for the 1332.5 keV
98 photons of ^{60}Co). Soil samples were analyzed by INAA according to Řanda and Kučera (2004).
99 Variable counting geometries optimized for individual analytical modes were applied.
100 Neutron irradiation was carried out in the LVR-15 reactor of the Nuclear Research Institute Řež plc.
101 (Czech Republic) at fluence rates of 8×10^{13} n cm^{-2} s^{-1} and 3×10^{13} n cm^{-2} s^{-1} for thermal and fast
102 neutrons, respectively. Measurements were carried out at the CANAM infrastructure of the NPI ASCR
103 Řež supported through the project No. LM2011019 (Ministry of Education, Youth and Sports of the
104 Czech Republic). In order to verify the quality of chemical analysis, standard reference material SRM
105 NIST 1566b (Oyster Tissue) was used throughout the study.
106 As the INAA determination of total Cd in soils does not provide reliable data, soil samples
107 were acid-digested in a microwave oven similarly as described in Gryndler et al. (2012) and Cd was
108 determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 5100). In
109 order to inspect the "mobile", acid soluble fraction of selected elements in soil, 1M HNO_3 extraction

110 was conducted as described in Borovička et al. (2014). Element concentrations in the extracts were
111 determined by inductively coupled plasma quadrupole based mass spectrometry (ICP-QMS, X Series
112 2, Thermo Scientific).
113
114 *2.3. Molecular methods*
115
116 The complete procedure of molecular identification of fungal species in ECM samples was
117 described in Kubrová et al. (2014). All obtained ITS rDNA sequences were identified to species/genus
118 level by querying the GenBank database, using the nucleotide–nucleotide (blastn) BLAST search
119 option, available through the National Center for Biotechnology Information
120 (http://blast.ncbi.nlm.nih.gov). Quality sequences were accessioned into the EMBL-Bank database
121 (Supplementary Table S2). Several collections of macrofungi used in this study were deposited in
122 herbarium of the Mycological department, National Museum, Prague (PRM).
123 In order to quantify fungal biomass in ECM roots, two cultivable ECM species forming
124 morphologically recognizable ECMs were selected: *Boletus badius* and *Amanita muscaria*. ECMs of
125 both species were collected from roots of *Picea abies* growing in Oe soil horizon at the investigated
126 site in September 2014 in close vicinity of fruit-bodies. They were cleaned in water under
127 stereomicroscope, and kept frozen until lyophilization followed by DNA extraction. Only the fresh,
128 turgescent, rigid ECMs with light apices and compact hyphal mantles were used in this study.
129 For the purposes of calibration of qRT-PCR data against gravimetric fungal biomass, the
130 mycelial cultures of *B. badius* (explanted from a basidiocarp collected directly at the studied locality,
131 PRM 923859, EMBL-Bank LN877746), and *A. muscaria* (isolate AM17, maintained by the Laboratory
132 of Fungal Biology, Institute of Microbiology ASCR, Prague; EMBL-Bank LN877747) were used. The
133 samples of both ECM roots and mycelia were weighed and DNA was extracted in the same way as
134 described above. In order to determine the extraction efficiency, 2×10^{10} gene copies of an internal
135 standard (linearized plasmid carrying fragment of cassava mosaic virus, GenBank accession

136 AJ427910) were added into each sample before extraction and quantified in the DNA extract as
137 described in Thonar et al. (2012).

138 We used qRT-PCR with two specific primer pairs for each species. Primers ITS1F, ITS2, and
139 ITS3 were used according to White et al. (1990) and Gardes and Bruns (1993). Specific primers for
140 *B. badius* designed in this study were BadiusF (forward, 5'-GGA AGG ATC ATT ATC GAA CAA GAA-3')
141 and BadiusR (reverse, 5'-AAG GCC TTG CTT GTC CAC-3'). For *A. muscaria*, specific primers AmusF1
142 (forward, 5'-TCT CTT GCT TGT TTC TTC A-3') and AmusR1 (reverse, 5'-CAA CAA TTG TTC ATG TAT GTA
143 AAT-3') were designed in this study.

144 Primers recognizing ITS rDNA of *B. badius* have been designed based on the motifs present
145 only in the sequences belonging to *B. badius* (GenBank accession numbers LK932098 and
146 HQ207696). These sequences were not found in the ITS of related relevant species of the genera
147 *Boletus*, *Buchwaldoboletus* and *Xerocomus* s.l.: *B. reticulatus* (KC422620), *B. edulis* (HM579927),
148 *B. pinophilus* (GU198987), *B. lignicola* (HM003619), *X. chrysenteron* (HQ207694), *X. communis*
149 (EF493247), *X. ferrugineus* (HQ207698), *X. porosporus* (HM190086), *X. pruinatus* (HQ207695),
150 *X. rubellus* (EF644119), and *X. subtomentosus* (DQ131632). qPCR with primer pairs ITS3/BadiusR and
151 BadiusF/ITS2 produced the amplicons of expected size of 230 bp and 240 bp, respectively.

152 Primers specific for ITS of *A. muscaria* have been designed on the basis of unique motifs
153 found in GenBank-deposited sequences of *A. muscaria* (EU071912, EU071897, EU071920, AB080983,
154 AB080984, AB080777, and AB080778) and absent from the sequences of closely related *A. regalis*
155 (unpublished sequence, collection PRM 860899), *A. gemmata* (unpublished sequence, collection
156 PRM 922166), *A. pantherina* (AB096046), and *A. eliae* (JF907763); however, the primer pair
157 AmusF1/AmusR1 was able to amplify DNA extracted from *A. regalis*. As expected, the length of qPCR
158 products obtained with primer pairs AmusF1/ITS2 and ITS1F/AmusR1 were 180 bp and 202 bp,
159 respectively.

160 DNA samples extracted from ECM tip of *B. badius* or *A. muscaria* were used as templates in
161 standard PCR with specific primer pairs BadiusF/BadiusR or AmusF1/AmusR1 with PCR conditions as

described in Borovička et al. (2011a). A few samples with none or weak signal (indicating possible PCR inhibition) were excluded from further processing. In total, 19 samples of *B. badius* ECM and 11 samples of *A. muscaria* ECM were then subjected to qRT-PCR. According to Janoušková et al. (2015), 10x diluted DNA sample (2 µl) was added to a 18 µl of Hot FirePol®EvaGreen® qPCR Supermix (Solis BioDyne, Tartu, Estonia) prepared according to the manufacturer's instructions (0.4 µl primer 1, 0.4 µl primer 2, 13.2 µl H_2O, 4 µl EvaGreen). The cycling conditions in the StepOnePlus instrument (Applied Biosystems) were: initial denaturation for 12 min at 95 °C, followed by 40 cycles of 15 s denaturation at 95 °C, annealing for 20 s at 55 °C, and synthesis for 20 s at 72 °C. The results for each sample are based on 3 replicates and have been corrected for DNA extraction efficiency using the quantitation of the internal standard added to the ECM samples before the DNA extraction as described above.

3. Results and discussion

3.1. Ectomycorrhizal diversity

Despite being heavily polluted by toxic metals, the investigated forest plantation is not poor in ECM macrofungi (Borovička et al., 2006; 2010b). However, only 11 distinct operational taxonomic units (OTUs) were identified from ECMs. The OTUs corresponding to *Thelephora terrestris*, *B. badius*, and *Paxillus involutus* were the ones most frequently detected. Notably, a large portion of the total ECM colonization by Thelephoraceae was also observed in other studies (Hrynkiewicz et al., 2008; Nieto and Carbone 2009).

The relatively low number of detected species can stem from three causes: i) We have collected samples from the Oe horizon under Norway spruce. ECM species are distributed throughout the soil profile with preference of particular soil horizons (Rosling et al., 2003). Thus, only species preferring the Oe horizon could have been isolated. ii) the belowground ECM community

188 typically consists of a few common species, colonizing 50-70 % of the available fine roots, and a
189 larger number of rarer species (Erland and Taylor, 2003). Morphotypes forming insufficient number
190 of ECMs could have not been sampled for chemical analysis and were thus omitted. This sampling
191 strategy may also influence the overall species composition of the dataset. iii) The ECM fungal
192 community does contain small number of species only. Similar numbers of OTUs corresponding to
193 ECM fungi have been reported by Lothamer et al. (2014) who extrapolated, using the Chao1
194 estimator, the richness of ECM fungal OTUs in *Quercus macrocarpa* ectomycorrhizosphere. They then
195 reported the ECM OTUs numbers lower than 10, independently of the sampling season. Our data are
196 thus not so far from those obtained using extensive molecular studies and can reflect the real
197 number of ECM fungal species present at the locality.
198
199 *3.2. Fungal biomass in ectomycorrhizae*
200
201 Quantitative real-time PCR is a useful tool to determine the biomass of individual fungal
202 species in soil (Landeweert et al., 2003). This method has been successfully used for quantification of
203 mycelia of ECM and saprotrophic (SAP) fungi both in field studies (Guidot et al., 2002, 2003; Hortal et
204 al., 2008; de la Varga et al., 2012, Borovička et al., 2014) and under artificial conditions (Parladé et al.,
205 2007; Kurth et al., 2013). Recently, Gryndler et al. (2013) detected and quantified mycelium of ECM
206 *Tuber aestivum* in soil and from non-mycorrhizal roots of *Carpinus betulus*. To the best of our
207 knowledge, no qRT-PCR data for the assessment of the proportion of mycelial biomass in ECMs has
208 been reported so far.
209 The quantities of fungal biomass in ECMs of *B. badius* and *A. muscaria* are presented in Table
210 1A and 1B, respectively; Table 1C depicts statistical analysis of this data. We detected considerably
211 varying concentration of fungal biomass in ECMs but median values were close to 5 % (w/w) in both
212 species. This value is smaller relative to the data presented by Antibus and Sinsabaugh (1993) who
213 reported mean concentration of fungal biomass in ECMs of *Betula populifolia* and *Pinus sylvestris*

214 within the range of 18-34%. Slightly higher values were reported for ECMs of *Tuber borchii* on *Tilia*
215 *platyphyllos* (Zeppa et al., 2000).

216 The reason for the lower fungus mean proportions observed in our samples remains unclear.
217 It appears reasonable to expect that the amount of the mycobiont in ECMs will be affected by many
218 environmental factors as well as by the physiological state of the partners in the symbiosis. However,
219 with a single exception of the age of ECMs, there are no relevant reports that would support this
220 notion. Zeppa et al. (2000) noted remarkably higher proportion of the symbiotic mycelium in young
221 ECMs; however, the difference they reported (51% in young and 35% in old ECMs) cannot fully
222 explain prevailing low values in our ECM samples.

223 The investigated site is characterized by extremely high level of pollution by heavy
224 metals/metalloids, with Pb concentrations of lower units of % (w/w) in the Oe horizon (Ettler et al.,
225 2004). A question appears whether such a toxic environment may explain the low fungal
226 concentrations in our samples. Furthermore, non-homogeneity of soil (the patchiness of pollutant
227 distribution) may, at least partly, explain the high variation in the proportion of fungal biomass in
228 ECMs. The very few high concentrations noted both in *B. badius* and *A. muscaria* might represent a
229 "normal level" of colonization whereas the prevailing low concentrations might be attributed to the
230 stress exerted by onsite pollution. Regrettably, no comparable literature data on fungal biomass
231 concentration in ECMs under conditions of varying stress (which would support the validity of this
232 explanation) are available.

233 Furthermore, the discrepancy between our data and the data of Antibus and Sinsabaugh
234 (1993) and Zeppa et al. (2000) might also stem from different methodologies as, unlike this, other
235 studies relied on the use of ergosterol as the biochemical marker for fungi (Wallander et al., 2013).
236 However, the molecular quantification of fungal biomass in ECMs proved to be highly consistent with
237 the use of two different primer pairs per each of the fungal species under study. The choice of the
238 primers used in qRT-PCR did not affect the results, as the differences in fungal biomass
239 concentrations obtained by the two independent primer pairs are negligible. Furthermore, the

concentration of the fungus in ECMs seems to be independent on sample weight, as no significant correlation between these two parameters was observed: correlation coefficients (R^2) were 0.1343 and 0.0442 for *B. badius* and *A. muscaria* ECMs, respectively (Table 1). Considering this, we assume that the observed proportion of mycobionts reflects different extent of the root colonization, i.e. the density of fungal structures inside the ECMs.

3.3. Element accumulation in ECMs

Element concentrations in ECMs, roots, soil extracts and soils are presented in box plots (Fig. 2); Cl soil mobile fractions were not determined. Complete data for ECMs can be found in the Supplementary Table S3 together with concentrations of Co, Cs, Rb, Se, Th, and U – these were very low and therefore not presented in the box plots. Full data set for roots and soils can be found in the Supplementary Tables S4, S5, and S6. Identification of the analyzed ECMs based on the obtained ITS rDNA sequences, including their identities with EMBL entries, is described in Supplementary Table S2; 6 samples remained unidentified.

Macrofungi are known to accumulate high levels of elements in their fruit-bodies. This ability is often species- or genus-specific and does not primarily depend on element concentrations in the environment; for discussion on factors influencing the element uptake in fungi see Falandysz and Borovička (2013). More recently, Kubrová et al. (2014) demonstrated that accumulation of Ag, Pb, U and Th in fruit-bodies does not depend on concentration and mobility of those metals in soils.

Silver is known to be hyperaccumulated in certain *Amanita* species and is also very effectively accumulated in both ECM and SAP macrofungi (Borovička et al., 2007; 2010b). In the latter study, the authors reported the concentrations of tens to hundreds of mg Ag kg^{-1} in fruit-bodies of various ECM species collected from Lhota near Příbram.

Despite being the least mobile element (similarly as reported in Kubrová et al., 2014), Ag was very highly accumulated in ECMs, with the highest concentrations in those formed by *B. badius* (47.7-

385 mg kg^{-1}). Curiously, this species is a rather weak Ag-accumulator with mean concentration of 14.2 mg kg^{-1} in fruit-bodies from the investigated site (Borovička et al., 2010b). Concentrations of Ag in fruit-bodies of *A. muscaria* (mean 31.3 mg kg^{-1}) from Lhota near Příbram are twice higher but in the analyzed ECMs of *A. muscaria*, Ag levels were lower than those in *B. badius*. Unfortunately, ECMs of the Ag-accumulating *Amanita submembranacea* were not detected in the Oe horizon, despite being searched in close vicinity of its fruit-bodies. The highest (but obviously outlying) value of Ag was found in a single ECM tip of *T. terrestris* (544 mg kg^{-1}). Silver concentrations in roots were mostly lower than total Ag levels in soils which indicates exclusion of this element by *Picea abies*.

Concentrations of **arsenic** in ECMs were mostly higher than those in the fine roots but, on the other hand, mostly lower than those in soils. Arsenic concentrations found in ECMs among the fungal species were very variable (e.g. 3.98-376 mg kg^{-1} in *B. badius*); rather low values were generally found in most ECMs of *P. involutus*. Arsenic is known to be accumulated by macrofungi (Stijve et al., 1990; Slekovec and Irgolic, 1996) and methylated As compounds are usually present in fruit-bodies (Kuehnelt et al., 1997a-b; Nearing et al., 2014). However, the ability of macrofungi to biosynthesize these compounds has not yet been demonstrated and their occurrence in fungal tissues is thus considered a probable consequence of activity of associated microbiota (Nearing et al., 2015).

In spite of its high toxicity, **cadmium** is strongly accumulated in saprotrophic macrofungi, particularly in *Agaricus* species of the section *Arvenses* (Cocchi et al., 2006). Concentrations of Cd in fruit-bodies of symbiotic fungi are much lower and well-documented from many unpolluted sites e.g. in *B. badius* (Kalač and Svoboda, 2000; Malinowska et al., 2004) and *P. involutus* (Brzostowski et al., 2011a-b); in both species, Cd concentrations only rarely exceed 1 mg kg^{-1}. For both species from the vicinity of Lhota near Příbram, Kalač et al. (1991) reported mean Cd contents of 2.7 and 1.6 mg kg^{-1}, respectively, and *A. muscaria* was the most efficient Cd accumulator (mean 29 mg kg^{-1}). Very similar results for macrofungi from Lhota near Příbram were reported by Lepšová and Král (1988) and Komárek et al. (2007).

291 Despite the Cd concentrations in ECMs varying in a rather large range of 10-333 mg kg^{-1},
292 median concentration was 47 mg kg^{-1}. Thus, besides Cl, cadmium was the most accumulated
293 element. Values exceeding 100 mg kg^{-1} were found at least in ECMs of 6 fungal species, with the
294 highest in levels in *B. badius*. Corresponding results were reported by Krupa and Kozdrój (2004) in
295 ECMs of *Betula* growing in an industrial desert with a similar level of Cd pollution (20 mg Cd kg^{-1} in
296 soil). On the other hand, very low concentrations (mean 4.6 mg kg^{-1}) were reported in various ECM
297 morphotypes from a Norwegian region described as "with high surface soil Cd levels" (Berthelsen et
298 al., 1995). With median value of 11.8 mg kg^{-1}, cadmium concentrations in roots were much lower
299 than those in ECMs.
300 **Copper** is known to be accumulated in ECM fungi but concentrations in fruit-bodies of
301 *B. badius* (Malinowska et al., 2004) and *A. muscaria* (Vetter 2005) are usually below 50 mg kg^{-1};
302 higher tens of mg kg^{-1} are reported from *P. involutus* (Brzostowski et al., 2011a-b). Concentrations of
303 Cu in fruit-bodies from the investigated area are not known. In the analyzed ECMs, we found Cu
304 concentrations of up to 162 mg kg^{-1} which indicate bioexclusion of Cu. When compared to fine roots,
305 Cu is only slightly enriched in ECMs. The highest contents were found in ECMs of *B. badius* (mean 120
306 mg kg^{-1}) and rather low values were observed in *P. involutus* (mean 67.3 mg kg^{-1}). This observation is
307 contrary to what is observed in the fruit-bodies. Notably, our results are strikingly different from
308 those published by Berthelsen et al. (1995) from polluted soils in Norway who reported significant
309 accumulation of Cu in ECMs (176-790 mg kg^{-1}).
310 The investigated locality is heavily polluted by **antimony**, with the concentrations in Oe
311 horizon commonly reaching higher hundreds of mg kg^{-1}. This is in agreement with Ettler et al. (2007)
312 who investigated its available soil contents in the same area. Antimony concentrations in ECMs and
313 fine roots are similar and much lower than those in soils, Sb is apparently bioexcluded. Only *Suillus*
314 and *Chalciporus* species are known to accumulate Sb (Borovička et al., 2006) and none of those
315 genera was covered in our study. According to the review by Pierart et al. (2015), nothing is known
316 about how ectomycorrhizal fungi adsorb and/or transform Sb at the soil-fungal-plant interface.

317 With a single exception, **vanadium** concentrations in ECMs and fine roots are similar and
318 generally lower than both total and extractable V in soils. This indicates effective bioexclusion of this
319 element. However, *A. muscaria* is known to accumulate V in fruit-bodies (Koch et al., 1987; Vetter
320 2005) and V was found at distinctly elevated levels of 26.4 and 61.2 mg kg^{-1} in its ECMs. In the fruit-
321 bodies of *A. muscaria*, V is deposited into amavadine, an organometallic compound of unknown
322 biological function (Garner et al., 2000; Hubregtse et al. 2005). High V contents in ECMs of
323 *A. muscaria* should be attributed to fungal activity but whether or not amavadin is synthesized in
324 mycelium and present in ECMs remains to be investigated.
325 With median value of circa 100 mg kg^{-1}, concentrations of **zinc** in fruit-bodies of ECM fungi
326 from pristine sites usually do not exceed 200 mg kg^{-1}. Concentrations of Zn in fruit-bodies of
327 *B. badius*, *P. involutus* and *A. muscaria* from such habitats are more or less similar and vary in the
328 range of 70-240 mg kg^{-1} (Vetter, 2005; Borovička and Řanda, 2007); Brzostowski et al. (2011a)
329 reported slightly higher values for *P. involutus*. Zinc is apparently accumulated in ECMs and its
330 concentrations are the highest we detected among the investigated metals (maximum 1,280 mg kg^{-1}).
331 However, the bioaccumulation factor is not high when compared with Ag or Cd as the total soil Zn
332 content varies within the range of 182-646 mg kg^{-1}. In this particular case, our results fit those of
333 Berthelsen et al. (1995) who reported Zn concentrations in ECMs in the range of 105-1,090 mg kg^{-1}.
334 In the fine roots, Zn was lower with concentrations in the range of 67-415 mg kg^{-1}.
335 Ectomycorrhizal and saprotrophic macrofungi are known to produce chlormethane (Redeker
336 et al., 2004; Anke and Weber, 2006) and various chlorinated compounds (Takahaski et al., 1992;
337 Hatanaka et al., 1998; Drehmel and Chilton, 2002). Limited data on **chlorine** concentrations in
338 macrofungal fruit-bodies indicate species-dependent uptake of Cl which is highly accumulated
339 especially in *Amanita* species (Stijve, 1984; Petrini et al., 2009); low concentrations are found e.g. in
340 *P. involutus* (Hedrich, 1988; Řanda et al., 2005). In contrast to the fine roots with median Cl
341 concentration of 962 mg kg^{-1}, chlorine is apparently greatly accumulated in ECMs (median 2,700 mg
342 kg^{-1}). However, the results do not copy the pattern known from the fruit-bodies. In Table 2, results

343 for Cl concentrations in fruit-bodies (unpublished data from our archive, fruit-bodies of various
344 origins) and ECMs of selected species from Lhota near Příbram are presented. As seen in *P. involutus*,
345 low concentrations in fruit-bodies were not followed in ECMs. The highest Cl concentrations were
346 found in the ECMs of *B. badius* and Cl levels in the ECMs of Cl-accumulator *A. muscaria* were rather
347 low (but only two samples were analyzed).

348 Concentrations of other analyzed elements in ECMs were rather low and often below the
349 detection limit of INAA. **Uranium** and **thorium** are not accumulated in macrofungi (Borovička et al.,
350 2011b) and their concentrations in ECMs did not exceed 0.55 and 0.65 mg kg^{-1}, respectively. The U
351 values in ECMs and fine roots are similar to those reported by Kubrová et al. (2014) for unpolluted
352 sites. Median concentration of **cobalt** in a representative dataset of fruit-bodies of symbiotic
353 macrofungi was 0.16 mg kg^{-1} (Borovička and Řanda, 2007). In ECMs, lower units of mg Co kg^{-1} were
354 detected, with only one outlying value of 14.8 mg kg^{-1} in *B. badius*. However, in contrast to fine roots
355 (median 0.67 mg kg^{-1}), concentrations of Co were slightly elevated (median 1.70 mg kg^{-1}). **Selenium** is
356 an element known to accumulate in certain ECM species, especially *Scutiger pes-caprae* and the
357 group of *Boletus edulis* (currently *Boletus sensu stricto*); common concentrations in ECM species are
358 approximately in the range of 0.15-1.50 mg kg^{-1} (Stijve et al. 1998, Borovička and Řanda 2007).
359 Selenium levels in ECMs were usually below detection limit of INAA, i.e. mostly lower than 2 mg kg^{-1}.
360 ECM species are also well-known accumulators of **rubidium** and **caesium**; accumulation of the latter
361 causes the uptake of radiocaesium in macrofungi (Horyna and Řanda, 1988; Vinichuk et al., 2010).
362 Caesium was generally below 1.5 mg kg^{-1} in ECMs but probably higher than in the fine roots (median
363 0.05 mg kg^{-1}). Concentrations of Rb in ECMs reached 22 ppm (with median value of circa 7 mg kg^{-1})
364 and were generally higher than those in the fine roots (median circa 2.3 mg kg^{-1}). It should be noted
365 that both *B. badius* and *P. involutus* included in the dataset (31% of all samples) are species with a
366 very high Rb/Cs accumulating ability in fruit-bodies (Klán et al., 1988).

367 Despite the fact that ENAA is considered suitable method for determination of Cs, Rb, Se, Th,
368 and U in biological samples (Chisela and Brätter, 1986; Chisela et al., 1986; Řanda et al., 2005),

369 concentrations of those elements were often near or below the detection limits. This was caused by
370 unexpectedly high levels of Sb in our samples, resulting in high activity of ^{124}Sb and subsequent
371 Compton effect which increased the detection limits. Concerning quality of our data, the results
372 obtained for the SRM NIST 1566b (Supplementary Table S7) were mostly in good agreement in the
373 certified values; slightly lower results were noted for Cu and slightly higher for V.
374 According to Kottke et al. (1998), ECMs of *B. badius* showed a higher potential to store N, P,
375 K, Mg, Fe, and Zn than other ECM types. In our study, *B. badius* appeared to be the most efficient
376 accumulator of Ag, Cd, Cu, and Cl. On the other hand, concentrations of Zn (mean 537 mg kg^{-1}) were
377 similar to those detected in *P. involutus* (mean 529 mg kg^{-1}) and *T. terresteris* (mean 492 mg kg^{-1}).
378
379 *3.4. Metal sequestration capacity of mycelia*
380
381 Metal tolerant ECM fungi can enhance fitness of the host plant in a metalliferrous
382 environment not only by improving the nutrition but also by altering the physiological response of
383 colonized roots to and actively protecting the plant against the toxicity of heavy metals (Urban, 2011;
384 Colpaert et al., 2011; Ma et al., 2014). Vinichuk (2013) recently reported a stepwise increase in the
385 concentration of Cd, Zn and Cu from the unpolluted soil to extraradical mycelia (respectively, 5-, 2.4
386 and 2 -fold increase relative to bulk soil) and to the fruit-bodies of ECM fungi (1.8-, 1.4 and 1.8-fold
387 compared to mycelia, respectively). However, there was no information about the metal content in
388 the mycelia directly colonizing plant roots. It is generally accepted that the protective metal barrier
389 function of the mycobiont can be achieved by biosorption onto cell wall and cellular uptake in the
390 hyphal mantle and Hartig net and/or exclusion of metals from ECMs (Bellion et al., 2006; Gadd, 2007;
391 Colpaert et al., 2011). The observation that ECMs tend to accumulate higher concentrations of
392 metals than non-mycorrhizal fine roots (Fig. 2) may indicate the sequestration of metals within the
393 hyphal structures.

394 The concentrations of Ag, Cd, Zn and Cu were markedly increased in ECMs of *B. badius* and
395 *A. muscaria* ECMs (Table 3). The expected concentrations of metals in the mycobionts shown in
396 Table 3 were calculated considering the mean proportion of the fungus in ECMs (Table 1) and the
397 difference in the levels of metals in ECMs and fine roots as accounting for the metal associated with
398 hyphae. Noteworthy, the mean Zn concentration calculated for *B. badius* are in good agreement with
399 2,600 mg Zn kg^{-1} reported in the fungal mantle of *S. luteus*/*P. sylvestris* ECM from metal polluted site
400 (Turnau et al., 2001), whilst the levels of Cd and Cu in both the *B. badius* and *A. muscaria* ECMs were
401 more than an order of magnitude higher.
402 Although the Cd, Zn and Cu concentrations calculated for *B. badius* are remarkably high, they
403 are still below or in similar range as the foliar concentrations accumulated and tolerated in
404 hyperaccumulating vascular plants – e.g., 9 to 19.6 g Zn kg^{-1} and 1.4 to 5.0 g Cd kg^{-1} in *Sedum alfredii*
405 (Xiong et al., 2004; Long et al., 2009) and 4.3 to 20 g Cu kg^{-1} in *Eleocharis acicularis* (Sakakibara et al.,
406 2011). Among macrofungi, the highest concentrations of these metals have been reported in wild-
407 grown fruit-bodies of *Russula atropurpurea* (1062 mg Zn kg^{-1}; Borovička and Řanda, 2007), *Agaricus*
408 *urinascens* (124 mg Cd kg^{-1}; Cocchi et al., 2006), and *Lycoperdon perlatum* (505 mg Cu kg^{-1}; Svoboda
409 et al., 2000). The highest concentration of Ag accumulated in a eukaryote under the natural
410 conditions was reported in fruit-bodies of *Amanita strobiliformis* (maximum 1,253 mg kg^{-1}; Borovička
411 et al., 2007).
412 It has been well documented that ECM fungi have efficient metal detoxification mechanisms
413 at their disposal that lie in the sequestration of excess heavy metals by compartmentalization or
414 intracellular complexation by cysteinyl-containing peptides that give rise to metal-thiolate
415 coordination ensuing on tight metal binding. Studies have indicated that the detoxification of Cd
416 (Blaudez et al., 2000; Ott et al., 2002; Sácký et al., 2014) and Zn (Turnau et al., 1995; Bucking and
417 Heyser, 1999; Ruytinx et al., 2013) in mycelia of ECM fungi largely relies upon the safe deposition of
418 the metals into vacuoles. In addition, *Hebeloma cylindrosporum* and *H. mesophaeum* can target Zn
419 into small non-vacuolar vesicles resembling yeasts zincosomes (Blaudez and Chalot, 2011; Sácký et

al., 2014). Among the family Boletaceae, *B. edulis* produces Cd-binding phytochelatins (PCs; [γ-Glu-Cys]$_2$Gly and [γ -Glu-Cys]$_3$Gly (Collin-Hansen et al., 2007), known as the major Cd-detoxification peptides in plants and some yeasts in which the cytosolic Cd-PC complex is transported into vacuoles for the maximum detoxification (Clemens and Simm, 2003).

Accumulating evidence suggests that the elimination of free Cd and Zn ions can also involve binding of the metals with the cytosolic, cysteine-rich metallothionein (MT) peptides produced in a response to the metal stress in various ECM species (Bellion et al., 2007; Ramesh et al., 2009; Osobová et al., 2011; Leonhardt et al., 2014; Sácký et al., 2014; Reddy et al., 2014, 2015). For example, binding with MT-like RaZBP, but not the compartmentalization, is a dominant Zn sequestration mechanism in Zn accumulating *Russula atropurpurea* (Leonhardt et al., 2014). *H. mesophaeum* has the specific Cd/Zn- inducible HmMT1 that complemented the compartmentalization of metals in mycelium exposed to high concentrations of Zn and Cd (Sácký et al., 2014). The detoxification of Ag in this species is dominated by other, Ag-inducible MTs, and MTs were identified as ligands that play a pivotal role in the sequestration of the intracellular Ag also in *Amanita submembranacea* (Borovička et al., 2010) and *A. strobiliformis* (Osobová et al., 2011). Although little is known about the handling of excess Cu in ECM fungi, studies have revealed that *P. involutus* (Bellion et al., 2007), *H. cylindrosporum* (Ramesh et al., 2009), *Laccaria bicolor* (Reddy et al., 2014), *Pisolithus albus* (Reddy et al., 2015), and *A. strobiliformis* (Hložková et al., 2015) highly express at least one MT gene when subjected to Cu exposure. The notion that binding with MTs could be involved in handling of the excess cellular Cu in ECM fungi is reinforced by the existence of Cu-MT complexes in *L. laccata* and *P. involutus* (Howe et al., 1997); moreover, the expression of the *P. involutus* Pi*MT1* conferred high Cu tolerance upon the transgenic *H. cylindrosporum* (Bellion et al., 2007).

Considering the metal tolerance of ECM fungi and a large surface offering cell wall chitin, glucans and proteins for biosorption of metals (Naja and Volesky, 2011), it appears reasonable to assume that the mycobiont may serve as a sink for the metals at the soil/ECM interface. However,

446 while there is the evidence that established ECM symbiosis can really reduce uptake of Cd, Zn or Cu
447 by the colonized plant (Adriaensen et al., 2005, 2006; Kozdrój et al., 2007; Krznaric et al., 2010;
448 Mrnka et al., 2012; Ma et al., 2014; Reddy et al., 2015), there are also studies that report the
449 opposite (Sell et al., 2005; Baum et al., 2006; Sousa et al., 2012).

450 In their elegant study, Ma et al. (2014) documented that the mycorrhization of the *Populus* ×
451 *canescens* with *P. involutus* elevated the expression of host genes coding for plasma membrane and
452 vacuolar transporters and PC synthase implicated in Cd uptake, detoxification, and flux towards
453 xylem for translocation to aboveground tissues; it was followed by a 20 to 30% increase in Cd
454 concentrations in the aerial parts of plants grown in the presence of 50 µM Cd. Considering this, we
455 cannot exclude the possibility that the elevated metal concentrations observed in *P. abies* ECMs
456 could be, at least in part, due to mycobiont-promoted flux of Ag, Cd, Zn and Cu into the root tissues.

457 In contrast, Reddy et al. (2015) recently reported that myccorhization of *Ecalyptus*
458 *tereticornis* with *Pisolithus albus*, in which Cd and Cu induced expression of the Ps*MT1* gene, reduced
459 the uptake of Cd and Cu in host tissues 3-fold, indicating metal barrier function of the mycobiont
460 facilitated by MT production. These studies thus indicate that the performance of ECM apparently
461 depends on both the metal and the host species and fungal isolates.

462 Ectomycorrhizae from polluted sites thus represent interesting subject for further studies.
463 Especially, micro-PIXE analysis might contribute to the knowledge of element distribution within
464 ectomycorrhizal tips (Turnau et al., 2001) and cultivable ECM species such as *P. involutus* are suitable
465 for studies of element-fungus-plant interactions in pot experiments (van Schöll et al., 2006).

466

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468

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Appendix A. Supplementary data

Supplementary data related to this article can be found at …….

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807 **Figure and Table captions**

808

809 **Fig. 1.** Investigated area at Lhota near Příbram (Czech Republic) and the sampling sites.

810

811 **Fig. 2.** Element concentrations (mg kg^{-1} in dry weight) in ectomycorrhizae, non-mycorrhizal fine roots,
812 nitric acid soil extracts and total soils from Lhota near Příbram presented in box plots.

813

814 **Table 1.** Concentrations of fungal biomass (% w/w of dry weight) in ectomycorrhizae of *Boletus*
815 *badius* (A) and *Amanita muscaria* (B) calculated from results of qRT-PCR (corrected for DNA
816 extraction efficiency) with a statistical summary (C).

817

818 **Table 2.** Concentrations of Cl in fruit-bodies (mg kg^{-1} in dry mass) from various sites and in
819 ectomycorrhizae from Lhota near Příbram presented as arithmetical mean and standard deviation.

820

821 **Table 3.** Concentrations of elements (mg kg^{-1} in dry mass) in roots and ectomycorrhizae and
822 calculated element concentrations in mycorrhizae-forming mycelia of *Boletus badius* and *Amanita*
823 *muscaria* at Lhota near Příbram.

824 **Table caption**. Expected concentrations of fungal biomass in ectomycorrhizae of *Boletus badius* and
825 *Amanita muscaria* were 10 and 7 % (w/w), respectively.

Table 1

A: *Boletus badius*				**B: *Amanita muscaria***			
weight	mass % biomass in ECM root			weight	mass % biomass in ECM root		
mg	ITS3-BadiusR	BadiusF-ITS2	average	mg	AmusF1-ITS2	ITS1F-AmusR1	average
1.39	20.5	16.9	18.7	3.38	9.25	7.54	8.40
3.18	1.46	1.37	1.42	2.36	23.5	18.4	21.0
2.73	22.6	22.0	22.3	0.79	7.97	7.04	7.51
4.02	0.56	0.44	0.50	1.79	4.63	2.70	3.67
14.1	2.20	2.10	2.15	1.29	5.24	4.39	4.81
11.4	5.87	5.60	5.73	0.68	9.23	6.57	7.90
3.44	12.7	11.5	12.1	5.50	2.68	2.04	2.36
1.74	3.81	1.81	2.81	3.14	13.3	9.62	11.4
3.89	11.5	10.1	10.8	1.93	0.91	0.71	0.81
3.76	38.4	35.2	36.8	3.47	1.58	1.46	1.52
9.38	4.98	4.26	4.62	10.7	3.92	3.68	3.80
13.9	1.53	1.26	1.40				
3.91	1.35	1.08	1.22				
2.54	15.4	12.8	14.1				
4.76	3.27	1.35	2.31				
6.56	15.6	12.3	13.9				
0.95	20.3	23.3	21.8				
1.37	6.03	3.99	5.01				
2.98	7.74	5.87	6.81				

C: Statistics, mass % biomass in ECM roots		
	Boletus	*Amanita*
minimum	0.50	0.81
median	5.73	4.81
mean	9.71	6.65
maximum	36.8	21.0

Table 2

species	fruit-bodies n	fruit-bodies Cl (mg kg^{-1})	ectomycorrhizae n	ectomycorrhizae Cl (mg kg^{-1})
Amanita muscaria	10	7021 ± 3229	2	1956 ± 261
Boletus badius	7	1339 ± 422	9	3632 ± 1267
Paxillus involutus	17	171 ± 70	7	2541 ± 292
Russula ochroleuca	4	2527 ± 626	1	3711
Thelephora terrestris	2	1340 ± 302	17	2799 ± 829

Table 3

	Roots			Ectomycorrhizae			ECM mycelium (calculated)		
	minimum	mean	maximum	minimum	mean	maximum	minimum	mean	maximum
B. badius	(n = 6)			(n = 9)					
Ag	0.69	7.25	9.95	47.7	175	358	402	1680	3767
Cd	6.10	19.3	34.5	22.1	170	333	166	1511	3157
Cu	<19.4	76.7	170	62.8	120	162	21	401	1105
Zn	129	284	415	224	537	713	695	2668	4683
Cl	586	819	1259	3651	4413	5614	30034	37127	44804
A. muscaria	(n = 2)			(n = 2)					
Ag	1.54	-	14.7	31.0	-	56.4	422	-	610
Cd	9.85	-	21.4	43.6	-	79.2	492	-	837
Cu	<24.3	-	27.0	37.6	-	38.7	178	-	354
Zn	191	-	324	236	-	321	281	-	2120
Cl	507	-	725	1695	-	2217	17478	-	22039
V	0.73	-	0.80	26.4	-	61.2	367	-	864

Figure 1
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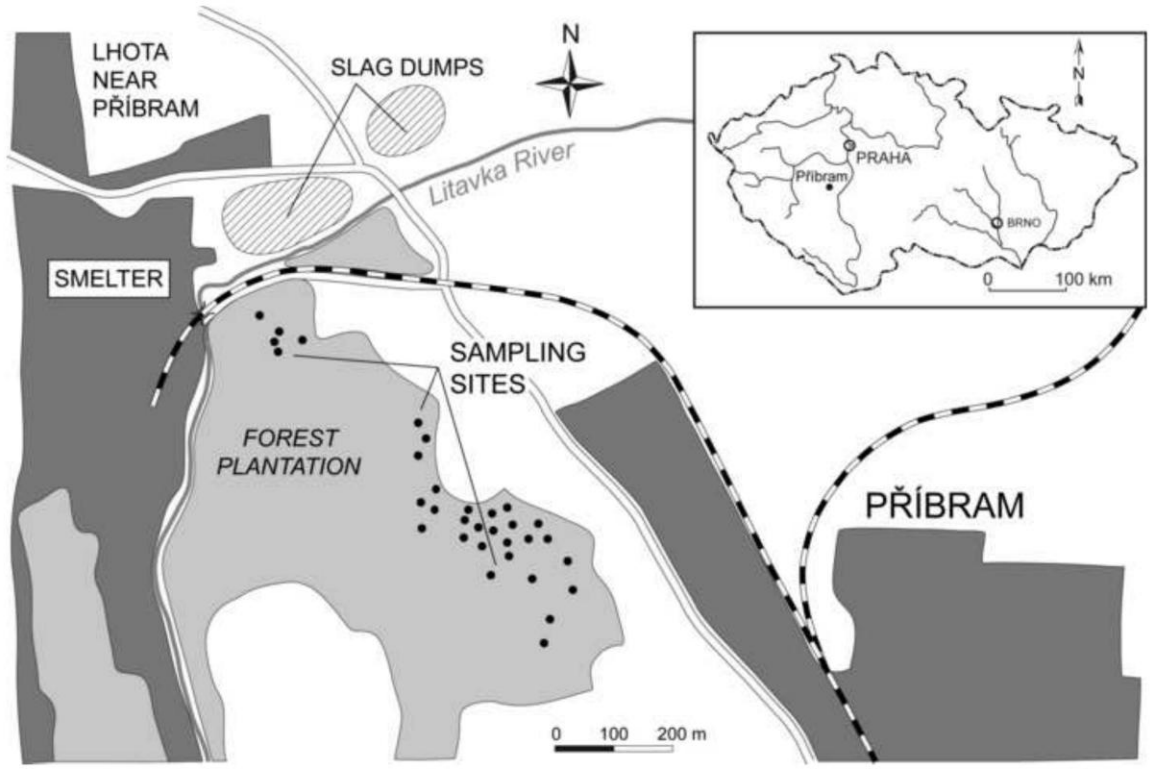

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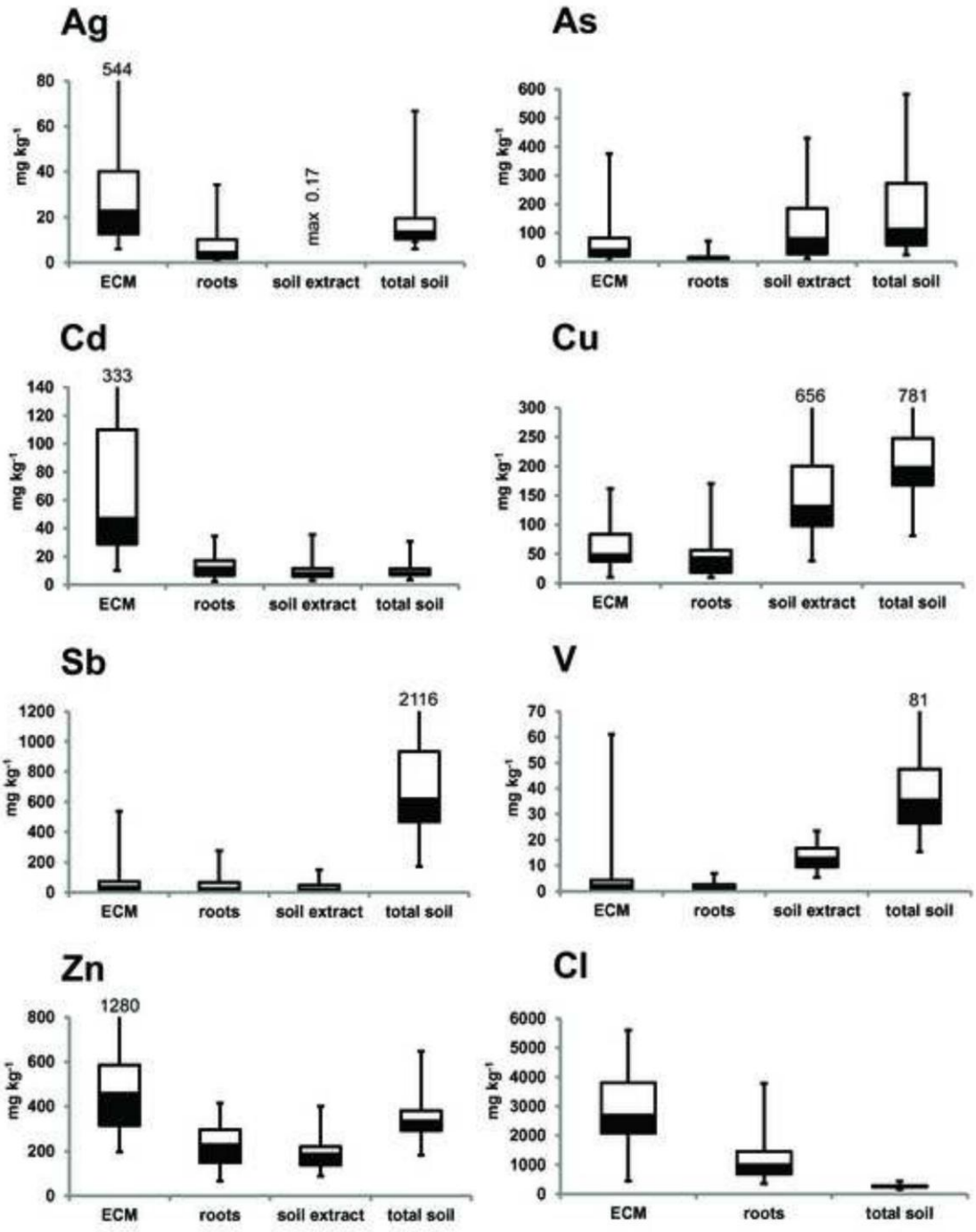

SUPPORTING INFORMATION

Bioaccumulation of heavy metals, metalloids, and chlorine in ectomycorrhizae from smelter-polluted area

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Supplementary Tables S1, S2, S3, S4, S5, S6, and S7.

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Supplementary Table S1. GPS coordinates of sites where samples of ectomycorrhizae, fine roots, and soils were collected. Approximate distribution of the sampling points within the area at Lhota near Příbram is depicted in Fig. 1.

Sampling points	GPS coordinates
313	N49 42.262 E13 59.507
314	N49 42.262 E13 59.485
316	N49 42.282 E13 59.483
317	N49 42.289 E13 59.471
318	N49 42.273 E13 59.456
319	N49 42.266 E13 59.461
320	N49 42.450 E13 59.211
321	N49 42.463 E13 59.154
334	N49 42.255 E13 59.458
335	N49 42.244 E13 59.529
336	N49 42.222 E13 59.520
355	N49 42.474 E13 59.129
356	N49 42.456 E13 59.151
358	N49 42.456 E13 59.149
359	N49 42.307 E13 59.429
361	N49 42.287 E13 59.468
368	N49 42.261 E13 59.532
369	N49 42.265 E13 59.509
370	N49 42.263 E13 59.506
371	N49 42.252 E13 59.501
372	N49 42.214 E13 59.514
373	N49 41.881 E13 58.688
375	N49 42.279 E13 59.476
376	N49 42.278 E13 59.414
377	N49 42.268 E13 59.389
378	N49 42.286 E13 59.324
379	N49 42.315 E13 59.341
389	N49 42.330 E13 59.298
390	N49 42.343 E13 59.319
391	N49 42.334 E13 59.331
392	N49 42.324 E13 59.353
393	N49 42.305 E13 59.332

Supplementary Table S2. Analyzed samples of ectomycorrhizae from the investigated site at Lhota near Příbram with indicated sample weight (mg dry mass). Closest BLAST matches of the ITS rDNA sequences, EMBL-Bank accession numbers and names of detected symbiotic fungal species are indicated.

weight (mg)	ID	EMBL-Bank	species	BLAST similarity
16.2	ECM 313a	-	*Thelephora terrestris*	91%, GU550115
11.8	ECM 313b	-	*Thelephora terrestris*	91%, GU550115
6.77	ECM 314a	LN877721	*Boletus badius*	100%, HM190050
6.61	ECM 316	LN877722	*Tomentella ellisii*	99%, HQ406823
8.59	ECM 317a	LN877723	*Thelephora terrestris*	100%, JX907820
6.31	ECM 318a	LM992869	*Thelephora terrestris*	100%, JQ711980
5.52	ECM 318b	LM992869	*Thelephora terrestris*	100%, JQ711980
9.81	ECM 319a	LN877724	*Thelephora terrestris*	100%, JQ711980
13.5	ECM 320a	LN877725	*Boletus badius*	100%, AJ889926
9.62	ECM 320b	LN877725	*Boletus badius*	100%, AJ889926
5.75	ECM 321a	LN877726	*Amphinema byssoides*	100%, JN943928
7.35	ECM 321b	LN877726	*Amphinema byssoides*	100%, JN943928
3.81	ECM 334/1	-	unidentified	-
9.66	ECM 334/2	-	unidentified	-
8.96	ECM 335	LN877727	*Thelephora terrestris*	100%, GQ267490
9.96	ECM 336a	LN877728	*Thelephora terrestris*	100%, FJ532478
12.7	ECM 336b	LN877728	*Thelephora terrestris*	100%, FJ532478
8.97	ECM 355a	LN877729	*Boletus badius*	99%, AJ889926
9.22	ECM 355b	LN877729	*Boletus badius*	99%, AJ889926
5.66	ECM 356/1	LM992873	*Boletus badius*	100%, HQ207696
8.46	ECM 356/2	LN877730	*Thelephora terrestris*	99%, JQ711981
14.8	ECM 358a	LN877731	*Boletus badius*	99%, AJ889926
10.7	ECM 358b	LN877731	*Boletus badius*	99%, AJ889926
4.90	ECM 359	LN877732	*Paxillus involutus*	99%, AJ438984
8.34	ECM 361	LN877733	*Tomentella* sp.	100%, FJ013069
6.62	ECM 368	LM992874	*Paxillus involutus*	100%, EU819416
9.01	ECM 369	LN877734	*Thelephora terrestris*	100%, HM189958
2.88	ECM 370	LN877735	*Thelephora terrestris*	100%, FJ532478
4.56	ECM 371/1	-	*Russula ochroleuca*	-
8.81	ECM 371/2a	LM992875	*Paxillus involutus*	99%, AJ438984
5.56	ECM 372	LN877736	*Paxillus involutus*	100%, EU078732
11.9	ECM 373	LN877737	*Thelephora terrestris*	100%, FJ532478
7.28	ECM 375a	-	unidentified	-
8.23	ECM 375b	-	unidentified	-
8.19	ECM 376	LN877738	*Thelephora terrestris*	100%, JQ711980
1.75	ECM 377/1	LM992876	*Cortinarius* aff. *olivaceofuscus*	98%, AY669585
4.75	ECM 377/2	-	unidentified	-
6.09	ECM 378	-	probably *Phiaocephala* sp.	-
2.47	ECM 379/1	LM992877	*Amanita muscaria*	99%, AB080983
9.22	ECM 379/2b	LN877739	*Tomentella* sp.	99%, DQ822832
0.51	ECM 389/2	LN877740	*Thelephora terrestris*	100%, JQ711980
2.67	ECM 389/3	LN877741	*Thelephora terrestris*	100%, JQ712012
4.25	ECM 389/4	-	unidentified	-
7.96	ECM 390/1	LN877742	*Tomentella sublilacina*	99%, HM189976
3.59	ECM 390/2	LM992871	*Paxillus involutus*	100%, JN673723
4.13	ECM 390/3	LN877743	*Tomentella sublilacina*	99%, HM189976
4.70	ECM 391/1	-	*Paxillus involutus*	91%, AJ438984
4.46	ECM 391/2	LN877744	*Boletus badius*	99%, AJ889926
5.61	ECM 391/3	-	*Paxillus involutus*	98%, AJ438984
3.61	ECM 392	LN877745	*Thelephora terrestris*	100%, HM189966
5.19	ECM 393 clean	LM992872	*Amanita muscaria*	100%, EF493267

Supplementary Table S3. Concentrations of elements (mg kg^{-1} in dry weight) in ectomycorrhizae from the investigated site at Lhota near Příbram.

ID	species	Ag	As	Cd	Cu	Sb	V	Zn	Cl	Co	Cs	Rb	Se	U	Th
ECM 313a	*Thelephora terrestris*	8.96	47.0	29.2	48.0	11.5	1.14	344	2441	1.55	0.09	5.36	< 0.72	0.05	< 0.05
ECM 313b	*Thelephora terrestris*	9.47	59.1	26.9	32.8	10.1	1.15	320	2548	1.18	0.13	5.71	< 0.91	0.05	< 0.06
ECM 314a	*Boletus badius*	181	376	86.8	102	539	8.10	713	3651	14.8	< 0.33	< 9.55	< 4.80	0.53	< 0.36
ECM 316	*Tomentella ellisii*	17.2	6.23	67.1	37.6	8.85	0.49	458	2269	1.17	< 0.12	6.76	< 1.60	0.11	< 0.11
ECM 317a	*Thelephora terrestris*	17.6	90.3	60.8	< 20.1	18.7	1.54	311	1914	1.25	< 0.10	< 2.14	< 1.30	0.10	< 0.09
ECM 318a	*Thelephora terrestris*	22.8	22.5	76.8	58.4	91.0	4.48	362	1436	2.85	< 0.17	< 4.84	< 2.39	0.21	< 0.18
ECM 318b	*Thelephora terrestris*	23.3	22.1	73.9	46.1	117	7.30	310	1658	2.63	< 0.21	< 5.26	< 2.83	0.23	< 0.21
ECM 319a	*Thelephora terrestris*	23.9	75.5	74.6	58.7	15.4	1.50	527	2822	3.11	0.12	7.19	< 1.17	0.10	< 0.08
ECM 320a	*Boletus badius*	47.7	40.6	239	96.9	48.1	1.55	593	3722	3.09	0.20	7.81	< 1.27	0.10	< 0.09
ECM 320b	*Boletus badius*	49.9	63.6	333	80.2	71.6	2.30	658	4037	3.49	0.18	8.20	< 1.74	0.10	< 0.13
ECM 321a	*Amphinema byssoides*	42.9	15.3	213	< 28.4	35.0	1.05	579	1764	2.11	< 0.16	< 4.31	< 2.05	0.11	< 0.15
ECM 321b	*Amphinema byssoides*	27.1	13.4	225	69.5	30.6	0.58	534	1831	2.36	< 0.13	< 3.52	< 1.78	0.09	< 0.12
ECM 334/1	unidentified	274	52.0	78.6	127	56.4	2.07	853	2311	3.35	< 0.32	12.6	< 3.60	0.12	< 0.26
ECM 334/2	unidentified	13.2	96.2	81.0	115	41.1	4.56	785	2774	3.59	< 0.11	6.03	< 1.42	0.12	< 0.10
ECM 335	*Thelephora terrestris*	9.49	28.3	36.0	24.4	7.04	0.77	323	2230	2.11	< 0.09	6.19	< 1.01	0.05	< 0.08
ECM 336a	*Thelephora terrestris*	30.1	53.0	26.9	< 28.9	8.99	2.40	496	2248	1.99	< 0.09	4.86	<1.07	0.10	< 0.08
ECM 336b	*Thelephora terrestris*	37.3	57.6	25.9	37.3	8.41	1.34	550	1987	2.62	< 0.08	5.60	< 0.91	0.10	< 0.07
ECM 355a	*Boletus badius*	385	119	169	155	269	3.19	469	4191	3.29	0.31	9.03	< 2.37	0.33	< 0.18
ECM 355b	*Boletus badius*	367	99.3	133	162	220	3.73	370	4495	2.82	0.24	7.44	< 2.16	0.29	< 0.17
ECM 356/1	*Boletus badius*	130	21.9	146	155	24.1	< 0.44	655	5614	1.57	0.20	8.44	2.61	< 0.05	< 0.12
ECM 356/2	*Thelephora terrestris*	22.3	107	119	84.7	28.5	< 0.41	1026	2875	1.29	< 0.09	6.83	< 1.11	< 0.05	< 0.08
ECM 358a	*Boletus badius*	160	26.2	162	134	49.3	1.77	621	4542	1.64	0.17	8.96	< 0.98	0.07	< 0.08
ECM 358b	*Boletus badius*	165	33.9	243	134	68.3	2.42	527	4674	1.70	0.18	6.38	1.33	0.09	< 0.10
ECM 359	*Paxillus involutus*	14.7	12.8	29.7	28.6	14.3	1.04	395	2359	0.66	0.22	10.1	< 1.61	0.11	< 0.09
ECM 361	*Tomentella* sp.	13.5	16.8	101	96.3	32.7	2.47	614	3780	1.54	0.19	10.2	< 1.10	0.13	< 0.08
ECM 368	*Paxillus involutus*	8.19	5.01	40.0	73.3	12.2	1.16	677	2381	2.63	0.17	8.32	< 1.21	0.06	< 0.07
ECM 369	*Thelephora terrestris*	7.44	124	24.6	33.2	5.75	< 0.42	446	4303	0.93	0.09	9.17	< 0.80	< 0.04	< 0.06
ECM 370	*Thelephora terrestris*	10.1	120	32.4	< 38.6	5.13	< 0.97	564	3214	1.70	< 0.20	< 5.81	< 2.32	< 0.09	< 0.18
ECM 371/1	*Russula ochroleuca*	11.1	16.7	76.2	68.5	36.3	2.53	558	3711	2.72	< 0.15	4.59	< 1.97	0.12	< 0.12
ECM 371/2a	*Paxillus involutus*	5.93	3.30	24.2	59.2	3.08	< 0.59	516	2078	0.78	< 0.07	7.30	< 0.78	0.06	< 0.06
ECM 372	*Paxillus involutus*	14.9	23.9	14.8	73.6	39.8	4.53	413	2683	2.28	0.28	8.11	< 1.74	0.26	0.42
ECM 373	*Thelephora terrestris*	6.08	52.2	10.0	32.0	24.4	9.17	239	3793	1.79	0.43	20.2	< 0.81	0.28	0.15
ECM 375a	unidentified	27.0	40.3	23.3	44.7	53.9	3.49	236	650	2.13	0.12	< 3.38	2.17	0.13	< 0.11
ECM 375b	unidentified	31.7	32.7	17.4	47.8	85.1	4.20	209	436	1.41	< 0.11	< 3.55	2.24	0.14	< 0.09
ECM 376	*Thelephora terrestris*	9.54	74.0	46.6	48.4	44.7	3.41	535	3998	1.51	0.19	6.99	< 1.16	0.10	< 0.10
ECM 377/1	*Cortinarius* aff. *olivaceofuscus*	25.6	36.4	41.2	< 45.5	91.8	9.85	285	4342	< 2.05	1.23	16.3	< 4.55	0.39	< 0.37
ECM 377/2	unidentified	19.6	43.6	67.7	50.6	101	6.96	349	5183	0.94	0.38	11.7	< 1.83	0.21	0.17
ECM 378	probably *Phiaocephala* sp.	20.7	18.5	42.2	29.8	85.5	5.25	308	812	1.07	< 0.13	< 4.45	1.73	< 0.07	< 0.14
ECM 379/1	*Amanita muscaria*	31.0	24.6	43.6	38.7	71.5	26.4	236	2217	2.00	0.28	< 8.76	< 3.27	0.15	0.61
ECM 379/2b	*Tomentella* sp.	17.4	59.3	32.6	81.7	25.7	2.28	415	2082	0.74	0.20	5.01	< 0.98	0.16	0.28
ECM 389/2	*Thelephora terrestris*	18.2	209	47.0	< 79.3	15.3	< 1.96	531	3365	5.48	< 1.04	< 20.3	< 12.1	< 0.30	< 0.73
ECM 389/3	*Thelephora terrestris*	544	118	181	75.8	318	5.41	1280	2937	5.74	0.54	< 9.73	< 5.81	< 0.19	< 0.39
ECM 389/4	unidentified	12.1	8.28	46.4	43.2	213	5.45	303	898	4.12	0.22	< 5.02	< 3.34	< 0.11	< 0.23
ECM 390/1	*Tomentella sublilacina*	9.80	170	18.1	45.7	85.3	3.29	239	888	0.86	0.13	< 2.58	1.78	0.15	0.17
ECM 390/2	*Paxillus involutus*	36.5	40.6	137	123	27.9	1.65	1031	2700	2.24	0.61	20.0	< 2.16	0.11	< 0.14
ECM 390/3	*Tomentella sublilacina*	36.6	151	138	115	58.6	3.59	867	3907	1.68	0.40	10.7	< 2.17	0.11	< 0.15
ECM 391/1	*Paxillus involutus*	18.0	4.60	40.3	67.9	14.5	< 0.50	458	3068	< 0.69	0.45	12.8	< 1.78	< 0.04	< 0.11
ECM 391/2	*Boletus badius*	90.8	3.98	22.1	62.8	6.59	< 0.50	224	4788	0.89	1.18	17.1	< 1.69	< 0.04	< 0.11
ECM 391/3	*Paxillus involutus*	10.9	3.24	20.4	45.7	11.9	0.86	216	2519	0.67	0.62	15.3	< 1.24	< 0.04	< 0.08
ECM 392	*Thelephora terrestris*	34.0	150	28.7	< 28.2	23.9	1.28	197	3823	< 0.91	< 0.16	5.25	< 1.96	< 0.08	< 0.14
ECM 393 clean	*Amanita muscaria*	56.4	24.8	79.2	37.6	34.7	61.2	321	1695	1.08	0.62	22.0	< 1.66	0.08	0.13

Supplementary Table S4. Concentrations of elements (mg kg^{-1} in dry weight) in non-mycorrhizal fine roots of *Picea abies* from the investigated site at Lhota near Příbram.

sample	weight (mg)	Ag	As	Cd	Cu	Sb	V	Zn	Cl	Cs	Rb	Co	Se	U	Th
KOR - 313	40.2	4.53	27.0	2.41	33.3	59.4	3.05	76,8	1031	< 0.05	3.32	0.42	< 0.65	0.16	< 0.05
KOR - 314	52.1	6.35	72.9	15.7	46.3	202	3.29	374	641	< 0.09	< 2.86	2.19	< 1.14	0.28	0.11
KOR - 316	43.3	18.2	7.73	19.9	44.0	29.9	1.32	340	1409	< 0.04	2.96	0.77	0,78	0.06	< 0.04
KOR - 317	45.7	14.1	10.8	11.4	52.3	37.4	2.51	337	1438	< 0.05	3.12	0.76	1,08	0.10	< 0.05
KOR - 318	47.2	10.0	18.5	11.9	86.5	162	5.26	228	1932	< 0.08	3.78	1.01	1,91	0.68	< 0.08
KOR - 319	58.8	6.99	7.91	20.5	51.6	12.1	0.67	355	792	0.04	1.42	1.37	< 0.31	0.03	< 0.03
KOR - 320	48.1	8.35	71.1	19.4	83.1	140	2.28	245	798	< 0.08	< 2.55	0.52	1,34	0.30	< 0.08
KOR - 321	59.0	1.96	6.47	33.3	34.3	16.4	0.47	296	901	< 0.03	1.36	0.58	1,29	0.03	< 0.03
KOR - 334	47.4	29.2	41.3	16.8	81.0	158	3.90	313	650	0.11	3.19	1.03	< 1.05	0.69	< 0.09
KOR - 335	54.5	13.2	41.7	3.85	61.1	165	5.82	163	569	0.09	< 2.66	1.44	1,06	1,32	0.12
KOR - 336	48.1	1.52	6.74	11.5	44.2	9.46	< 0.64	215	402	< 0.03	3.22	0.94	0,34	0.04	< 0.03
KOR - 355	35.1	9.35	44.8	23.2	170	277	4.52	334	700	0.12	< 3.00	1.15	3,37	0.40	0.16
KOR - 356	59.5	8.84	7.33	17.0	49.0	18.9	0.53	207	1259	0.03	1.84	0.40	0,57	0.02	< 0.02
KOR - 358	42.8	9.95	13.4	34.5	92.1	9.95	2.06	415	586	0.06	< 1.76	0.65	2,06	0.09	0.07
KOR - 359	38.7	4.80	50.3	17.4	99.0	168	6.89	276	489	0.05	< 1.48	0.88	1,64	0.25	0.16
KOR - 361	61.9	34.3	6.72	4.08	49.2	32.2	2.73	101	994	0.05	1.67	0.42	1,27	0.15	0.04
KOR - 368	46.0	11.5	9.57	11.7	< 21.6	20.0	< 0.43	293	2498	0.04	1.41	0.81	< 0.29	0.07	< 0.02
KOR - 369	58.3	10.3	5.92	12.0	29,7	5.17	< 0.60	214	3788	0.02	1.92	0.78	0,27	0.03	0.03
KOR - 370	41.8	1.07	6.03	7.38	< 27.7	11.0	0.73	145	3701	< 2.03	2.67	0.62	< 0.23	0.03	0.02
KOR - 371	37.3	1.90	1.80	5.36	70,0	5.49	< 0.80	101	3723	0.08	8.60	0.63	0,25	0.03	0.02
KOR - 372	50.5	0.52	5.56	2.16	< 19.8	2.79	< 0.45	66	1992	0.02	2.21	0.72	< 0.13	0.05	< 0.01
KOR - 373	50.2	0.85	9.76	4.41	43.5	4.27	0.87	204	3243	0.06	4.18	1.24	< 0.17	0.06	0.02
KOR - 375	46.4	3.10	12.5	12.3	< 27.1	17.2	1.51	241	1170	0.03	2.82	0.69	0,27	0.03	< 0.02
KOR - 376	44.6	4.58	11.9	6.04	27.7	22.8	4.73	152	2060	0.07	5.30	0.49	0,73	0.06	< 0.02
KOR - 377	48.0	33.5	13.2	10.5	71.2	72.1	1.44	167	1133	0.08	2.95	0.55	< 0.46	0.18	< 0.04
KOR - 378	41.4	3.81	8.38	9.75	34.3	24.0	1.90	240	777	0.05	2.28	0.34	0,88	0.02	< 0.02
KOR - 379	41.5	1.54	7.57	9.85	< 24.3	11.6	0.73	191	725	0.05	2.18	0.36	< 0.25	0.03	< 0.02
KOR - 389	46.0	1.85	9.71	7.14	< 21.6	25.2	0.67	113	359	0.05	2.25	0.37	0,69	0.02	< 0.02
KOR - 390	42.3	0.91	11.4	16.9	< 22.4	23.2	0.60	271	546	0.05	0.75	0.55	0,44	0.03	< 0.02
KOR - 391	42.9	0.69	8.66	6.10	< 19.4	9.76	< 0.37	129	930	0.05	2.70	0.31	< 0.23	< 0.01	< 0.02
KOR - 392	47.2	0.83	12.5	18.3	< 31.6	2.48	< 0.71	143	1269	< 0.01	1.81	0.66	< 0.16	< 0.01	< 0.11
KOR - 393	48.4	14.7	61.0	21.4	27.0	115	0.80	324	507	0.10	3.60	0.72	< 0.53	0.05	< 0.04

Supplementary Table S5. Total element concentrations (mg kg^{-1} in dry weight) in organic soil samples (Oe horizon) from the investigated site at Lhota near Příbram.

soil sample	Ag	As	Cd	Cu	Sb	V	Zn	Cl	Co	Cs	Rb	Se	U	Th
313	10.4	291	6.80	230	833	68.5	409	302	3.98	2.65	19.5	14.7	3.27	2.44
314	12.5	422	5.10	218	1053	60.3	335	265	4.98	2.94	29.0	7.54	3.75	3.36
316	10.2	32.9	10.2	137	300	21.7	247	292	1.83	0.82	7.05	6.34	0.79	0.84
317	13.9	90.2	8.50	170	471	35.6	305	273	3.20	1.68	14.7	9.00	1.62	1.71
318	14.5	130	10.2	207	537	44.3	350	260	3.69	1.97	17.4	11.6	1.43	2.07
319	8.46	463	8.50	260	1193	81.2	385	208	6.27	4.16	30.9	11.6	5.42	4.12
320	17.0	92.8	20.4	251	793	26.6	352	254	2.71	1.26	10.9	10.4	1.51	1.48
321	16.1	59.9	27.2	278	824	16.9	341	436	1.81	0.77	6.95	15.0	< 1.14	0.80
334	12.2	583	8.50	245	1120	63.6	458	318	5.20	3.04	21.9	9.77	4.56	3.35
335	9.19	278	5.10	149	566	71.5	285	250	4.99	2.64	25.3	6.69	3.54	2.82
336	10.6	302	5.10	158	620	61.9	368	342	4.87	2.26	20.1	7.66	4.54	2.58
355	66.7	304	17.0	781	2116	45.8	646	271	4.74	1.92	< 13.0	15.9	2.10	2.10
356	25.0	58.4	23.8	374	1008	17.1	328	287	2.02	0.80	< 8.81	16.7	< 0.73	0.70
358	42.2	112	30.6	679	1525	32.5	445	349	2.49	1.12	8.48	21.8	0.98	0.89
359	16.0	48.5	8.50	172	469	25.1	272	250	2.29	1.08	9.04	8.89	0.95	1.09
361	21.3	257	6.80	273	860	47.2	477	304	3.90	2.24	21.3	9.29	3.66	2.57
368	6.77	95.5	5.10	198	495	44.9	267	232	2.94	1.69	13.6	9.89	1.53	1.55
369	13.6	267	5.10	202	826	50.5	370	217	3.88	2.41	19.7	8.65	3.70	2.61
370	7.87	49.3	8.50	130	371	28.3	263	216	2.38	1.06	8.89	7.27	1.14	1.06
371	11.6	112	6.80	223	549	36.3	304	254	2.97	1.62	12.0	9.16	2.04	1.47
372	10.2	177	5.10	184	530	48.0	302	268	3.72	2.15	19.3	5.90	3.68	1.99
373	5.89	41.5	3.40	81	171	32.8	182	236	3.02	1.67	11.9	4.41	1.69	1.74
375	10.3	32.9	5.10	111	305	20.7	215	212	1.83	0.85	8.67	6.32	0.78	0.91
376	14.4	33.8	8.50	150	350	26.6	335	204	2.29	0.94	8.34	6.92	0.94	0.88
377	22.6	316	10.2	305	1183	50.0	432	292	4.17	2.52	15.1	7.60	5.35	2.49
378	20.6	79.2	10.8	241	584	36.9	545	345	2.63	1.54	9.85	13.0	1.07	1.09
379	12.4	183	7.65	188	656	33.1	247	180	3.50	2.00	16.8	9.91	1.94	2.06
389	20.0	96.6	8.50	220	679	28.9	315	278	2.43	1.35	15,4	11.6	1.08	1.39
390	12.9	55.7	13.6	166	533	18.1	319	262	1.87	0.88	6.20	9.66	0.91	0.95
391	37.3	226	10.2	333	1307	39.7	399	264	3.65	1.75	15.5	18.7	2.32	1.73
392	21.8	157	11.9	173	737	28.7	305	235	2.92	1.55	10.5	13.9	1.67	1.44
393	18.7	316	23.8	198	1277	33.1	364	387	3.62	1.53	< 11.0	7.59	1.46	1.83

Supplementary Table S6. Nitric acid-extractable fractions (mg kg^{-1} in dry weight) of elements in organic soil samples (Oe horizon) from the investigated site at Lhota near Příbram.

soil sample	Ag	As	Cd	Cu	Sb	V	Zn
313	0.02	187	4.10	166	78.3	23.5	130
314	0.10	298	4.06	137	94.3	14.2	112
316	0.07	17.7	8.80	87.0	5.14	9.00	202
317	0.15	55.9	8.03	102	12.9	11.0	186
318	0.03	88.1	7.88	139	18.2	15.1	190
319	0.02	316	4.86	203	149	20.7	109
320	0.04	53.4	20.5	198	13.1	9.57	236
321	0.02	24.5	32.0	212	11.6	6.57	268
334	0.04	430	5.73	203	113	18.4	159
335	0.02	190	2.88	94.4	51.9	18.4	89
336	0.05	229	4.46	113	60.2	19.0	143
355	0.17	198	15.8	656	85.3	20.0	215
356	0.03	28.0	28.5	288	15.9	7.01	255
357	0.04	80.6	14.6	120	9.18	6.66	288
358	0.09	56.5	35.5	538	41.4	16.7	306
359	0.02	24.8	8.28	129	10.9	10.4	176
360	0.01	10.4	8.58	65.7	3.82	5.44	223
361	0.03	185	5.79	217	36.2	16.8	198
368	0.01	53.9	5.74	123	19.8	18.8	146
369	0.06	187	5.47	149	42.4	16.5	151
370	0.02	26.7	9.51	78.9	8.62	11.1	192
371	0.04	66.0	7.33	121	21.7	12.8	158
372	0.10	103	4.33	89.6	21.6	13.9	134
373	0.00	17.7	2.94	38.0	3.50	11.2	102
374	0.08	79.5	7.13	123	11.6	12.8	190
375	0.04	14.9	7.10	69.9	5.19	7.93	159
376	0.10	13.9	10.5	87.7	7.26	12.7	284
377	0.10	214	9.81	245	62.1	21.1	197
378	0.07	41.5	12.6	155	18.1	15.9	401
379	0.04	128	7.92	88.7	17.6	8.37	116
389	0.06	60.0	8.94	137	13.6	9.29	169
390	0.01	24.6	15.1	116	7.58	6.67	245
391	0.07	103	8.42	225	38.1	14.1	132
392	0.05	93.4	7.30	132	20.5	10.1	116
393	0.05	180	27.0	140	55.3	10.5	216

Supplementary Table S7. Element concentrations (mg kg^{-1} in dry weight) obtained for the standard reference material NIST 1566b (Oyster tissue). The results are based on 4 independent measurements and presented as arithmetic mean with standard deviation.

element	certified value (mg kg^{-1})	results (mg kg^{-1})
As	7.65 ± 0.65	7.23 ± 0.70
Cd	2.48 ± 0.08	2.58 ± 0.24
Co	0.371 ± 0.009	0.39 ± 0.02
Cl	5140 ± 100	4934 ± 165
Cu	71.6 ± 1.6	65.1 ± 4.38
Rb	3.26 ± 0.14	3.05 ± 0.15
Se	2.06 ± 0.15	1.96 ± 0.06
Ag	0.666 ± 0.009	0.65 ± 0.03
Th	0.0367 ± 0.0043	0.0390 ± 0.002
V	0.577 ± 0.023	0.67 ± 0.03
Zn	1424 ± 46	1398 ± 40
	reference value (mg kg^{-1})	**results (mg kg^{-1})**
Sb	0.011 ± 0.002	0.0133 ± 0.003
U	0.255 ± 0.0014	0.259 ± 0.02