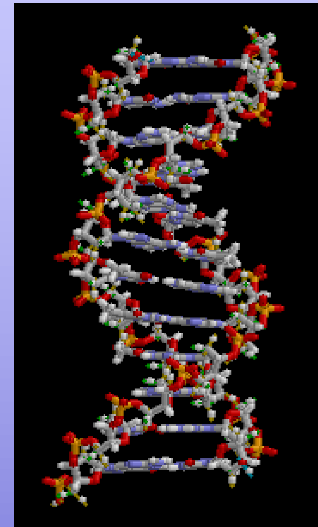
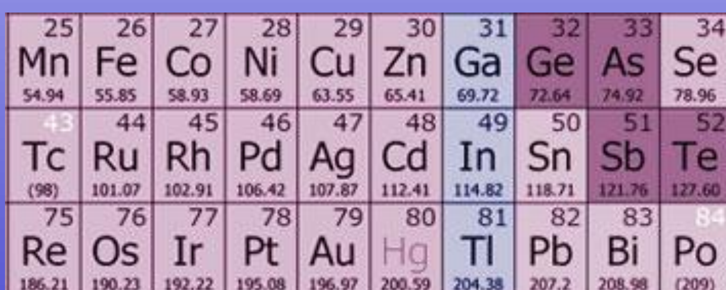
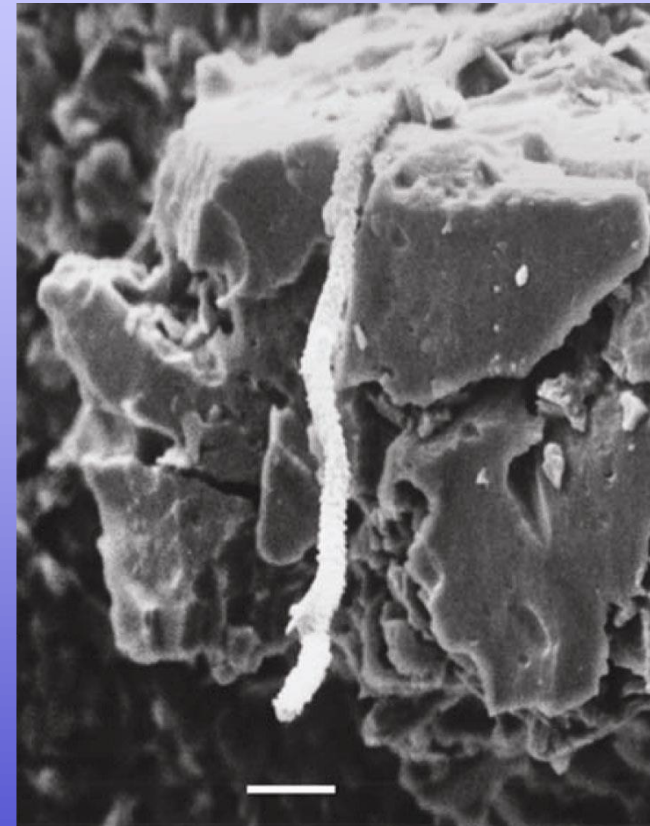
GEOMYKOLOGIE VI.

(houby a arzén)



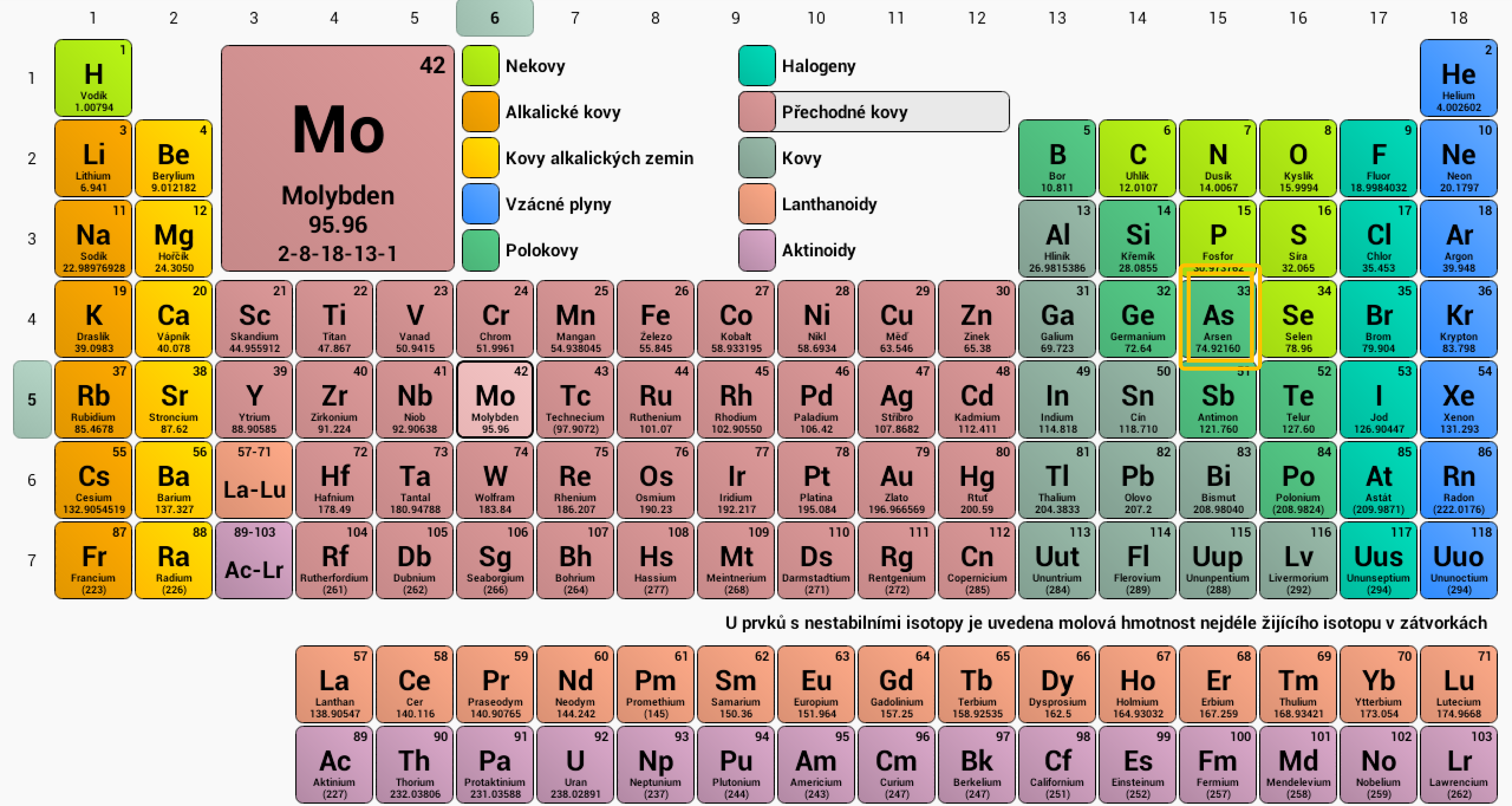


25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.41	31 Ga 69.72	32 Ge 72.64	33 As 74.92	34 Se 78.96
43 Tc (98)	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.76	52 Te 127.60
75 Re 186.21	76 Os 190.23	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po (209)



RNDr. Jan Borovička, Ph.D.

Ústav jaderné fyziky AV ČR / Geologický ústav AV ČR



Arzén je prvek patřící mezi POLOKOVY neboli METALOIDY.

Problematická skupina prvků kombinující vlastnosti kovů (pevnost a lesk) a nekovů (vyšší elektronegativita, tj. vyšší schopnost přitahovat vazebné elektrony)

ARZÉN / ARSEN

Chemická značka As, latinsky *Arsenicum*.

Chalkofilní prvek, polokov (metaloid).

V přírodě monoizotopní prvek (^{75}As, 100% zastoupení)

Lidstvu známý tisíce let – zejména díky své toxicitě.

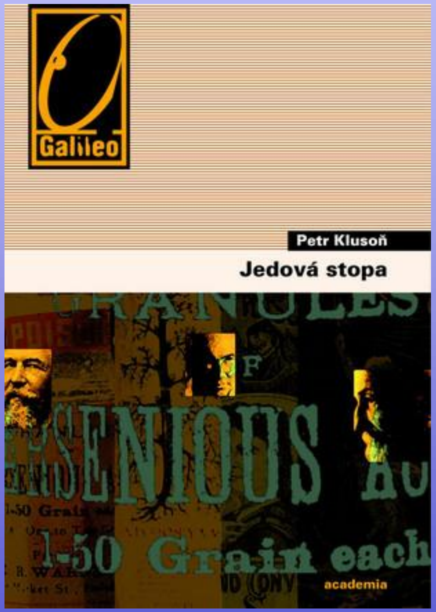
Problémy při zpracování rud: arzén se přidával k vylepšení tvrdosti mědi v oblastech, kde nebyl k dispozici cín. Vznik toxického těkavého As_2O_3.

Řecký bůh kovářství Hefaistos je často ošklivý a kulhá, že by důsledek chronické otravy arzénem?

Z historie travičství

Oxidu arsenitému (As_2O_3) staří Řekové říkali arsenikon, což v jejich jazyce znamenalo **mužný** nebo **silný**. Z této substance se v průběhu staletí stal jednoznačně nejzneužívanější jed lidské historie (otrušík, utrejch).

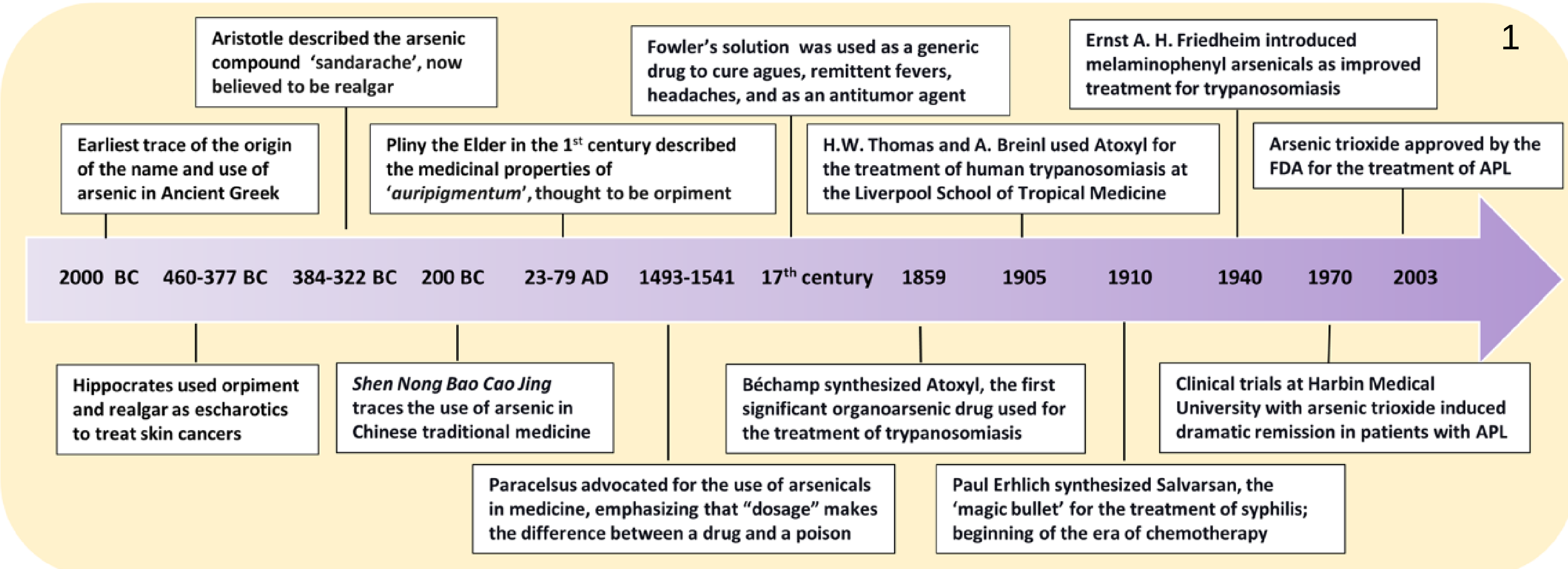
- nenápadnost (bílý prášek bez chuti rozpustný ve vodě i alkoholu)
- všeobecná dostupnost
- jednoduchá příprava
- smrtelná dávka pro člověka ~120 mg (orálně)
- s lékařskou pomocí lze přežít i 9 000 mg[1]



V období 1820–1860 bylo v Anglii, Německu, Španělsku a Francii na 1000 kriminálních případů otrav, 350 až 500 spojených přímo s oxidem arsenitým.

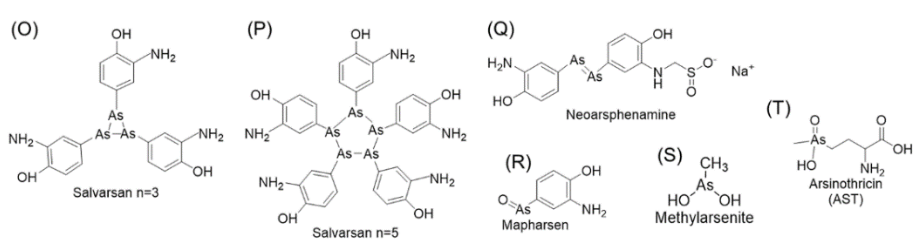
[1]Vantroyen et al. (2004) J Toxicol Clin Toxicol. 42: 889-895

Využití arzénu v medicíně



Využití v medicíně již od roku 2000 př.n.l., prakticky skončilo objevem antibiotik, ale existují i protinádorové účinky sloučenin arzénu.

3. Antibiotics/antimicrobials [1]



Arsfenamin (Salvarsan, sloučenina 606) – první moderní antimikrobiální chemoterapeutikum, účinná léčba syfilis.

[1] Paul et al. (2023) Biometals (2023) 36:283–301.

Arzén jako biogenní prvek?

Biogenní prvky jsou takové, které jsou nezbytné pro život.

FOSFOR: vyskytuje se v kostech, zubech, v ATP (adenosintrifosfát) a v nukleových kyselinách (DNA, RNA). **ARZÉN** má některé vlastnosti a chemické chování společné s fosforem.

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Nov. 29, 2010

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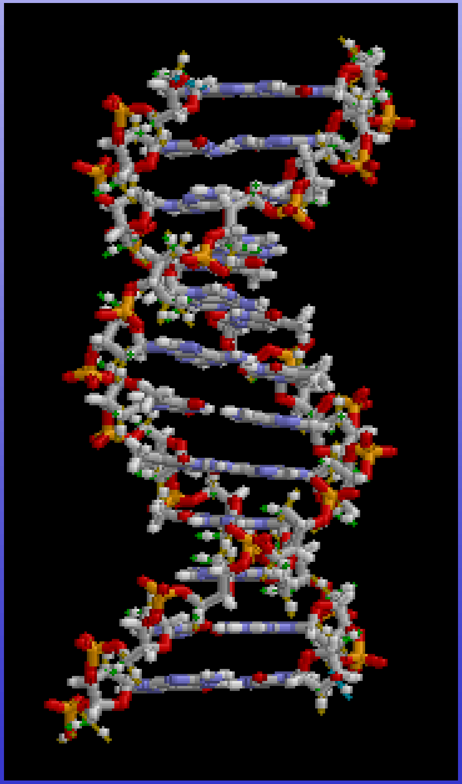
MEDIA ADVISORY : M10-167

NASA Sets News Conference on Astrobiology Discovery; Science Journal Has Embargoed Details Until 2 p.m. EST On Dec. 2

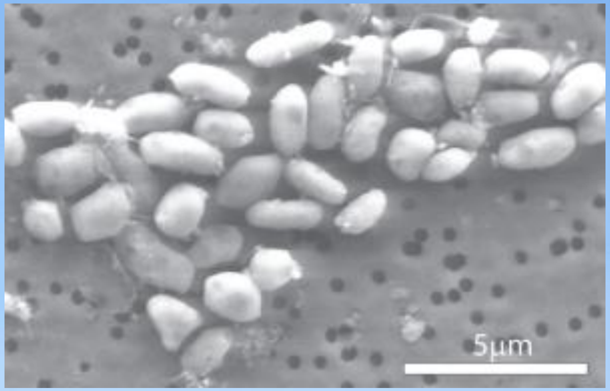
WASHINGTON -- NASA will hold a news conference at 2 p.m. EST on Thursday, Dec. 2, to discuss an astrobiology finding that will impact the search for evidence of extraterrestrial life. Astrobiology is the study of the origin, evolution, distribution and future of life in the universe.

The news conference will be held at the NASA Headquarters auditorium at 300 E St. SW, in Washington. It will be broadcast live on NASA Television and streamed on the agency's website at http://www.nasa.gov.

Participants are:
- Mary Voytek, director, Astrobiology Program, NASA Headquarters, Washington
- Felisa Wolfe-Simon, NASA astrobiology research fellow, U.S. Geological Survey, Menlo Park, Calif.
- Pamela Conrad, astrobiologist, NASA's Goddard Space Flight Center, Greenbelt, Md.
- Steven Benner, distinguished fellow, Foundation for Applied Molecular Evolution, Gainesville, Fla.
- James Elser, professor, Arizona State University, Tempe

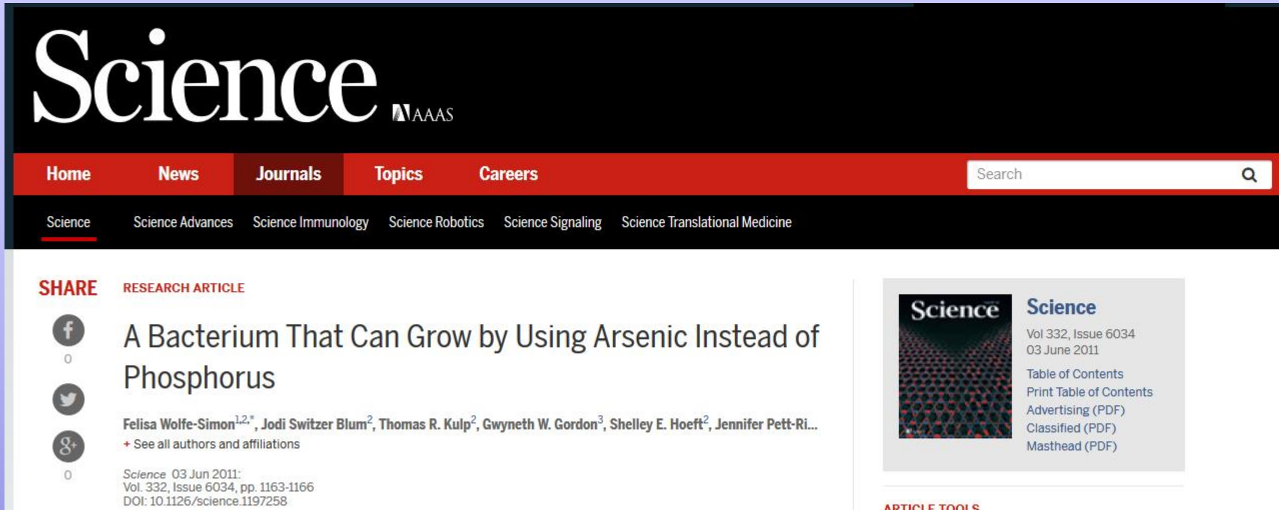


Jezero Mono
CA, USA

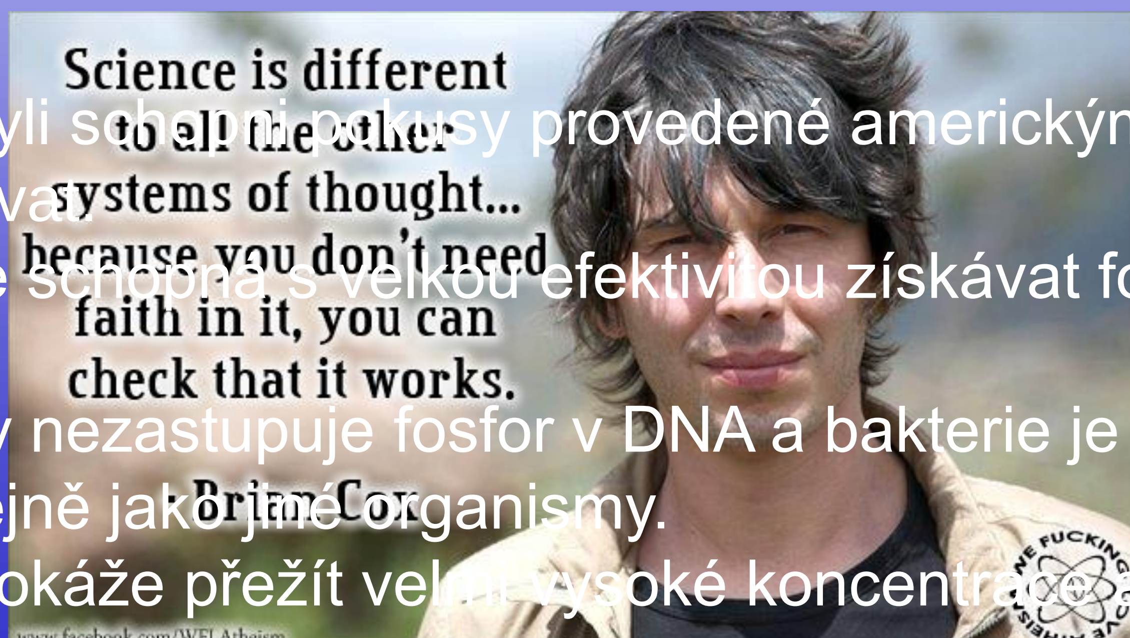


GFAJ-1 bakterie mohou žít při vysokých koncentracích As v prostředí

Arzén jako biogenní prvek?



Bakterie GFAJ-1 dokáže využít arzén jako prvek zastupující fosfor v DNA.



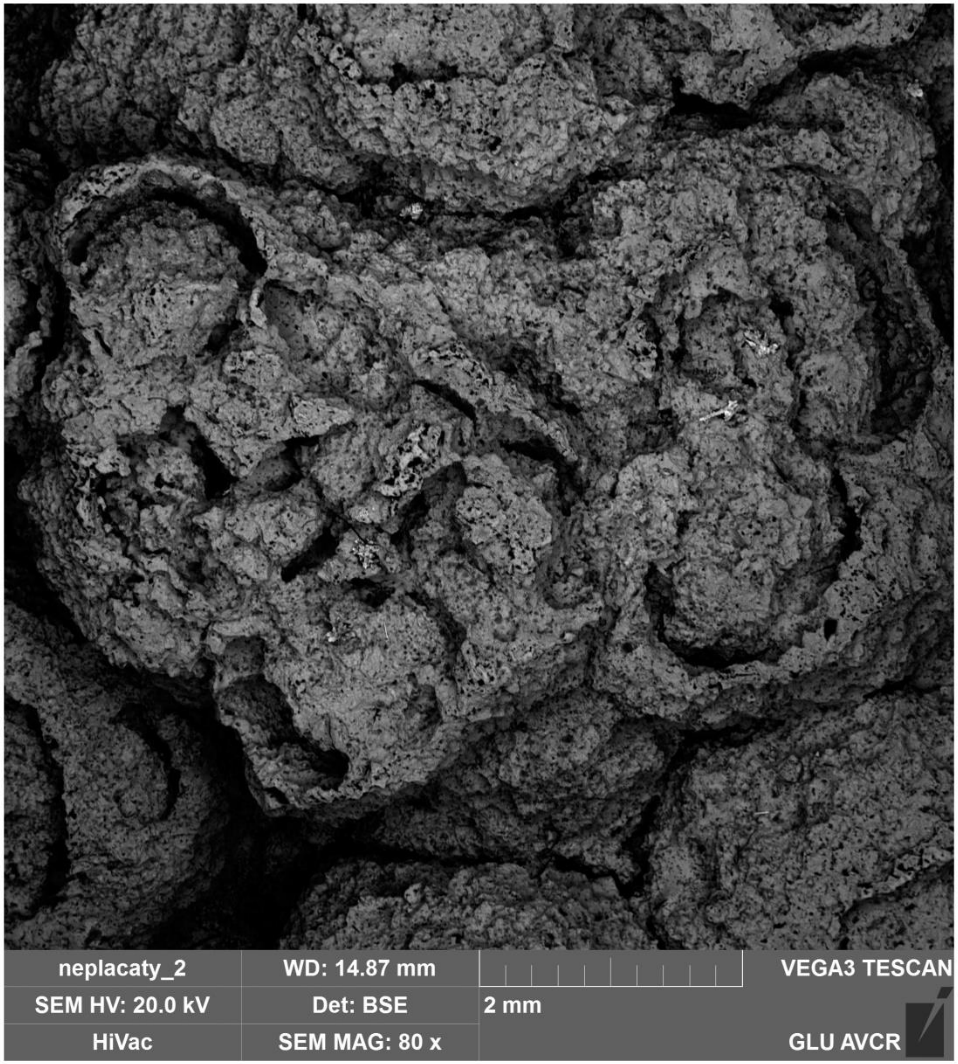
- Vědci nebyli schopni pokusy provedené americkým týmem reprodukovat.
- Bakterie je schopná s velkou efektivitou získávat fosfor z prostředí.
- Arzén tedy nezastupuje fosfor v DNA a bakterie je na fosforu závislá stejně jako jiné organismy.
- Bakterie dokáže přežít velmi vysoké koncentrace arzénu.

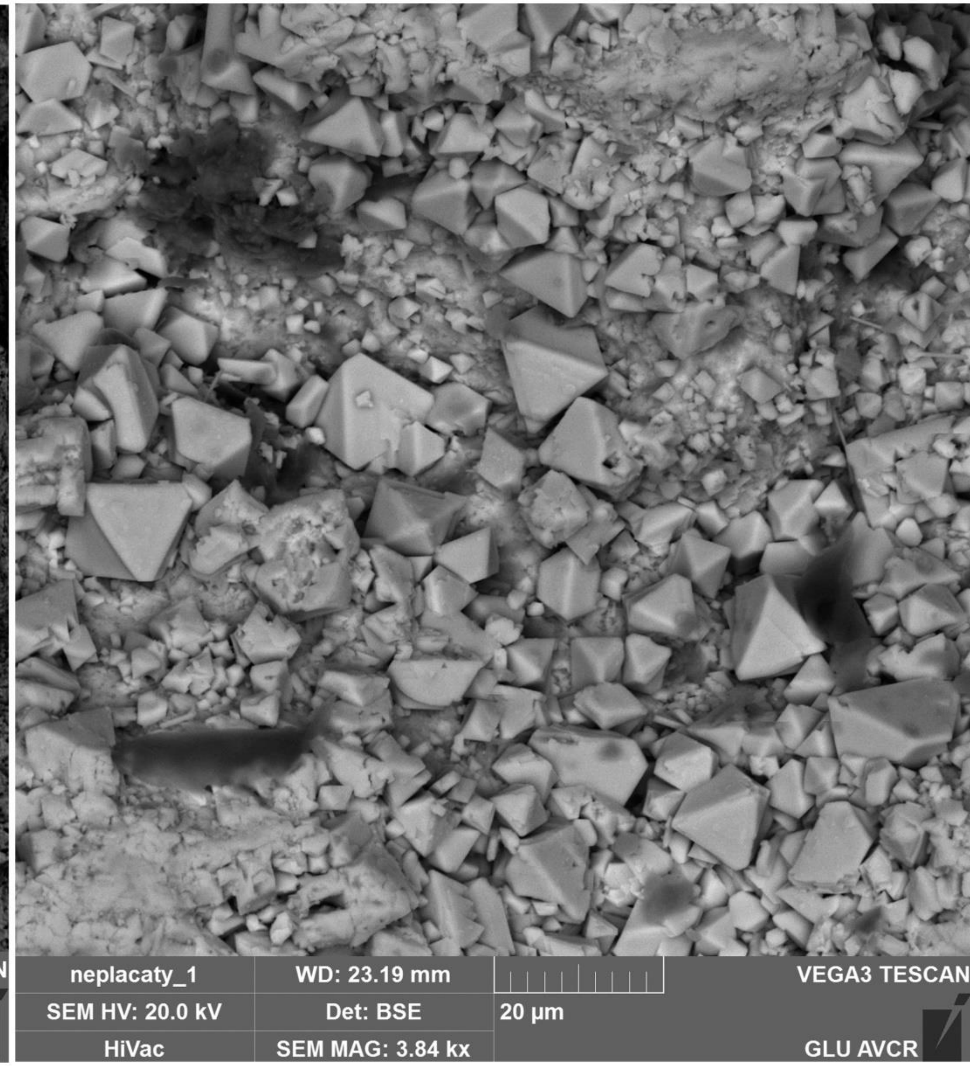
Česko – země arzénu zaslíbená

foto
Wikipedie

Pec pod Sněžkou, Kutná Hora (Kaňk), Roudný, Mokrsko, Příbram, Jáchymov, Jílové a mnoho dalších lokalit…

Vzorek k prohlídce: ryzí arzén





Výskyt arzén v životním prostředí

PŘIROZENÝ VÝSKYT

- Rudní minerály arzénu: arzenopyrit, stibarsen (allemontit), ryzí arzén, sekundární minerály (realgar)
- Rudní minerály s arzénem: pyrit, markazit, galenit, sfalerit
- **Uhlí (12-18[1], 40[2], 333[3] mg/kg).**
- Sopečná činnost (plyny)

ANTROPOGENNÍ ZDROJE

- Těžba a zpracování rud
- Energetika (tepelné elektrárny)
- Zemědělství (pesticidy, CCA[4])

[1] průměrný obsah v uhlí, [2] průměr ze Severočeské pánve, [3] průměr ze Sokolovské pánve
[4] chromated copper arsenate (chemické ošetření dřeva)

PRINCIP VŠUDYPŘÍTOMNOSTI PRVKŮ

Chemické prvky jsou všudypřítomné (kromě nestabilních radioaktivních izotopů), vše je otázkou koncentrace.

Základním předpokladem pro takové studium chemických prvků jsou rozvinuté metody analytické chemie: tj. možnost stanovit koncentrace arzénu.

Instrumentální metody:
AAS, ICPOES, ICPMS, INAA

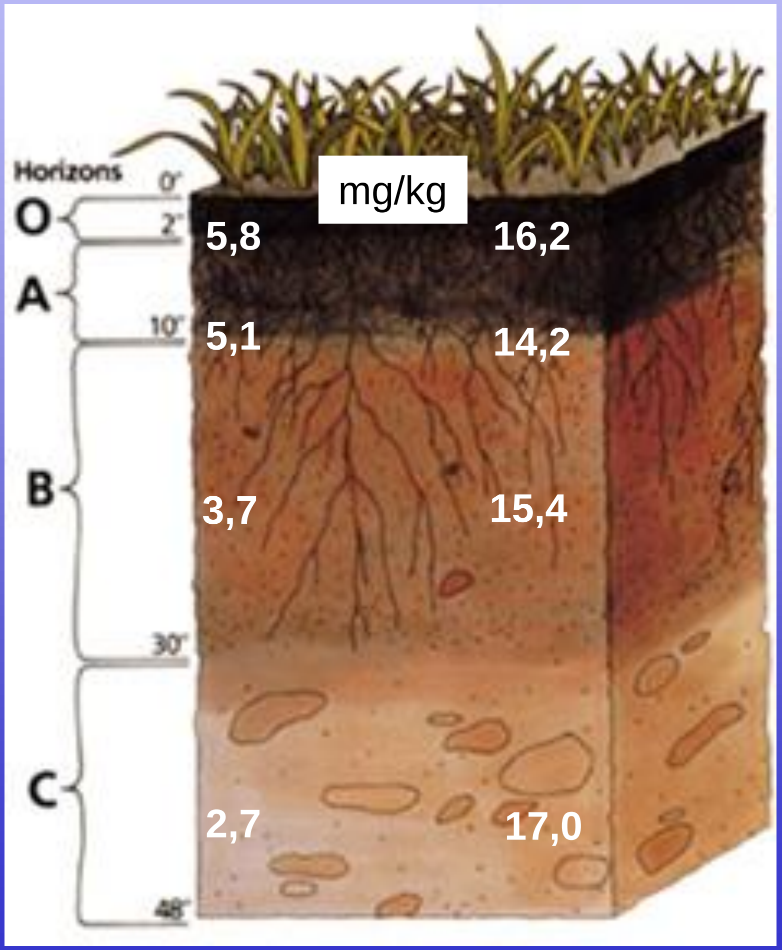
Arzén v životním prostředí

Distribuce a speciace prvků v biosféře je důsledkem procesů, jichž se zúčastňují živé organismy včetně člověka. Normální koncentrace **arzénu** ve složkách ŽP:

Zemská kůra: 1,8-2,5 mg/kg

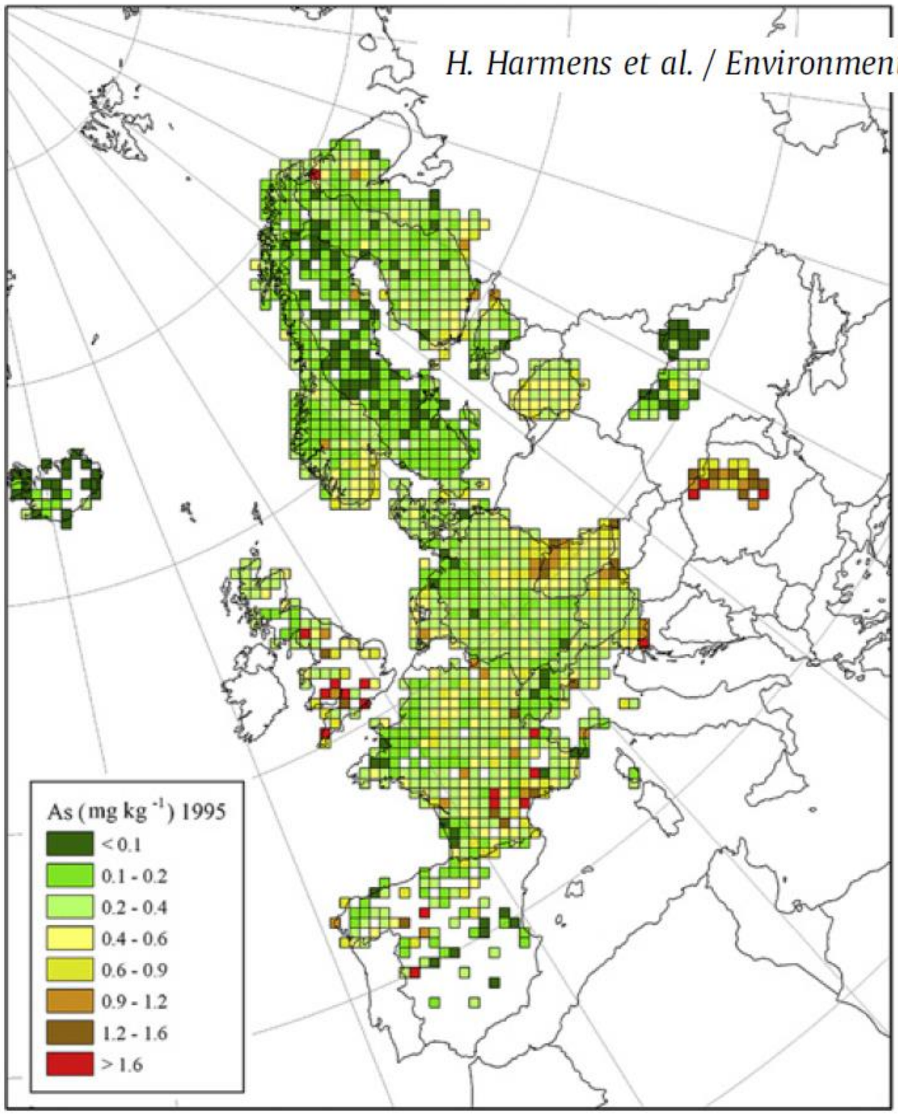
Žula: 1,5-2,5 mg/kg
Bazalt (čedič): 0,5-2,5 mg/kg
Vápenec: 1-2,5 mg/kg
Pískovec: 0,5-1,2 mg/kg

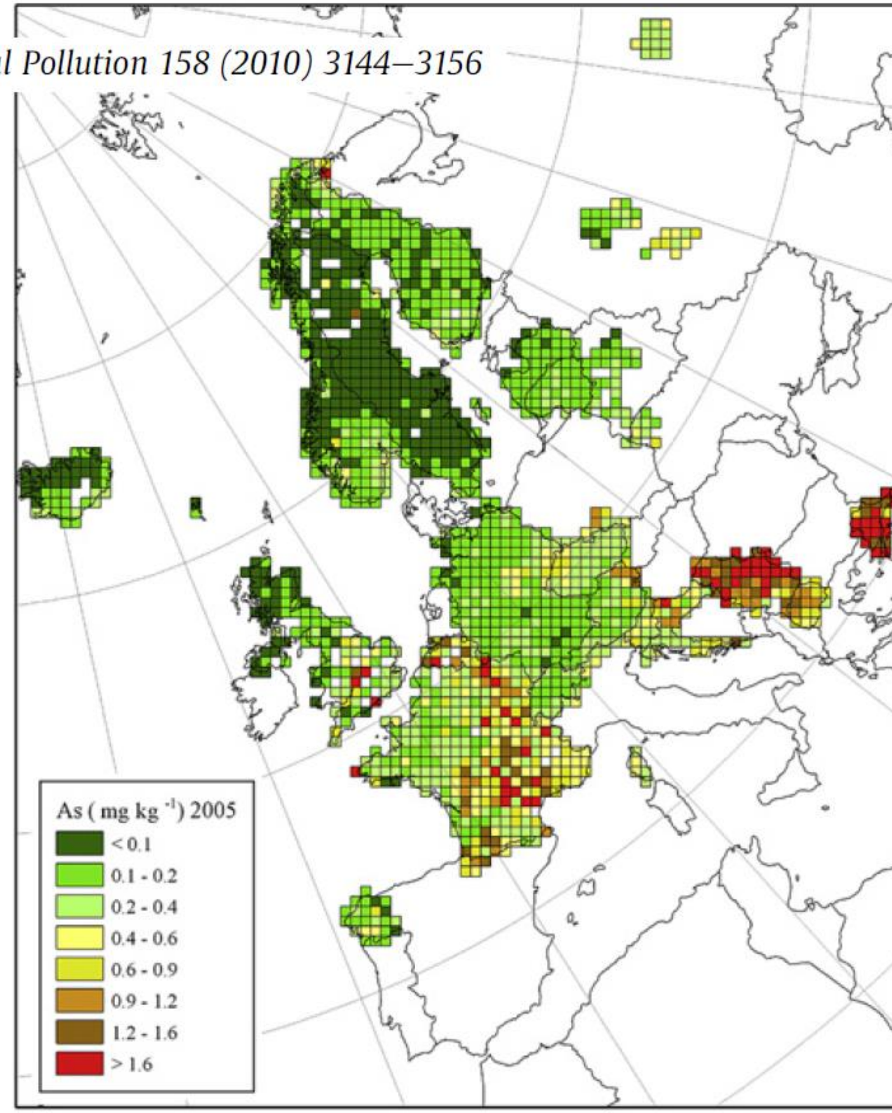
Půdy: 1-30 mg/kg



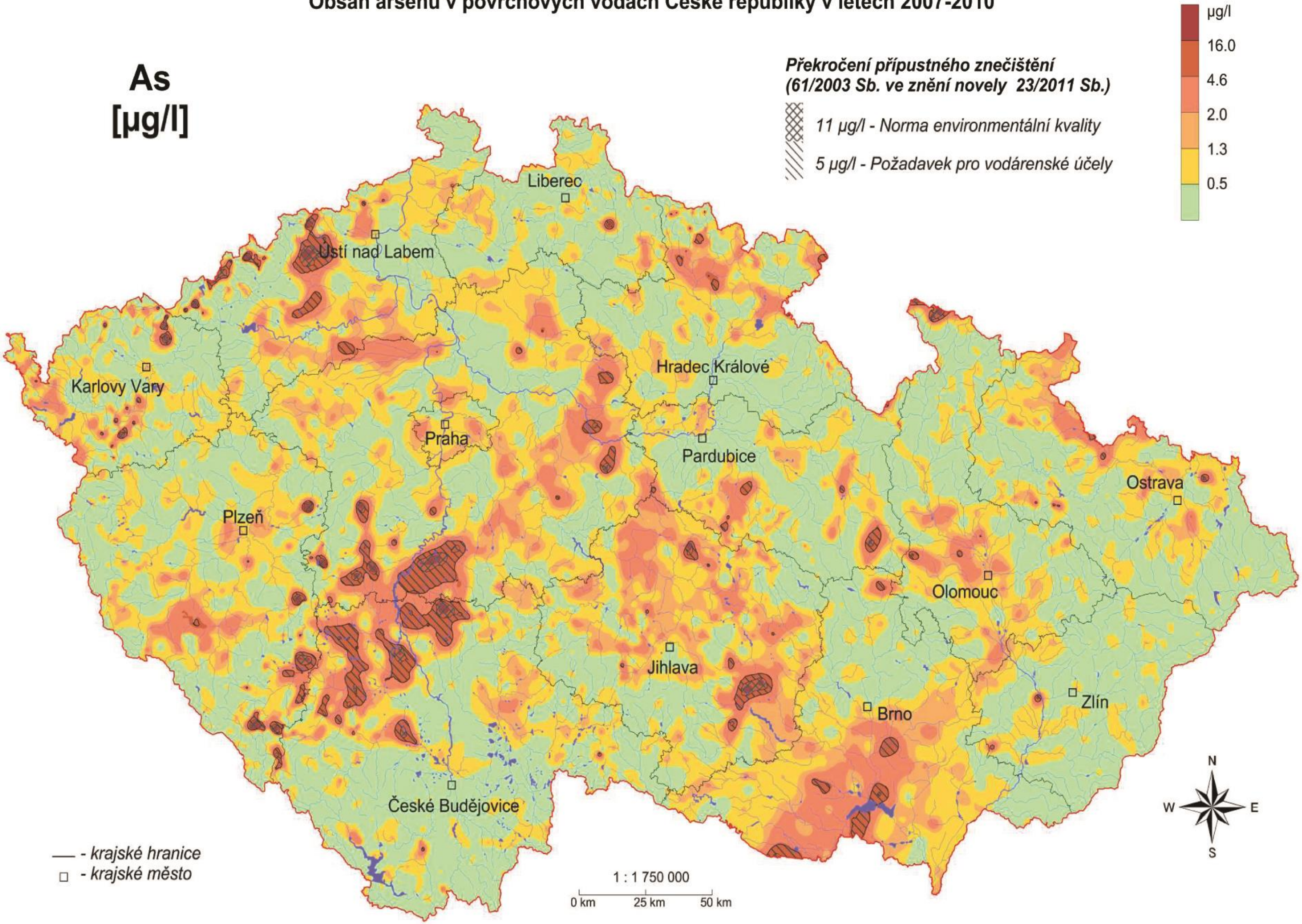
Geochemický monitoring: koncentrace As v meších v letech 1995 a 2005

H. Harmens et al. / Environmental Pollution 158 (2010) 3144–3156



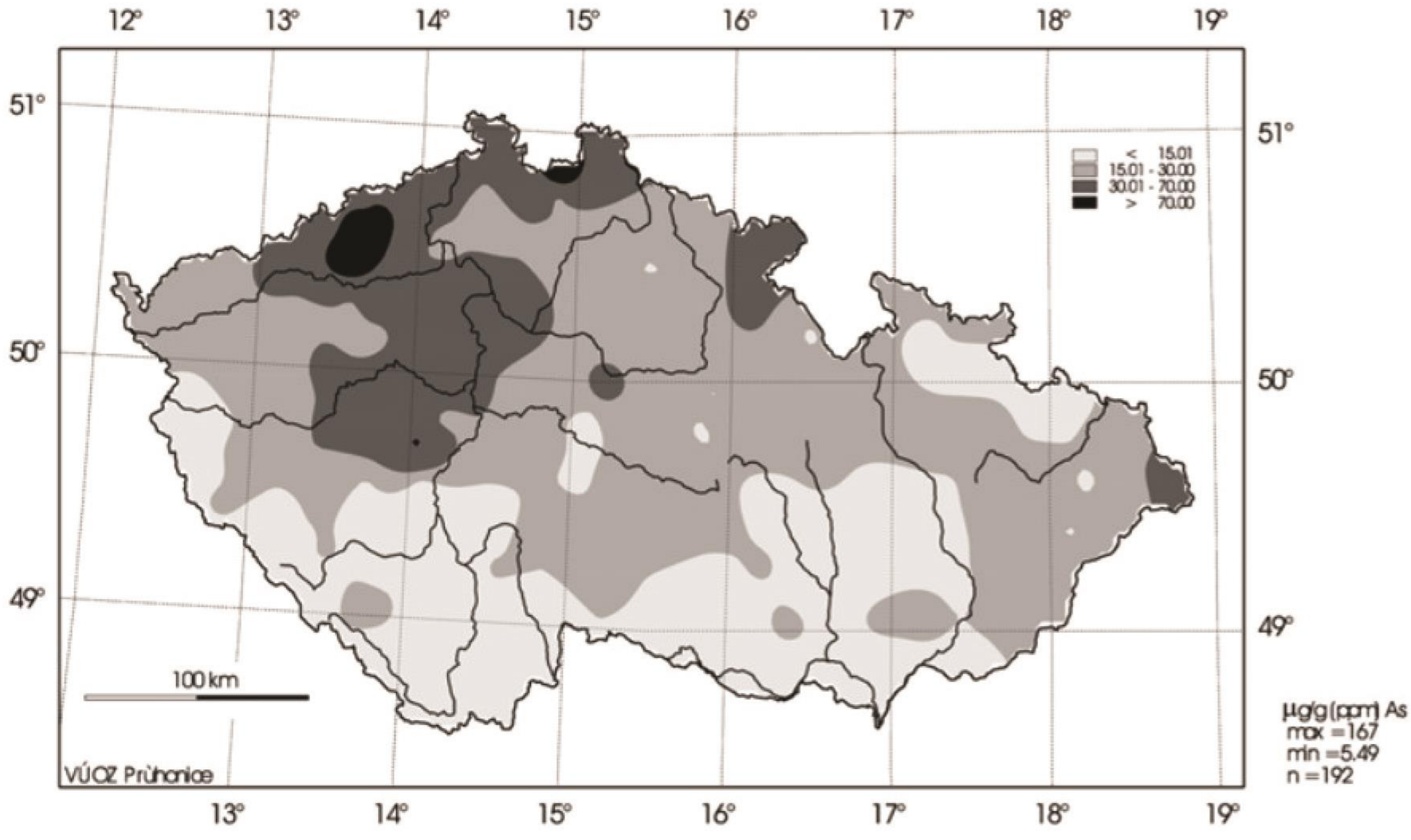


Obsah arsenu v povrchových vodách České republiky v letech 2007-2010



Majer a kol. (2012): Atlas chemismu povrchových vod České republiky, ČGS.

Arzén v humusu jehličnatých lesů v ČR v roce 1995



Suchara a Sucharová (2012)

Arsenic Content of Food and Forage Plants from Various Countries[a] (µg/kg)

Plant	Sample	Range/Mean
Barley	Grains	3–18
Oats	Grains	10
Wheat	Grains	3–10
Rice	Grains	0.5–10
Corn/maize[b]	Grains	1848
Snap beans	Pods	1.3–7
Cabbage	Leaves	20–69
Lettuce	Leaves	20–50
Carrot	Roots	10–80
Potato	Tubers	10–20
Potato[b]	Tubers	864
Tomato	Fruits	9–12
Orange	Fruits	11–50
Edible mushroom	Whole	280
Clover	Tops	20–160
Grass	Tops	280–330
		1000–5400[c]

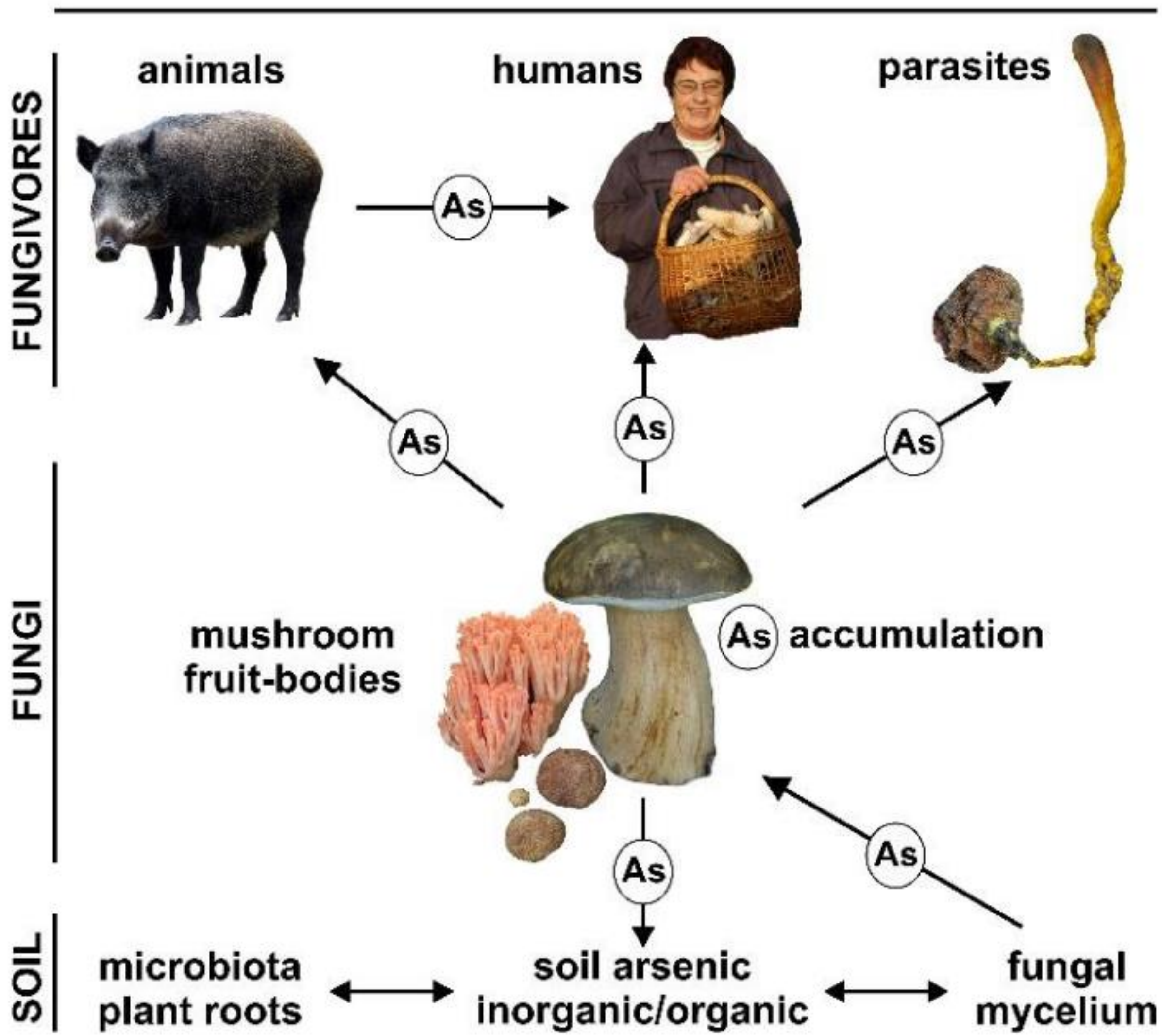
[a] Presented are common, possible background values from various sources, unless otherwise indicated.

[b] Plants from Chile, after Queirolo et al. (2000).

[c] Grass from climatic zone (Kazakhstan).[1131]

Kabata-Pendias (2012)

Arzén v „mykosféře“



Arzén v houbách

Půdy: 1-30 mg/kg As, průměrně 7 mg/kg.

Houby: akumulují As, a to hlavně saprotrofové. Data z dostupné literatury (2005) pro čisté lokality (mg/kg sušiny):

	SAP	ECM	SAP_{lig}
medián	**1.60**	**0.53**	**0.54**
aritmetický průměr	4.16	1.14	1.01
geometrický průměr	1.84	0.48	0.47
maximum	52.4	23.4	11.8
minimum	0.13	0.02	0.02
n	174	247	48

Arzén v houbách

V Evropě rostou tisíce druhů velkých hub, koncentrace As ve většině z nich nejsou známy.

V období let 2003-2018 jsme shromáždili asi 2000 vzorků (nikoliv druhů) hub, z nichž většina byla analyzována.

Byly odhaleny významné akumulace As až do hodnot přesahujících 10 000 mg/kg na čistých lokalitách.

V letech 2016-2018 probíhal výzkum (tříletý mezinárodní projekt ve spolupráci s Univerzitou ve Štýrském Hradci) zaměřený na chemické složení arzénu v houbách.

Arzén v houbách: faktory

Druhová závislost koncentrace.

Zvýšené koncentrace As v prostředí vedou ke zvýšeným koncentracím As v houbách.

Oblasti s přirozenou a antropogenní kontaminací.

Mokrsko

- ložisko zlata s vysokým výskytem arzenopyritu
- As v půdách často vyšší než 1000 mg/kg
- běžné houby mají As v průměru 10x zvýšený

Arzénové sloučeniny v houbách: záhada původu

V půdním roztoku jsou dominantní **anorganické sloučeniny arzénu**

- arseničnanové (V) ionty AsO_4^{3-}
- arsenitanové (III) ionty AsO_3^{3-}

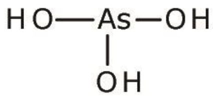
V houbách obvykle dominují **organické, metylované formy** arzénu (tj. s jednou či více vázaných $–CH_3$ skupin).

Metylace arzénu byla prokázána u bakterií a některých mikroskopických hub. Mycelia velkých hub pěstovaná *in vitro*, exponovaná arzénu [As(III) I As(V)], však ani při dlouhodobé expozici v drtivé většině testovaných případů nevykazují přítomnost methylovaných sloučenin arzénu.

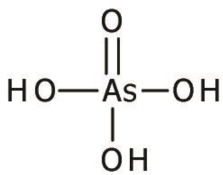
Hlavní formy arzénu v plodnicích hub

anorganické

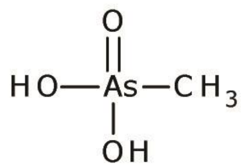
organické



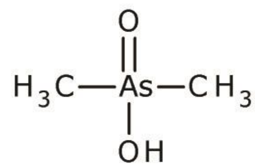
As (III)



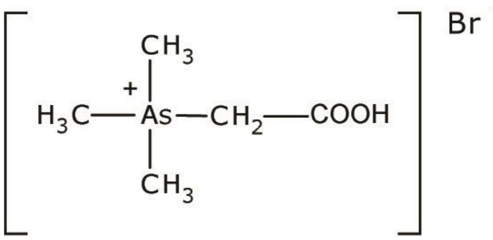
As (V)



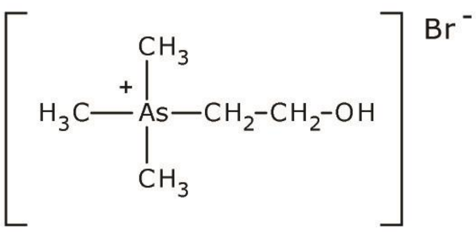
methylarsonová kys.



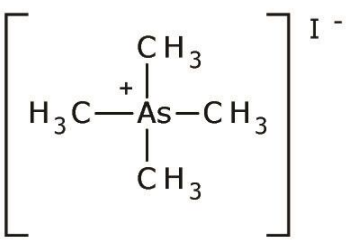
dimethylarsinová kys.



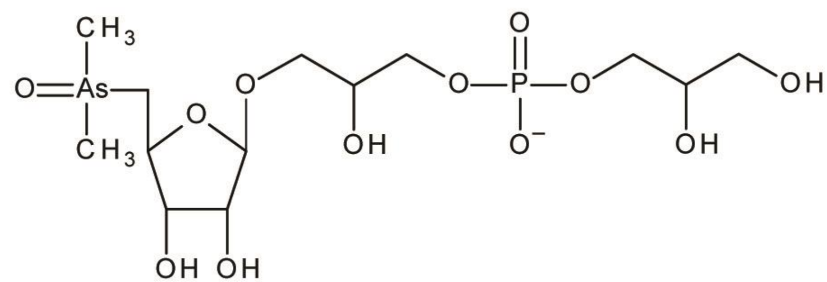
arsenobetain



arsenocholin

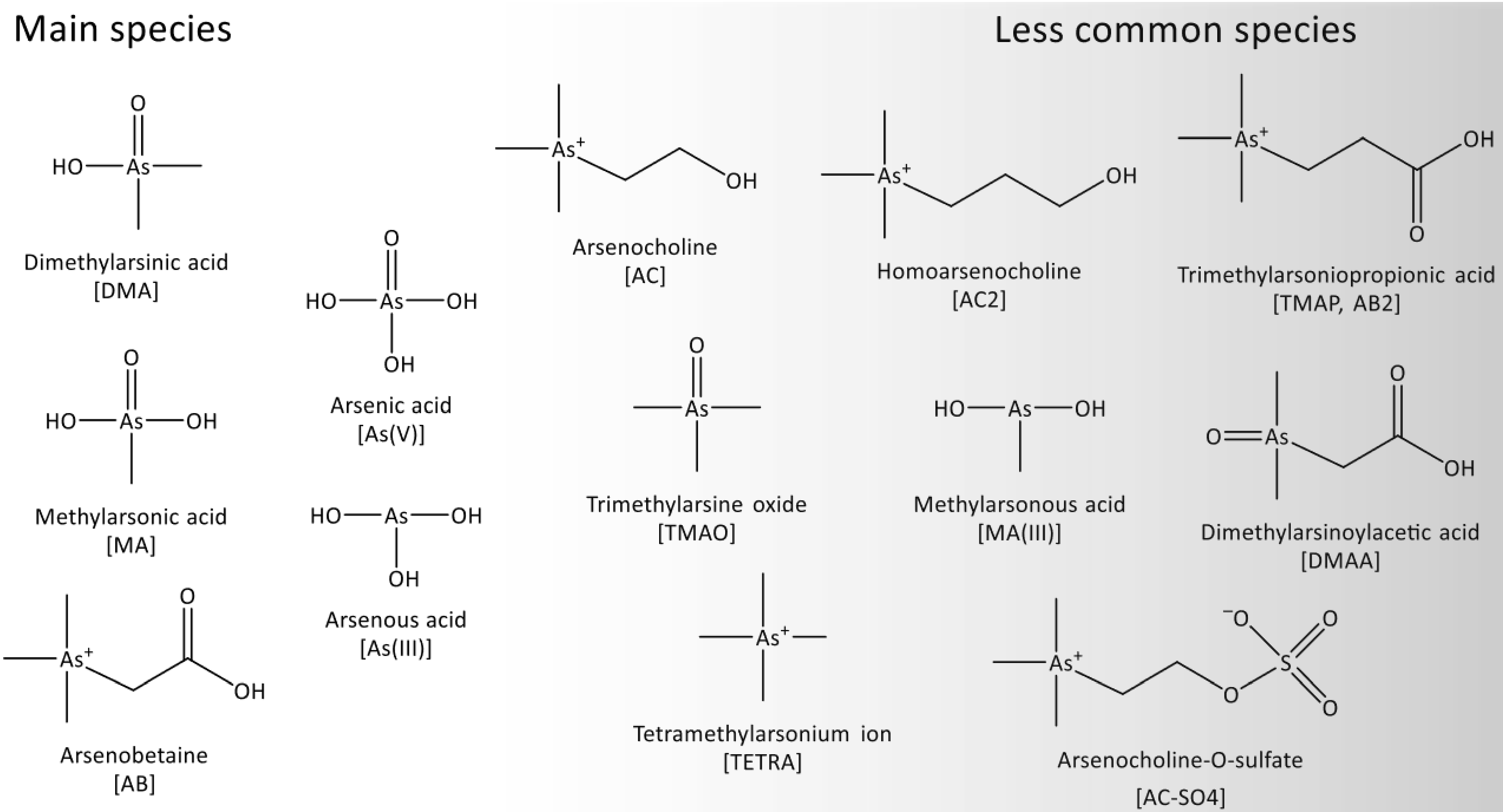


tetramethylarsoniový iont



arsenosacharid

Běžné a vzácné formy arzénu v houbách



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Uptake and transformation of arsenic during the vegetative life stage of terrestrial fungi

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Arsenic
Mycelium
Biotransformation
Concentration factor

A B S T R A C T

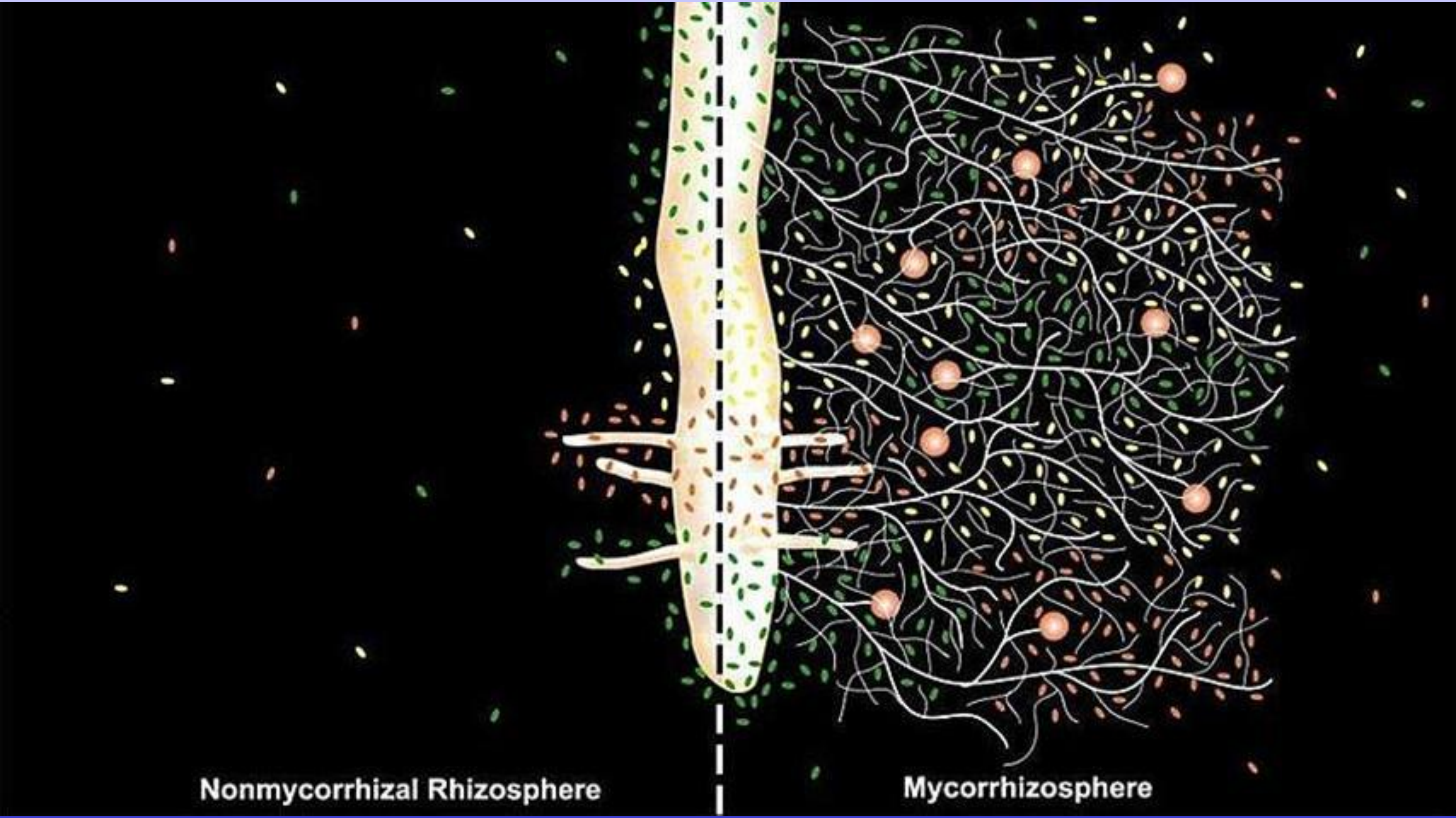
Many species of terrestrial fungi produce fruiting bodies that contain high proportions of arsenobetaine (AB), an arsenic compound of no known toxicity. It is unknown whether fungi produce or accumulate AB from the surrounding environment. The present study targets the vegetative life stage (mycelium) of fungi, to examine the role of this stage in arsenic transformations and potential formation of AB. The mycelia of three different fungi species were cultured axenically and exposed to AB, arsenate (As(V)) and dimethylarsinoyl acetic acid for 60 days. *Agaricus bisporus* was additionally exposed to hypothesized precursors for AB and the exposure time to As(V) and dimethlyarsinic acid was also extended to 120 days. The mycelia of all fungi species accumulated all arsenic compounds with two species accumulating significantly more AB than other compounds. Few biotransformations were observed in these experiments indicating that it is unlikely that the mycelium of the fungus is responsible for biosynthesizing AB.

Table 2
Average arsenic species concentration, column recovery (CR) and extraction efficiency (EE) of mycelium.

Fungus species	Treatment	As compounds (ug/kg wet weight)							Column recovery (%)	Extraction efficiency (%)
		As III	As V	MMA	DMA	TMAO	DMAA	AB		
A. bisporus	Control[A]	130 ± 9	nd	nd	nd	nd	nd	nd	140 ± 30	80 ± 20
	As(V)[A]	trace	70 ± 40	nd	nd	nd	nd	nd	120 ± 10	34 ± 10
	DMAA[A]	trace	nd	nd	nd	nd	190 ± 40	nd	110 ± 10	85 ± 8
	AB[A]	51 ± 60	nd	nd	nd	nd	nd	240 ± 40	90 ± 20	96 ± 2
	Control[B]	nd	nd	nd	nd	nd	nd	nd	NC	NC
	As(V) 60 days[B]	104.8 ± 0.8	80 ± 5	nd	nd	nd	nd	nd	110 ± 10	74 ± 20
	As(V) 90 days[B]	210 ± 20	150 ± 100	nd	nd	nd	nd	nd	80 ± 30	85 ± 11
	As(V) 120 days[B]	280 ± 100	150 ± 200	trace	trace	nd	nd	nd	90 ± 70	90 ± 3
	DMA 60 days[B]	nd	nd	nd	300 ± 9	nd	nd	nd	110 ± 4	73 ± 20
	DMA 90 days[B]	nd	nd	nd	500 ± 4	nd	nd	nd	100 ± 2	90 ± 4
	DMA 120 days[B]	nd	nd	nd	800 ± 20	nd	nd	nd	100 ± 20	68 ± 4
	Control[C]	nd	nd	nd	nd	nd	nd	nd	NC	NC
	MMA[C]	nd	nd	150 ± 10	nd	nd	nd	nd	110 ± 10	NC
	TMAO[C]	nd	nd	nd	nd	160 ± 100	nd	nd	92 ± 30	NC
	DMA 30 days[C]	nd	nd	nd	140 ± 40	nd	nd	nd	80 ± 10	NC
S. luteus	Control	trace	trace	nd	nd	nd	nd	nd	NC	82 ± 2
	As(V)	110 ± 40	trace	nd	nd	nd	nd	nd	83 ± 20	70 ± 10
	DMAA	trace	222*	nd	nd	nd	180 ± 80	nd	110 ± 20	90 ± 10
	AB	trace	nd	nd	nd	nd	nd	70 ± 30	100 ± 20	87 ± 10
S. crispa	Control	trace	60 ± 70	nd	nd	nd	nd	nd	140 ± 100	98 ± 3
	As(V)	trace	270 ± 10	nd	nd	31 ± 3	nd	nd	100 ± 7	93 ± 3
	DMAA	trace	165*	nd	nd	nd	110 ± 70	nd	110 ± 30	96 ± 1
	AB	nd	nd	nd	nd	nd	nd	580 ± 60	100 ± 7	97 ± 1

[A B C] Control mycelium (no As) corresponds to treatments with the same letter.
* indicates only observed in one replicate, nd = non-detect (<3 μg/kg), trace <5 μg/kg, NC = not calculated.
Column recovery = (Sum of arsenic compounds/Total As in extract)*100, Extraction Efficiency = (Total As in extract/Total As in mycelium)*100.

Pocházejí organosloučeniny As z půdy?



Akutní toxicita sloučenin arzénu

LD_{50} je v toxikologii označení pro dávku látky podané testovaným jedincům, která způsobí úhyn 50 % testovaných živočichů do 24 hodin od expozice. Udává se v mg/kg živé hmotnosti.

Sloučenina arsenu	Zkratka	LD_{50} (mg/kg)
Arsenitan	As^{III}	15 – 42
Arseničnan	As^{V}	20 – 800
Monometylarsonát	MMA	700 – 1800
Dimetylarsinát	DMA	1200 – 2600
Trimetylarsin oxid	TMAO	10600
Tetramethylarsoniový ion	TETRA	890
Arsenocholin	AC	6500
Arsenobetain	AB	> 10000

toxický

netoxický

Sloth et al. (2005): myši a krysy, orální podání.

Stanovení sloučenin As v houbách

Sloučeniny As v houbách jsou poměrně stabilní (na rozdíl od sloučenin Sb), lze je efektivně extrahovat.

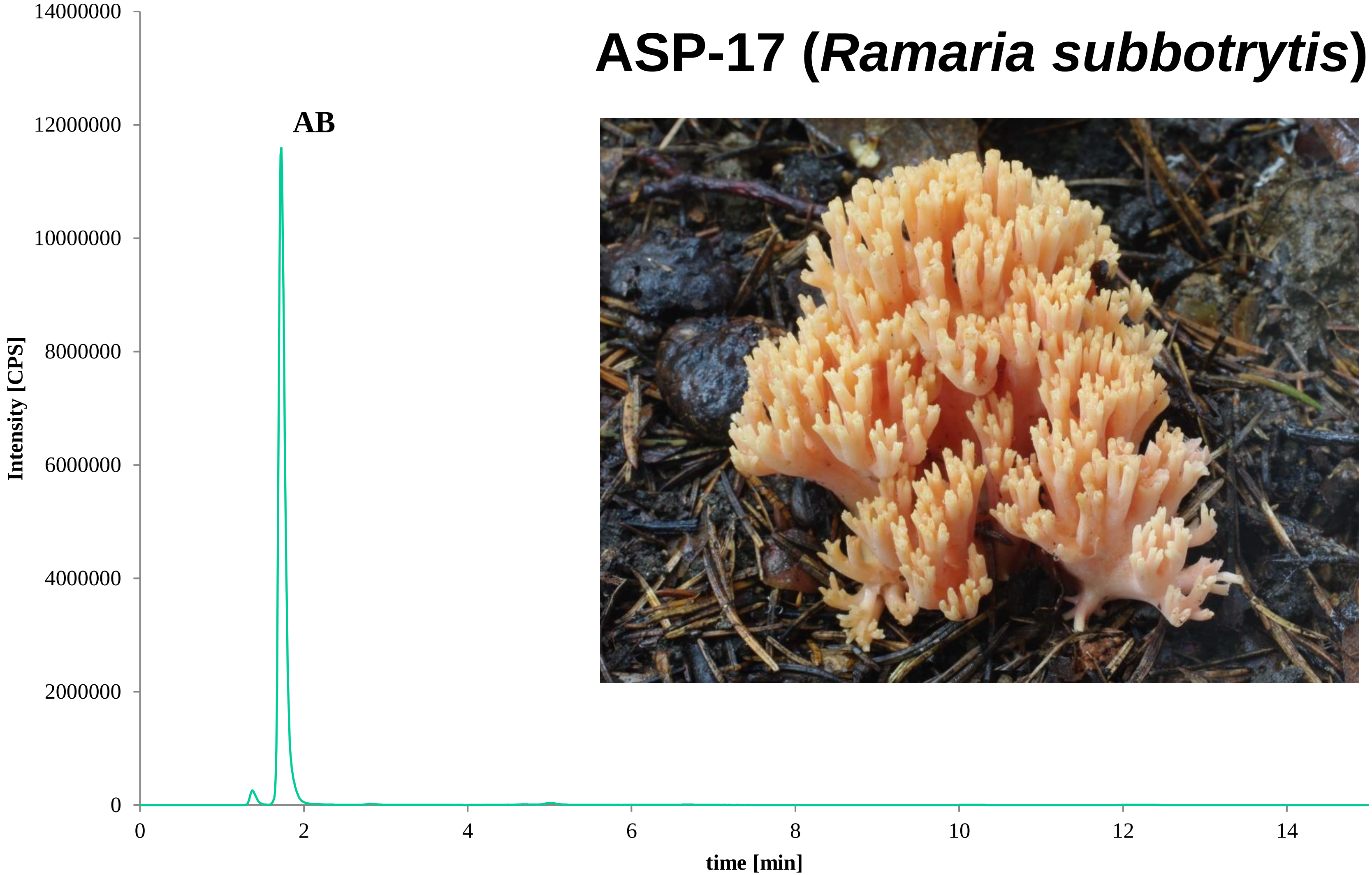
Extrakce: namletý vzorek (100 mg) sušené či lyofilizované houby se extrahuje ve vodě nebo v metanolu.

HPLC-ICPQQQMS

vysokotlaká kapalinová chromatografie kombinovaná s ICPQQQMS

Přítomnost látek s As + jejich identifikace.

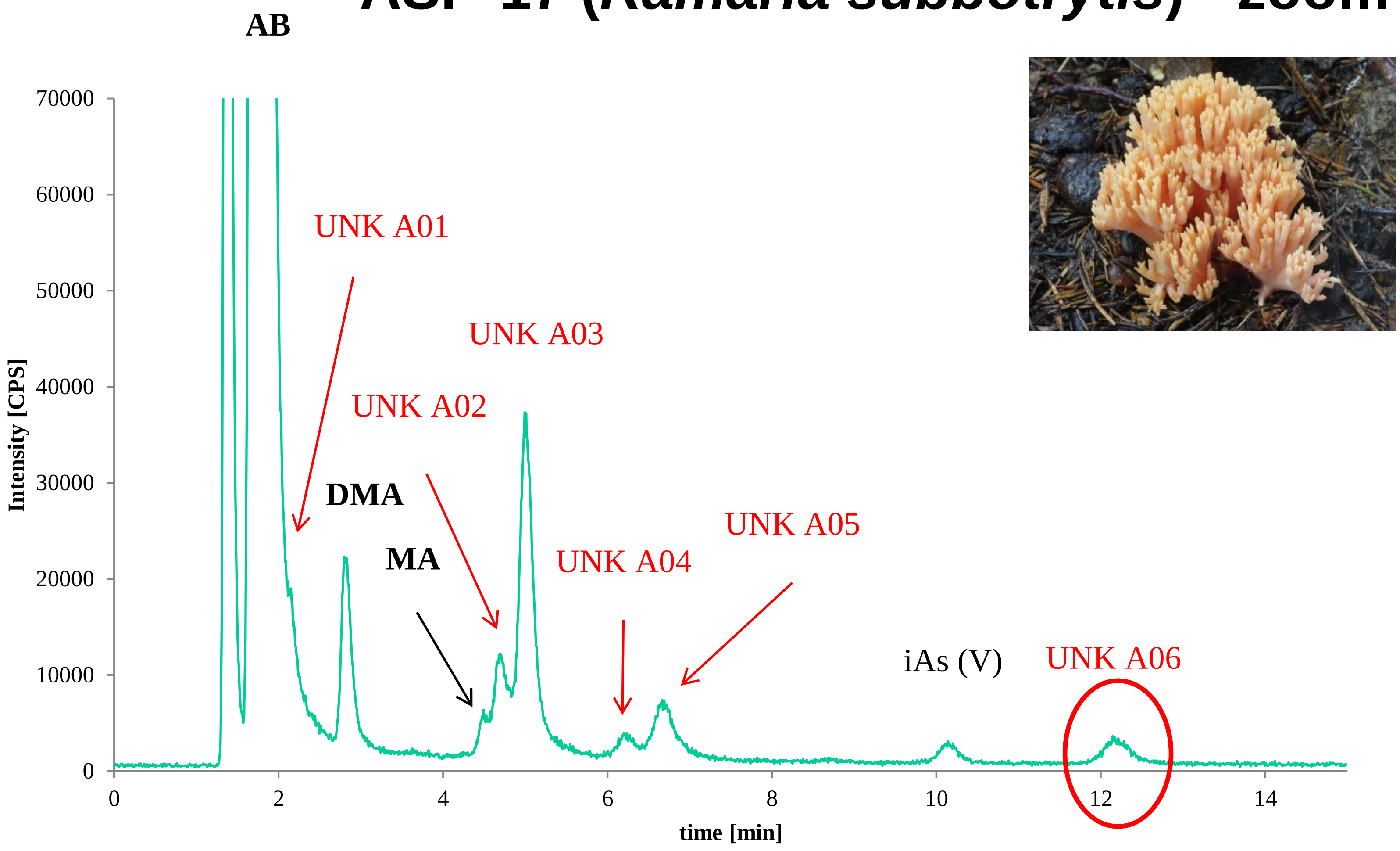
Stanovení sloučenin As v houbách

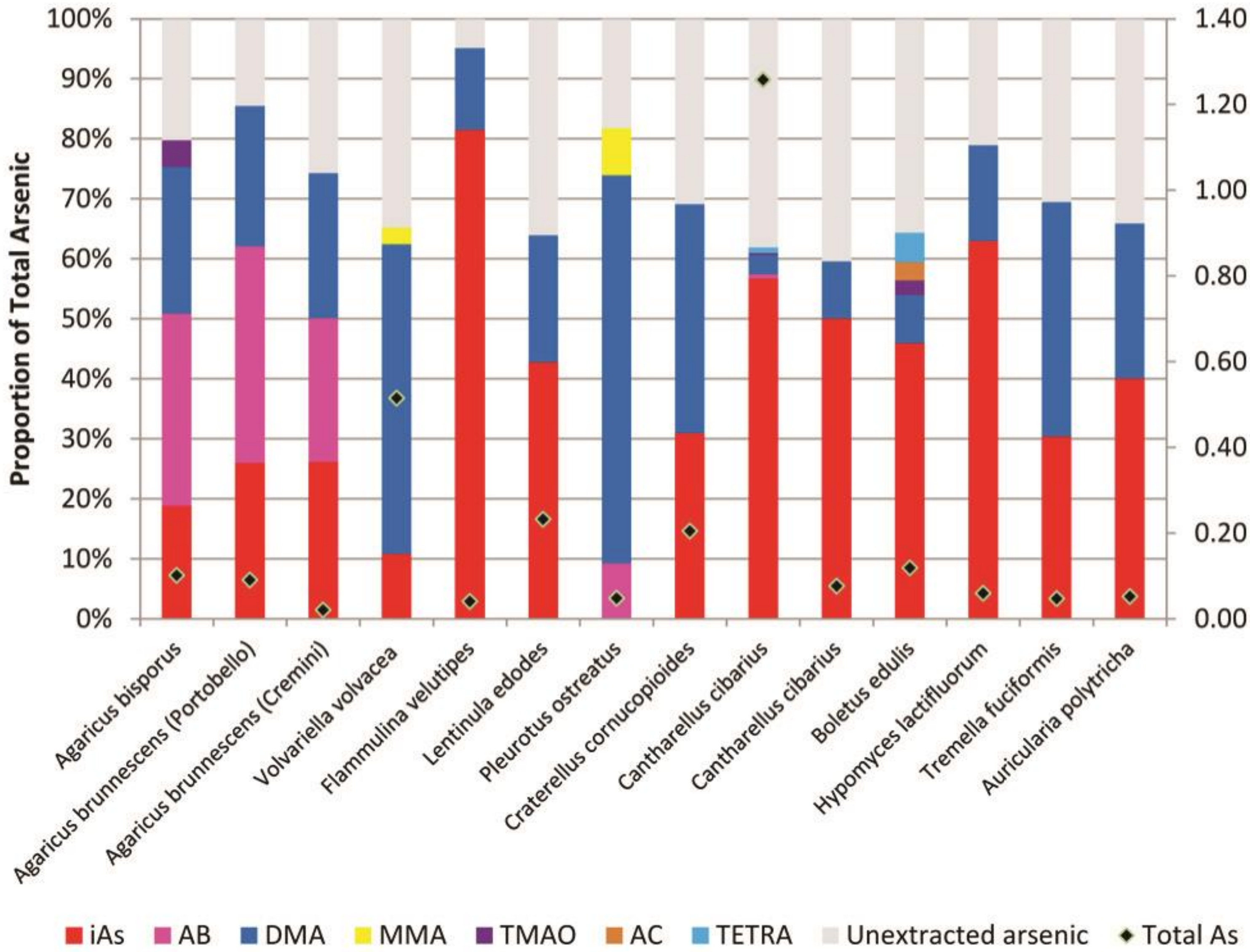


ASP-17 (*Ramaria subbotrytis*)

Stanovení sloučenin As v houbách

ASP-17 (*Ramaria subbotrytis*) - zoom





Nearing et al. (2014) Environmental Pollution 197: 108-115.

Průměrné zastoupení sloučenin As (levá osa), celkového arzénu (pravá osa + body) v tržních houbách z Kanady.

Pečárka lesní – *Agaricus sylvaticus*

Baňka velkokališná – *Sarcosphaera coronaria*

Lakovka ametystová – *Laccaria amethystina*


Plesňák zemní – *Thelephora terrestris*


Vláknice tuřínonohá – *Inocybe napipes*

Čirůvka větší – *Tricholoma matsutake*

Pavučinec sivonohý – *Cortinarius glaucopus*


Pavučinec proměnlivý – *Cortinarius polymorphus*

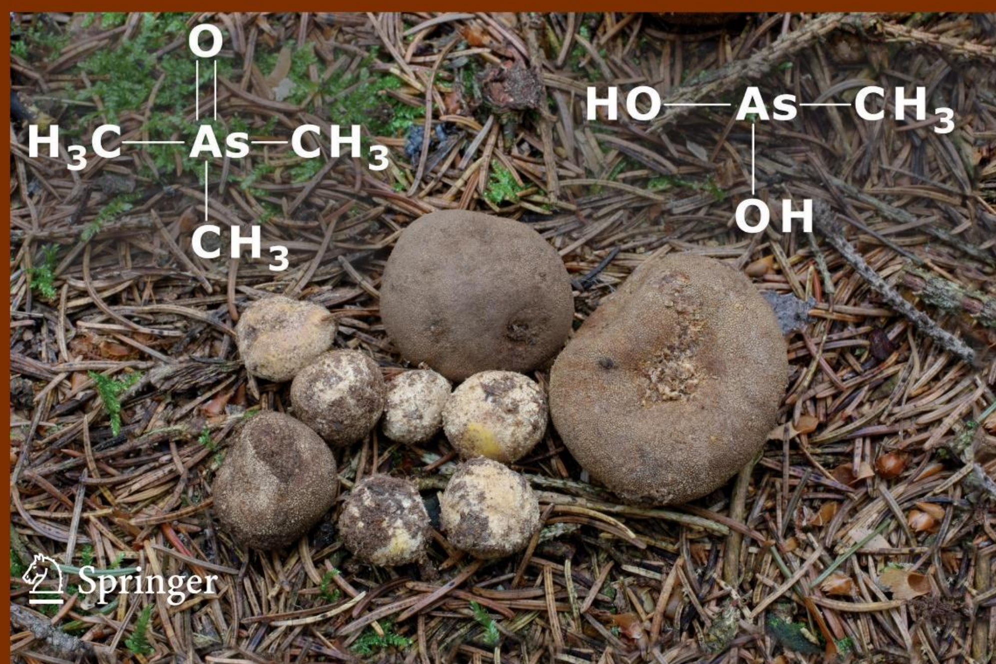

Olšovička zlatá – *Alnicola xanthophylla*


Pavučinec olšinný – *Cortinarius alnetorum*

Volume 410 · Number 9 · March 2018

ANALYTICAL & BIOANALYTICAL CHEMISTRY

ABC presents bioanalysis and environmental analysis
Flipped learning for delivery of analytical chemistry topics
Analytical challenges in sports drug testing
Unique arsenic speciation profile in *Elaphomyces* spp.



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ANALYTICAL & BIOANALYTICAL CHEMISTRY Vol 410 · No 9 · March 2018 · pp xxxx–xxxx

Jelenka pestrá – *Elaphomyces muricatus*


Jelenka obecná – *Elaphomyces granulatus*


Moje pracoviště…

Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-018-0903-3

COMMUNICATION

CrossMark

A unique arsenic speciation profile in *Elaphomyces* spp. ("deer truffles") —trimethylarsine oxide and methylarsonous acid as significant arsenic compounds

Simone Braeuer[1] · Jan Borovička[2,3] · Walter Goessler[1]

TMAO, MA, MA (III)*

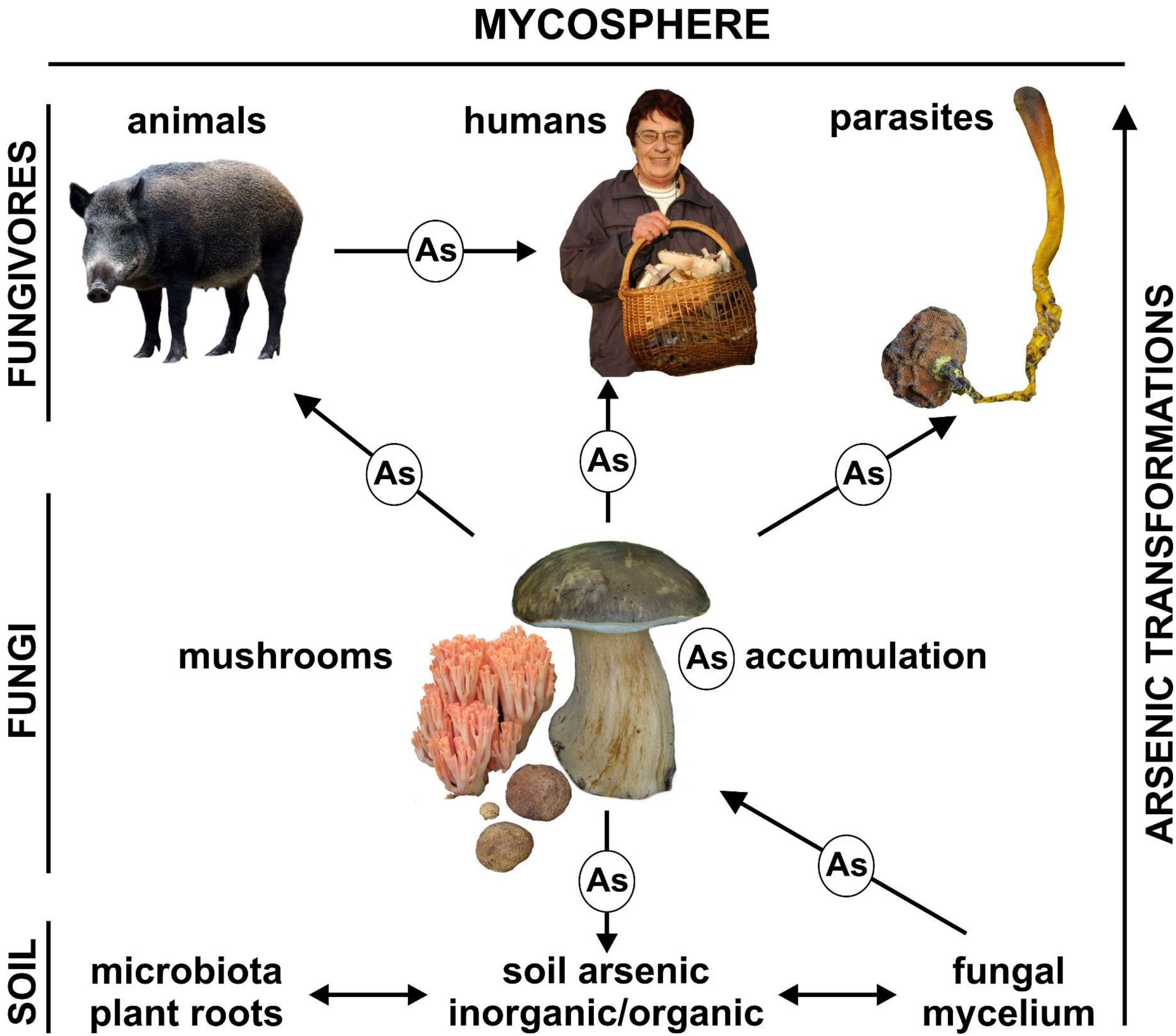
*vysoce toxická

Received: 31 October 2017 / Revised: 21 December 2017 / Accepted: 18 January 2018

Abstract

Arsenic and its species were investigated for the first time in nine collections of *Elaphomyces* spp. ("deer truffles") from the Czech Republic with inductively coupled plasma mass spectrometry (ICPMS) and high-performance liquid chromatography coupled to ICPMS. The total arsenic concentrations ranged from 12 to 42 mg kg^{-1} dry mass in samples of *E. asperulus* and from 120 to 660 mg kg^{-1} dry mass in *E. granulatus* and *E. muricatus*. These concentrations are remarkably high for terrestrial organisms and demonstrate the arsenic-accumulating ability of these fungi. The dominating arsenic species in all samples was methylarsonic acid which accounted for more than 30% of the extractable arsenic. Arsenobetaine, dimethylarsinic acid, and inorganic arsenic were present as well, but only at trace concentrations. Surprisingly, we found high amounts of trimethylarsine oxide in all samples (0.32–28% of the extractable arsenic). Even more remarkable was that all but two samples contained significant amounts of the highly toxic trivalent arsenic compound methylarsonous acid (0.08–0.73% of the extractable arsenic). This is the first report of the occurrence of trimethylarsine oxide and methylarsonous acid at significant concentrations in a terrestrial organism. Our findings point out that there is still a lot to be understood about the biotransformation pathways of arsenic in the terrestrial environment.

Keywords *Elaphomyces* · Fungi · Deer truffles · Arsenic speciation · Trimethylarsine oxide · Methylarsonous acid


MYCOSPHERE
FUNGIVORES
animals
humans
parasites
As
As
As
As
FUNGI
mushrooms
As accumulation
As
As
SOIL
microbiota
plant roots
soil arsenic
inorganic/organic
fungal
mycelium
ARSENIC TRANSFORMATIONS


Elaphomyces granulatus, Aim 2


Tolypocladium
ophioglossoides, Aim 2


E. muricatus with T. ophioglossoides
mycelial cords, Aim 2

Arsenocholine-*O*-sulfate: A novel compound as major arsenic species in the parasitic mushroom *Tolypocladium ophioglossoides*

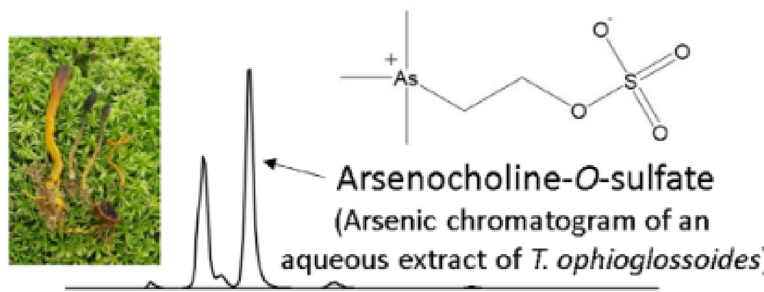
Simone Braeuer [a,*], Jan Borovička [b,c], Ronald A. Glabonjat [a], Lorenz Steiner [a], Walter Goessler [a]

[a] *Institute of Chemistry, University of Graz, Universitaetsplatz 1, 8010, Graz, Austria*
[b] *Nuclear Physics Institute of the Czech Academy of Sciences, Hlavní 130, 25068, Husinec-Řež, Czech Republic*
[c] *Institute of Geology of the Czech Academy of Sciences, Rozvojová 269, 16500, Prague 6, Czech Republic*

HIGHLIGHTS

- Arsenocholine-*O*-sulfate was discovered for the first time in nature.
- Arsenocholine-*O*-sulfate was the main As species in *Tolypocladium ophioglossoides*.
- Concentrations of As species and 35 elements were determined in *T. ophioglossoides*.
- Hosts, *Elaphomyces* spp., show different elemental profiles than *T. ophioglossoides*.

GRAPHICAL ABSTRACT



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Arsenic transformation
Arsenocholine
ICPMS

ABSTRACT

The As concentrations, along with 34 other elements, and the As speciation were investigated in wild-grown samples of the parasitic mushroom *Tolypocladium ophioglossoides* with inductively coupled plasma mass spectrometry (ICPMS) and high performance liquid chromatography coupled to ICPMS. The As concentrations were 0.070–3.44 mg kg^{-1} dry mass. More remarkable was the As speciation, where up to 56% of the extracted As were found to be an unknown As species, which was marginally retained under anion- and also cation-exchange conditions. After testing several different chromatographic settings, the compound was finally isolated and identified as 2-(sulfoxyethyl) trimethylarsonium ion (in short: arsenocholine-*O*-sulfate) with high resolution mass spectrometry. The compound was synthesized and further quantified in all investigated samples *via* ion-pair chromatography coupled to ICPMS. In addition to the high abundance of arsenocholine-*O*-sulfate in *T. ophioglossoides*, small amounts of this As species were also detected in one sample of the host mushroom, *Elaphomyces asperulus*. In a sample of another parasitic mushroom, *Ophiocordyceps sinensis*, arsenocholine-*O*-sulfate could not be detected, but the main species was another unknown compound that was oxidized to inorganic As(V) with hydrogen peroxide. This is the first discovery of arsenocholine-*O*-sulfate in nature. It is possible that it is present in many other organisms, at least in low concentrations, and just has not been detected there yet because of its unusual chromatographic behavior. The existence of arsenocholine-*O*-sulfate brings up questions again about the biotransformation pathways of As in the environment and the specific behavior of fungi.

Chemické složení plodnic jelenky drsné (ASP-059) a různých vzorků stromat housenice cizopasné

Unknown 1: arsenocholin sulfát

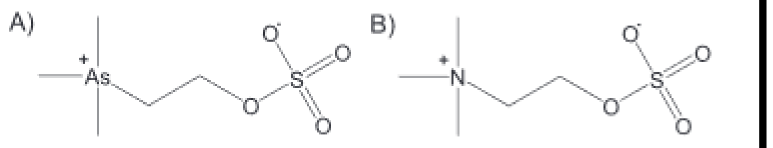
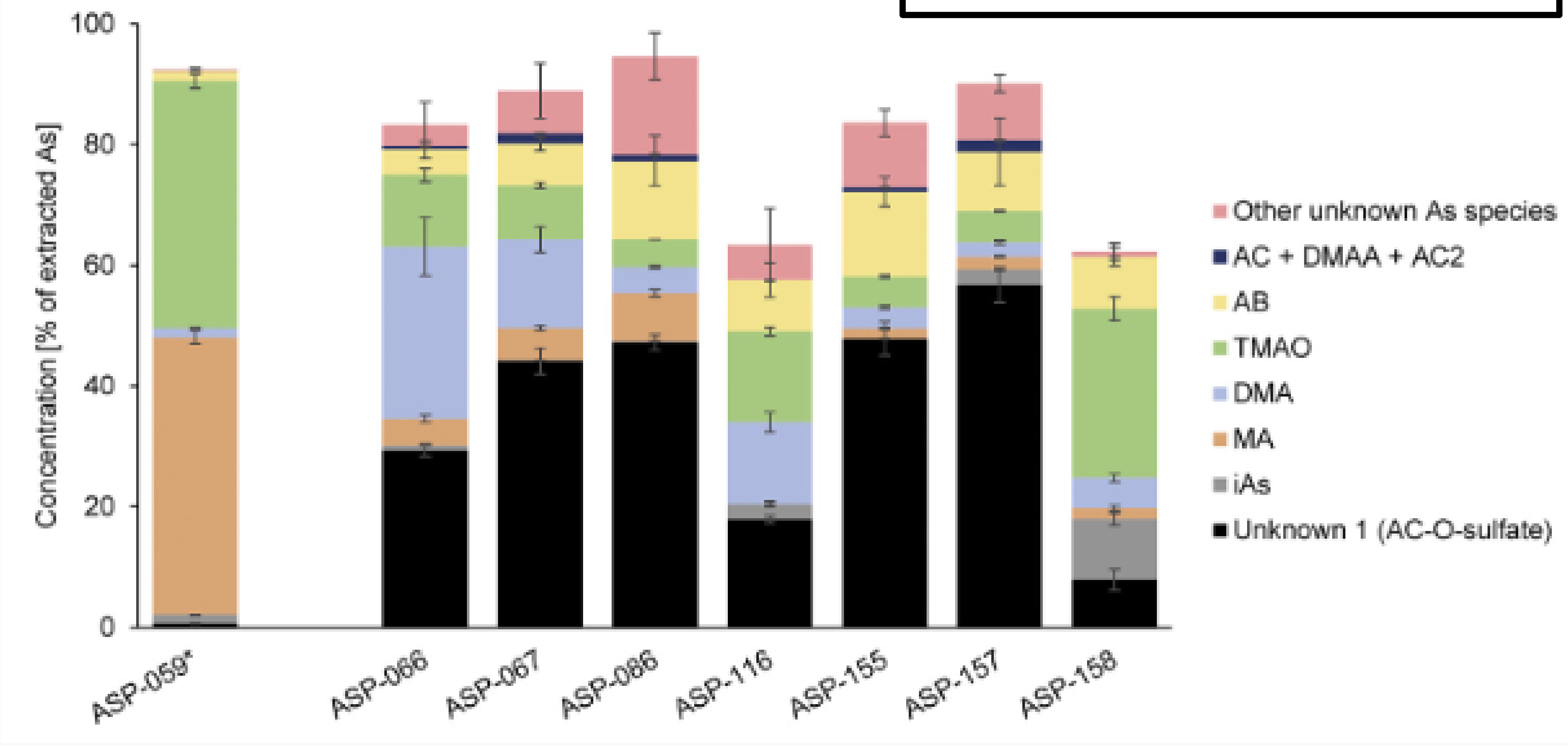


Fig. 3. Structures of AC-O-sulfate (A) and choline-O-sulfate (B).



Science of the Total Environment 648 (2019) 1570–1581

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Speciation analysis of elements accumulated in *Cystoderma carcharias* from clean and smelter-polluted sites

Jan Borovička [a,b,*], Simone Braeuer [c], Jan Sácký [d], Jan Kameník [b], Walter Goessler [c], Jakub Trubač [e], Ladislav Strnad [f], Jan Rohovec [a], Tereza Leonhardt [d], Pavel Kotrba [d]

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[b] *Nuclear Physics Institute, Czech Academy of Sciences, Hlavní 130, 25068 Husinec-Řež, Czech Republic*
[c] *University of Graz, Institute of Chemistry, Universitätsplatz 1, 8010 Graz, Austria*
[d] *Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Technická 3, 166 28 Prague, Czech Republic*
[e] *Institute of Geochemistry, Mineralogy and Mineral Resources, Faculty of Science, Charles University, Prague, Albertov 6, 12843 Prague 2, Czech Republic*
[f] *Laboratories of the Geological Institutes, Faculty of Science, Charles University, Albertov 6, 12843 Prague 2, Czech Republic*

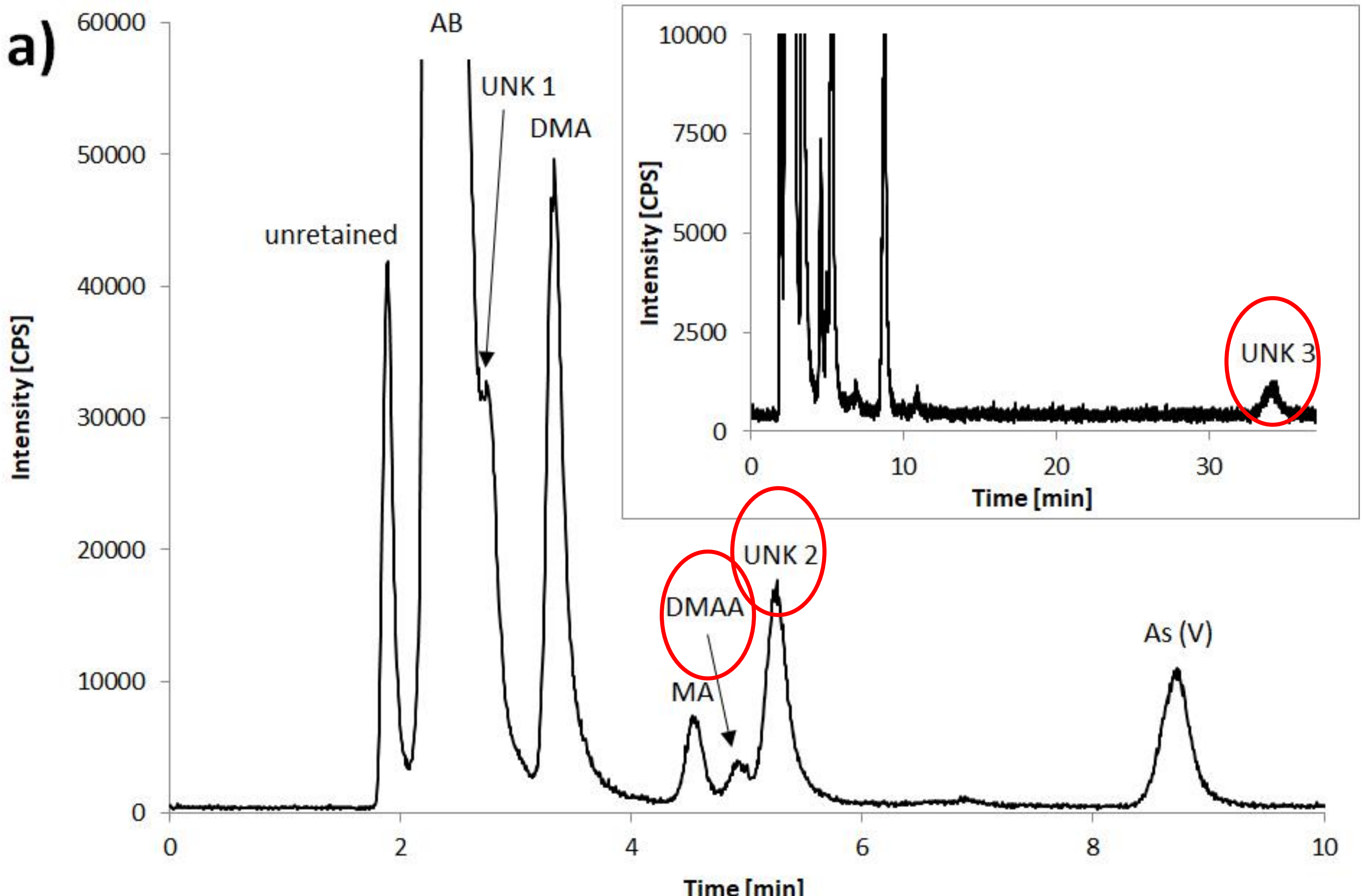
HIGHLIGHTS

- *Cystoderma carcharias* accumulates Cd, concentrations of up to 600 mg kg^{-1} were found.
- Pollution influences element concentrations but only to some extent As speciation.
- As speciation of *C. carcharias* consists of many compounds, several are still unknown.
- Arsenobetaine is the main As species in *C. carcharias*.
- Cysteinyl-rich peptides are involved in Cd and Cu binding in *C. carcharias*.

GRAPHICAL ABSTRACT

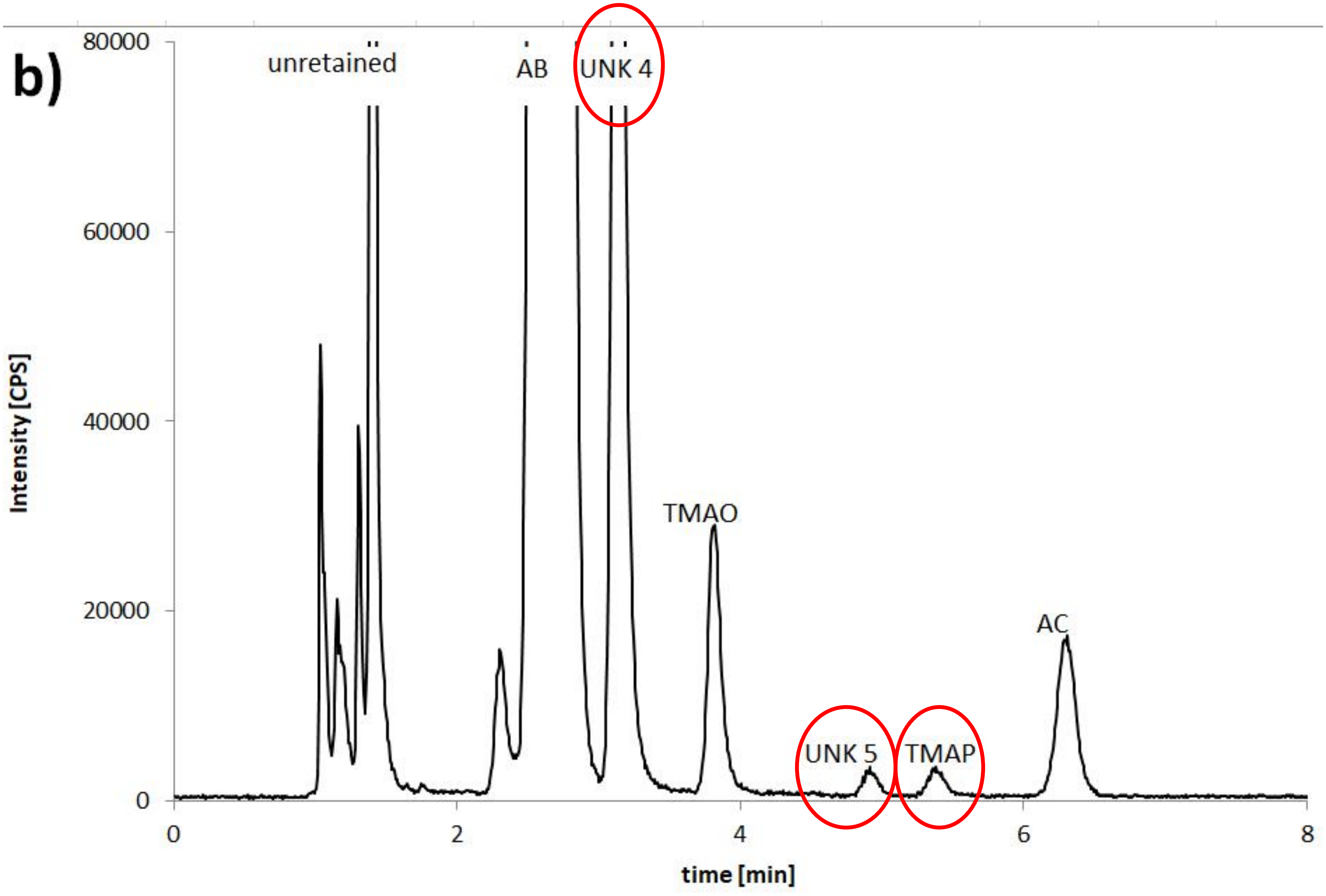
Zrnivka žraločí – *Cystoderma carcharias*

anion-exchange chromatogram

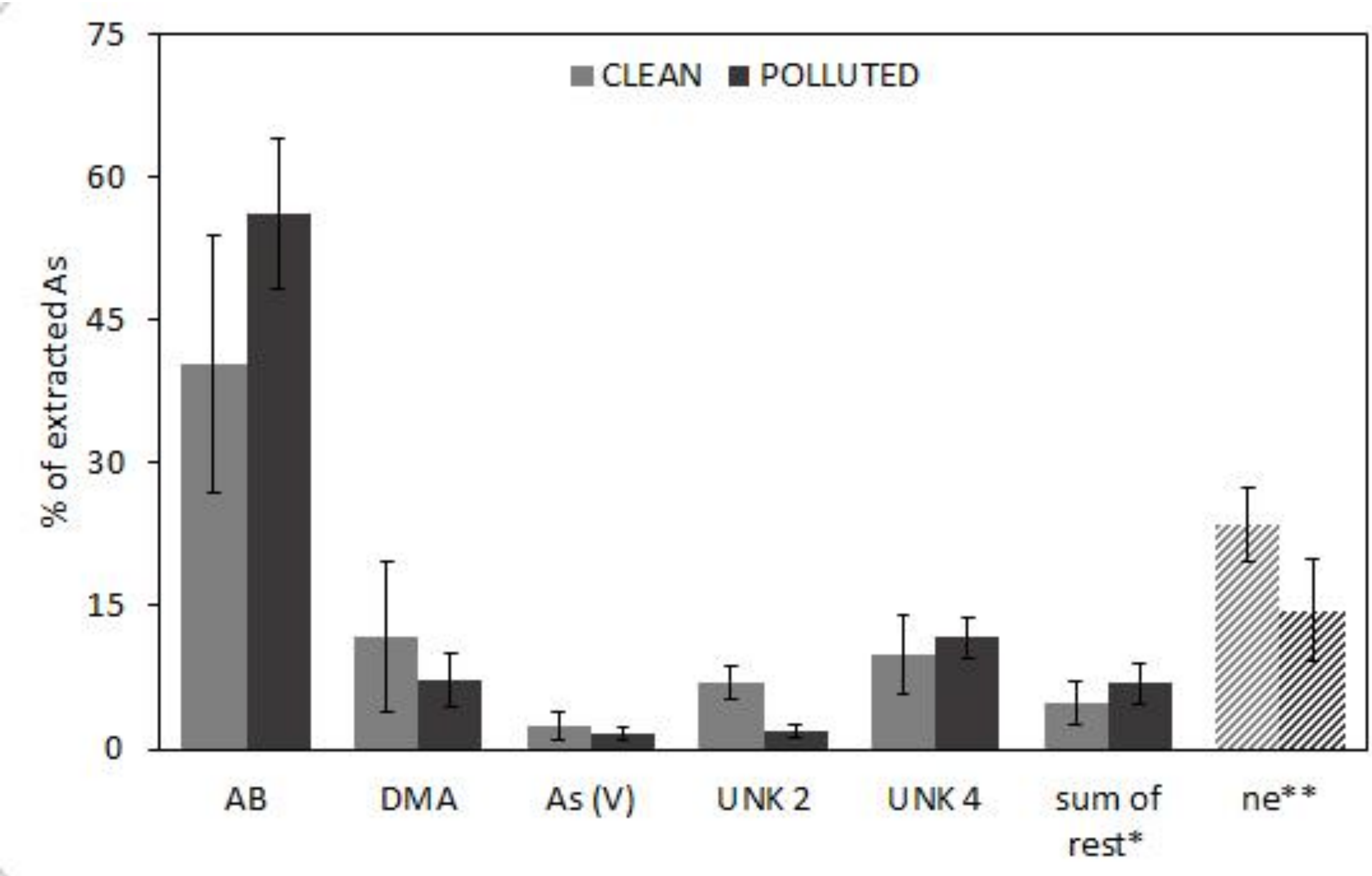


DMAA: dimethylarsinoylacetate

cation-exchange chromatogram



As a jeho sloučeniny v *C. carcharias*



Talanta 188 (2018) 107–110

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Homoarsenocholine – A novel arsenic compound detected for the first time in nature

Simone Braeuer[a], Jan Borovička[b,c], Toma Glasnov[a], Gema Guedes de la Cruz[a], Kenneth B. Jensen[a], Walter Goessler[a,*]

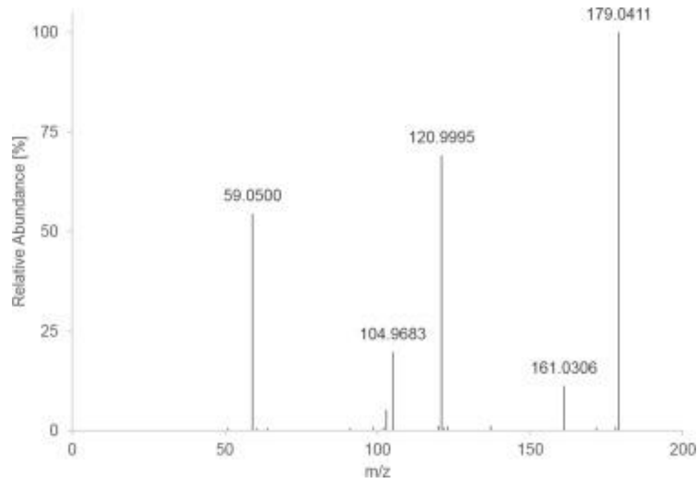
[a] *Institute of Chemistry, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria*
[b] *Nuclear Physics Institute, Czech Academy of Sciences, Hlavní 130, 25068 Husinec-Řež, Czech Republic*
[c] *Institute of Geology, Czech Academy of Sciences, Rozvojová 269, 16500 Prague 6, Czech Republic*

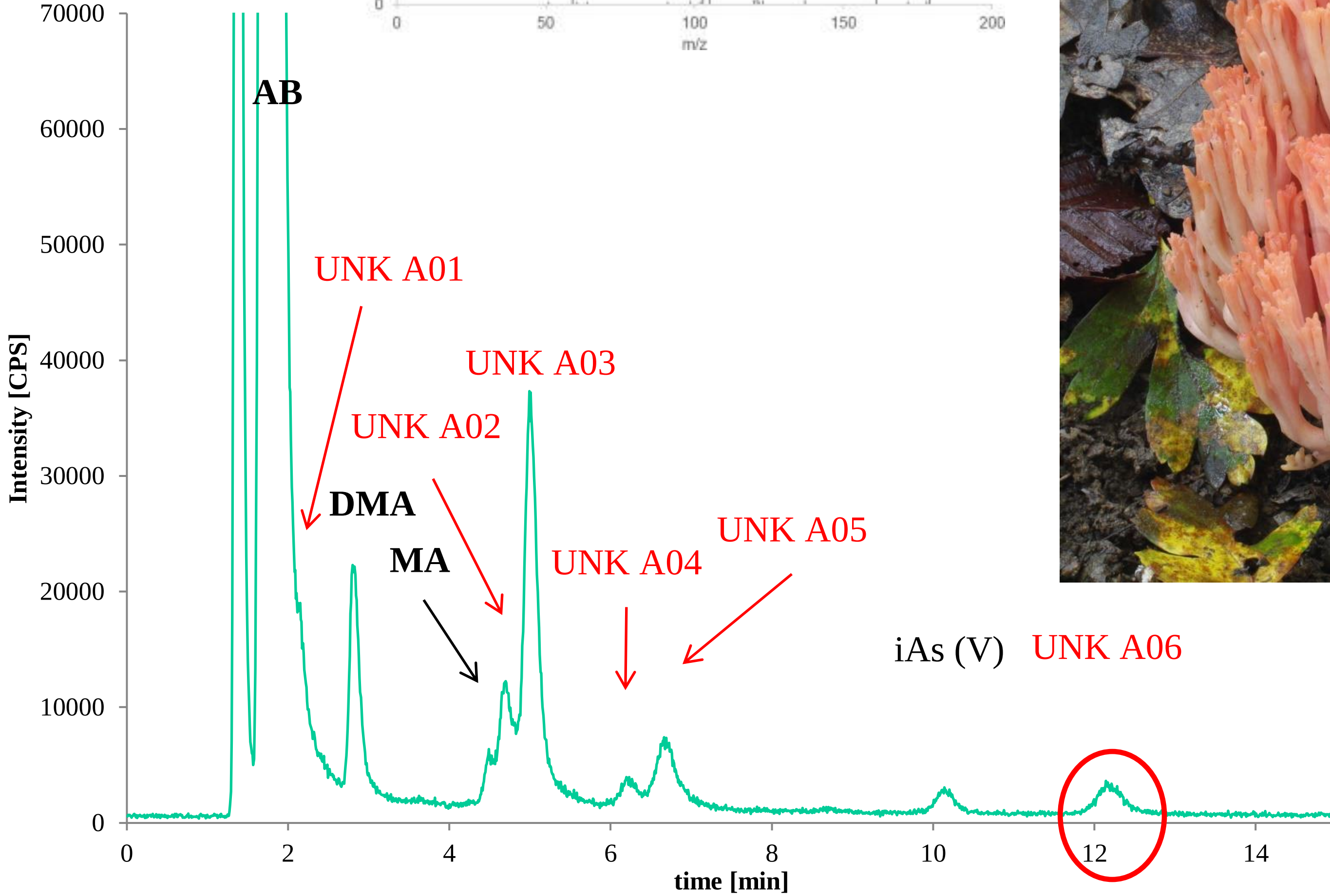
A R T I C L E I N F O

Keywords:
Arsenic speciation
Ramaria
Fungi
Homoarsenocholine
(3-hydroxypropyl) trimethylarsonium ion
ICPMS

A B S T R A C T

The arsenic speciation was determined in macrofungi of the *Ramaria* genus with HPLC coupled to inductively coupled plasma mass spectrometry. Besides arsenic species that are already known for macrofungi, like arsenobetaine or arsenocholine, two compounds that were only known from marine samples so far (trimethylarsoniopropanate and dimethylarsinoylacetate) were found for the first time in a terrestrial sample. An unknown arsenical was isolated and identified as homoarsenocholine. This could be a key intermediate for further elucidation of the biotransformation mechanisms of arsenic.


179.0411
120.9995
59.0500
104.9683
161.0306
Relative Abundance [%]
100
75
50
25
0
0
50
100
150
200
m/z


Intensity [CPS]
70000
60000
50000
40000
30000
20000
10000
0
AB
UNK A01
UNK A03
UNK A02
DMA
MA
UNK A04
UNK A05
iAs (V)
UNK A06
0
2
4
6
8
10
12
14
time [min]

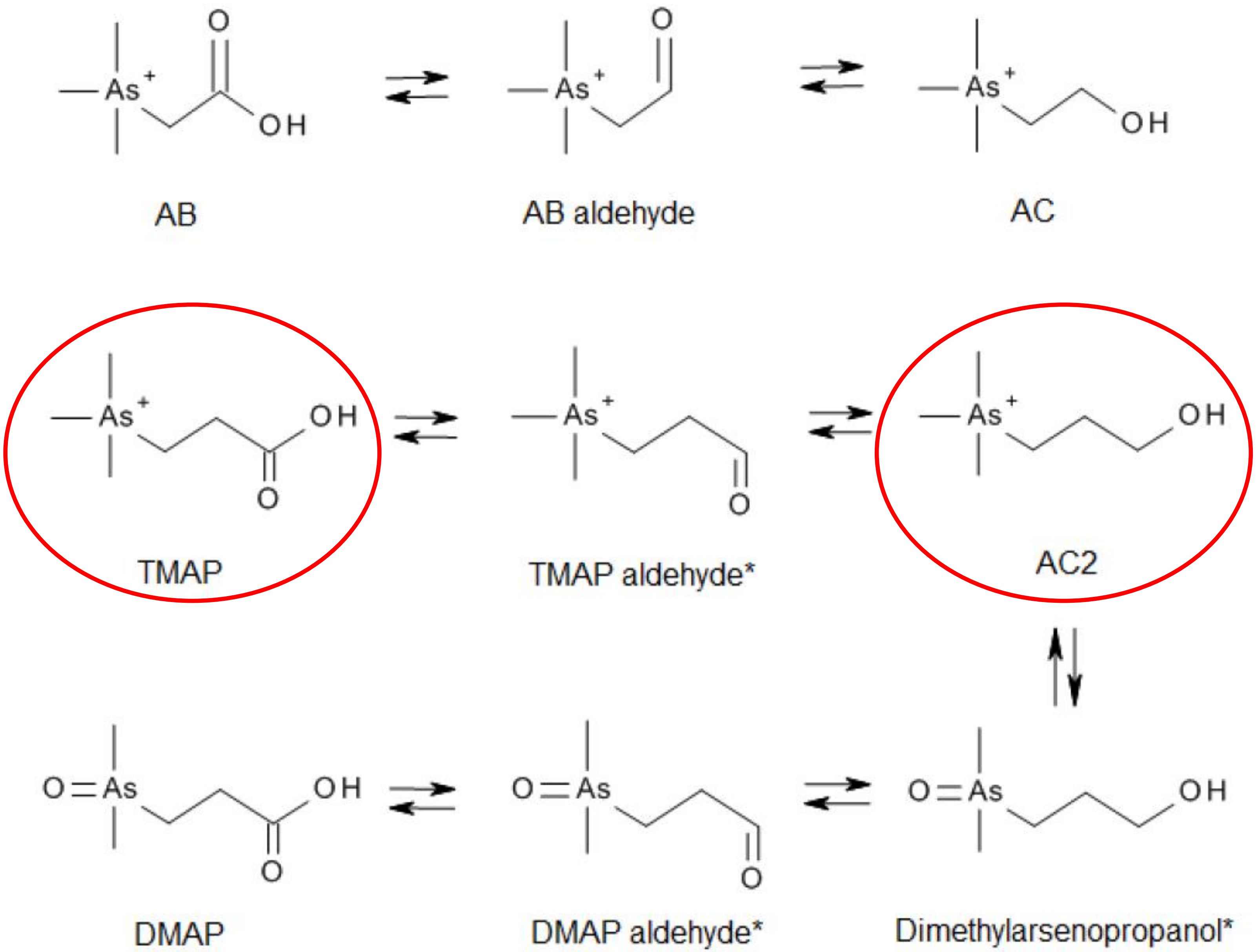

Kuřátka lososová

homoarsenocholin

trimethylarsoniopropanate

Table 1 Total As and extracted As concentrations in *Ramaria* samples [mg kg^{-1} dm] and detected As species [% of extracted As]. Other As species that were detected in small amounts (< 5 %) are: MA, DMA, As (V), TMAO, TETRA, DMAA and several unknown As species.

Sample ID	Species	origin	total As [mg kg^{-1}]	extr. As [mg kg^{-1}]	AB [%]	AC [%]	TMAP [%]	AC2 [%]
ASP-017	*R subbotrytis*	Slovakia	25 ± 2	22 ± 2	92 ± 7	1.6 ± 0.3	0.16 ± 0.02	0.27 ± 0.04
ASP-023	*R subbotrytis*	Czech Republic	61 ± 5	66 ± 4	91 ± 9	2.7 ± 0.4	0.3 ± 0.1	0.47 ± 0.02
ASP-068	*R subbotrytis*	Czech Republic	44 ± 4	39 ± 1	80 ± 20	1.7 ± 0.2	0.58 ± 0.01	0.47 ± 0.04
STM-107	*R* sp.	Austria	1.7 ± 0.1	1.4 ± 0.1	67 ± 2	4.1 ± 0.2	1.26 ± 0.1	0.82 ± 0.08
STM-108	*R* sp.	Austria	11.7 ± 0.2	9.5 ± 0.1	89 ± 1	1.57 ± 0.02	0.46 ± 0.02	0.37 ± 0.01
STM-109	*R* sp.	Austria	8.3 ± 0.3	6.9 ± 0.2	87 ± 2	2.4 ± 0.1	0.83 ± 0.03	0.93 ± 0.01


As+
O
OH
AB
AB aldehyde
AC
TMAP
TMAP aldehyde*
AC2
O=As
DMAP
DMAP aldehyde*
Dimethylarsenopropanol*

Analytical and Bioanalytical Chemistry (2024) 416:1399–1405
https://doi.org/10.1007/s00216-024-05132-z

RESEARCH PAPER

Arsenobetaine amide: a novel arsenic species detected in several mushroom species

Martin Walenta[1] · Andrea Raab[1] · Simone Braeuer[2] · Lorenz Steiner[3] · Jan Borovička[4,5] · Walter Goessler[1]

Received: 2 November 2023 / Revised: 13 December 2023 / Accepted: 18 December 2023 / Published online: 16 January 2024

Abstract
The total arsenic mass fraction as well as the arsenic speciation were studied in four different mushroom species with inductively coupled plasma mass spectrometry and high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry, respectively. Arsenic mass fractions detected in the mushrooms were covering a range from 0.3 to 22 mg As kg^{-1} dry mass. For the arsenic speciation, species like arsenobetaine, inorganic arsenic, or dimethylarsinic acid were found, which are commonly detected in mushrooms, but it was also proven that the recently discovered novel compound homoarsenocholine is present in *Amanita muscaria* and *Ramaria sanguinea*. Moreover, a previously unidentified arsenic species was isolated from *Ramaria sanguinea* and identified as trimethylarsonioacetamide, or in short: arsenobetaine amide. This new arsenical was synthesized and verified by spiking experiments to be present in all investigated mushroom samples. Arsenobetaine amide could be an important intermediate to further elucidate the biotransformation pathways of arsenic in the environment.

Keywords Mushrooms · *Ramaria sanguinea* · Arsenic speciation · Trimethylarsonioacetamide · HPLC-ICPMS · HR ESI-MS

Volume 416 · Number 6 · March 2024

ANALYTICAL & BIOANALYTICAL CHEMISTRY

SCS Swiss Chemical Society

Real Sociedad Española de Química

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ANALYTICAL & BIOANALYTICAL CHEMISTRY

Vol 416 · No 6 · March 2024 · pp xxxx–xxxx

Macro- and microplastic databases for high-throughput FTIR analysis

Wearable biosensors for human sweat glucose detection

Evaluating deep eutectic solvents in the synthesis of molecularly imprinted fibers

Arsenobetaine amide: a novel arsenic species detected in several mushroom species

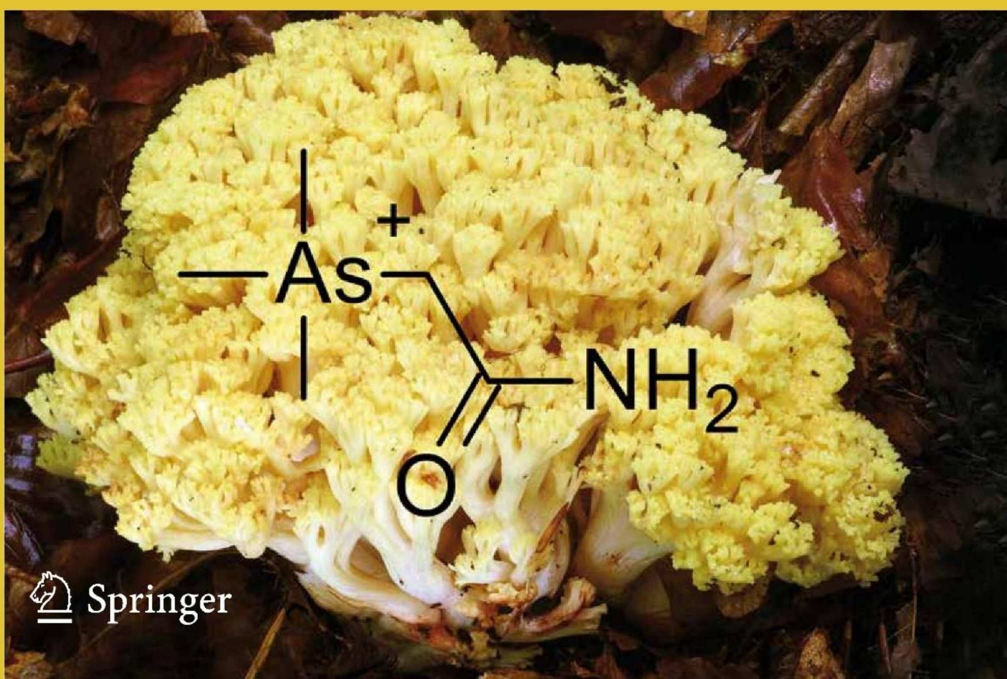
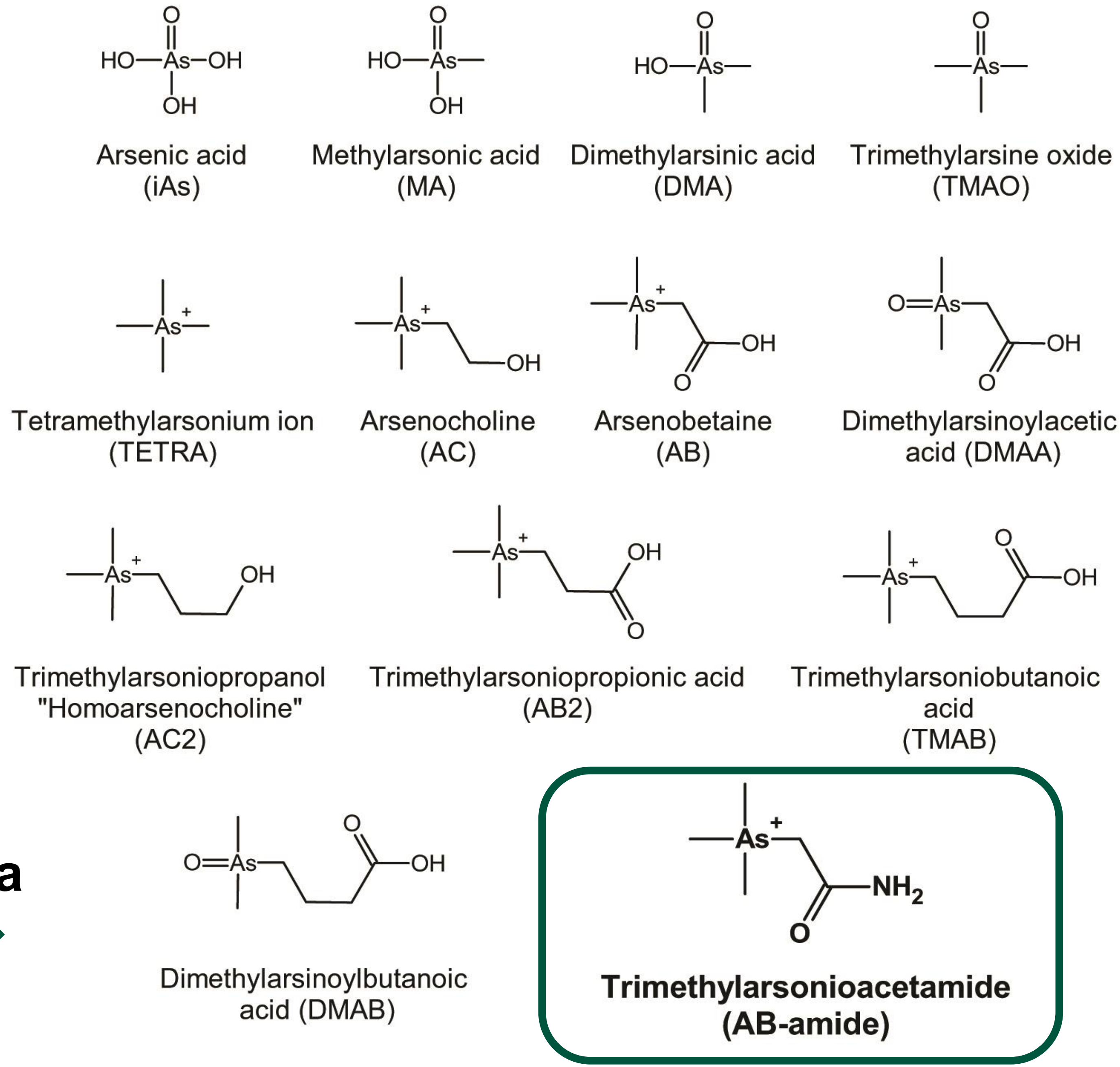


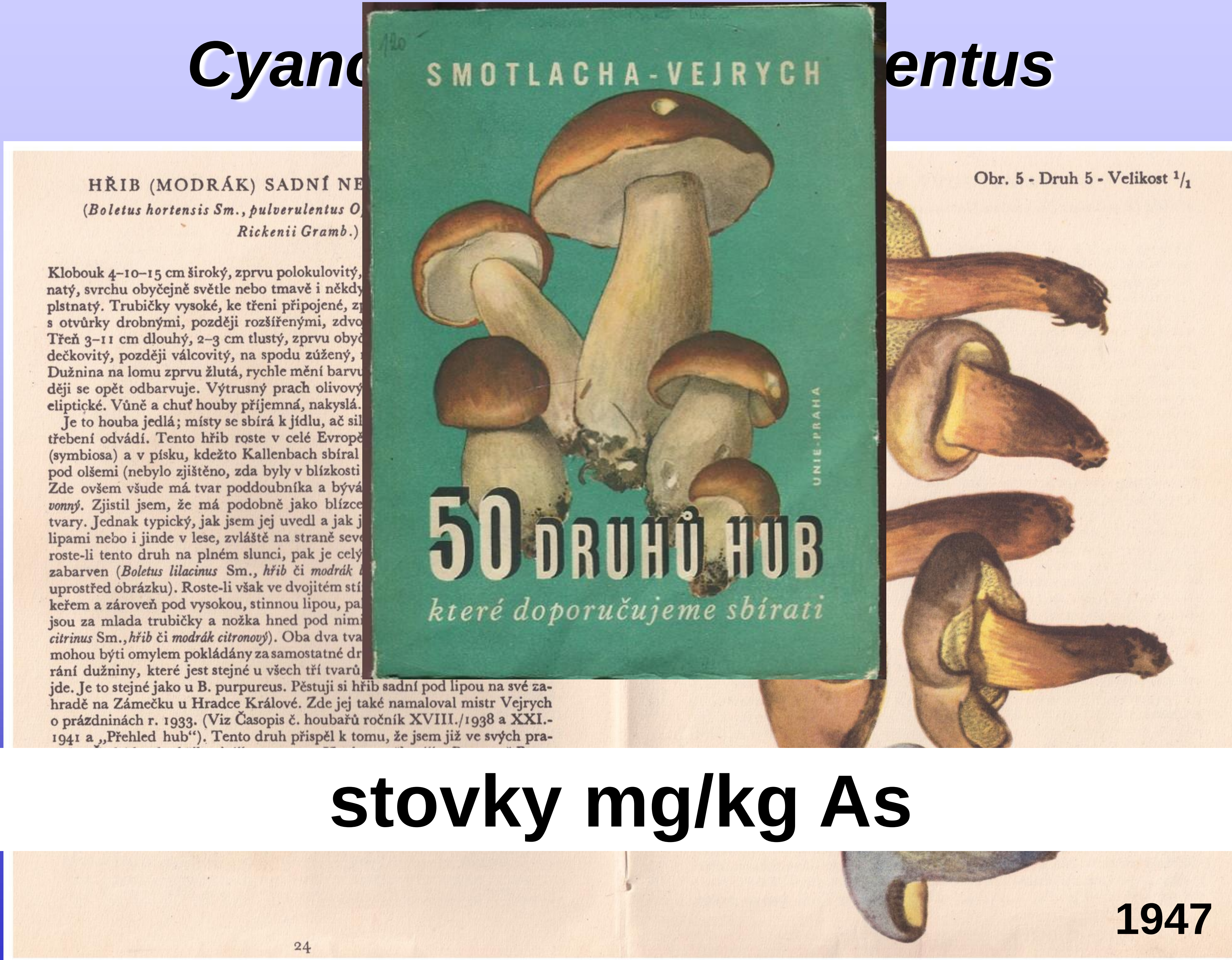
Fig. 1 Arsenic species relevant for this publication



Nová sloučenina As →

Hřib modračka – *Cyanoboletus pulverulentus*

Cyano entus


HŘIB (MODRÁK) SADNÍ NE

(Boletus hortensis Sm., pulverulentus O
Rickenii Gramb.)

Klobouk 4–10–15 cm široký, zprvu polokulovitý,
natý, svrchu obyčejně světle nebo tmavě i někdy
plstnatý. Trubičky vysoké, ke třeni připojené, z
s otvůrky drobnými, později rozšířenými, zdvo
Třeň 3–11 cm dlouhý, 2–3 cm tlustý, zprvu obyč
dečkovitý, později válcovitý, na spodu zúžený,
Dužnina na lomu zprvu žlutá, rychle mění barvu
ději se opět odbarvuje. Výtrusný prach olivový
eliptické. Vůně a chuť houby příjemná, nakyslá.
Je to houba jedlá; místy se sbírá k jídlu, ač si
třebení odvádí. Tento hřib roste v celé Evropě
(symbiosa) a v písku, kdežto Kallenbach sbíral
pod olšemi (nebylo zjištěno, zda byly v blízkosti
Zde ovšem všude má tvar poddoubníka a bývá
vonný. Zjistil jsem, že má podobně jako blízce
tvary. Jednak typický, jak jsem jej uvedl a jak j
lipami nebo i jinde v lese, zvláště na straně sev
roste-li tento druh na plném slunci, pak je celý
zabarven (*Boletus lilacinus* Sm., *hřib* či *modrák*
uprostřed obrázku). Roste-li však ve dvojitém stí
keřem a zároveň pod vysokou, stinnou lipou, pa
jsou za mlada trubičky a nožka hned pod nimi
citrinus Sm., *hřib* či *modrák citronový*). Oba dva tva
mohou býti omylem pokládány za samostatné dr
rání dužniny, které jest stejné u všech tří tvarů
jde. Je to stejné jako u B. purpureus. Pěstuji si hřib sadní pod lipou na své za-
hradě na Zámečku u Hradce Králové. Zde jej také namaloval mistr Vejrych
o prázdninách r. 1933. (Viz Časopis č. houbařů ročník XVIII./1938 a XXI.-
1941 a „Přehled hub"). Tento druh přispěl k tomu, že jsem již ve svých pra-

24

Obr. 5 - Druh 5 - Velikost 1/1

stovky mg/kg As

1947

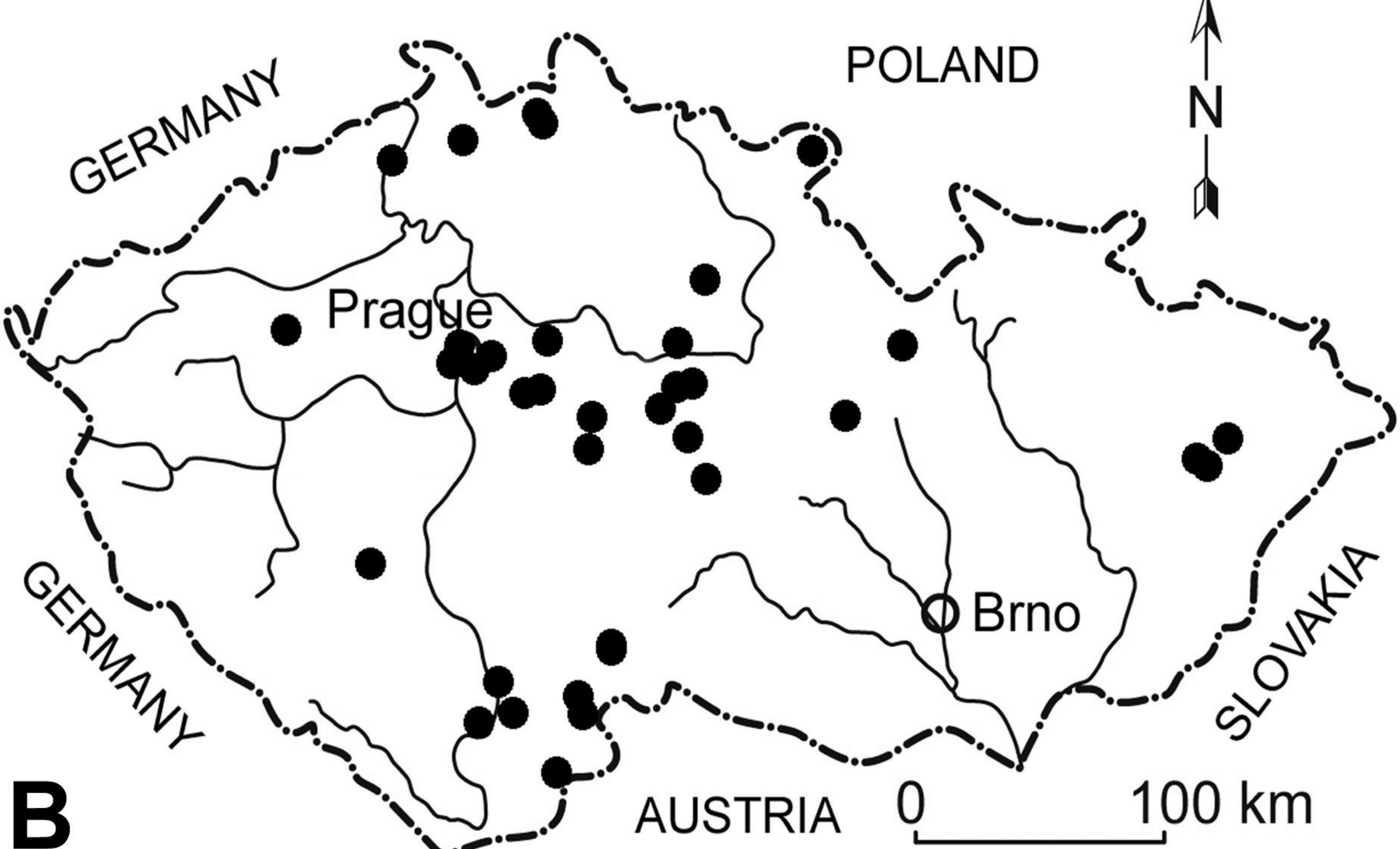
Cyanoboletus pulverulentus: otázky

1) Jaké
2) Je As
3) Jaká
4) Exist plodn
5) Exist půdá
6) Je hř

Je

Jak získat dost sběru pro výzkum?


Česká republika (35)
Francie (1)
Madeira (1)
USA (1)
Cyanoboletus pulverulentus



GERMANY
POLAND
N
Prague
GERMANY
Brno
SLOVAKIA
AUSTRIA
0
100 km
B

Food Chemistry 242 (2018) 225–231

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Arsenic hyperaccumulation and speciation in the edible ink stain bolete (*Cyanoboletus pulverulentus*)

Simone Braeuer[a], Walter Goessler[a], Jan Kameník[b], Tereza Konvalinková[c], Anna Žigová[d], Jan Borovička[b,d,*]

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This paper is dedicated to Tjakko Stijve on the occasion of his 80th birthday.

Keywords:
Edible mushrooms
Dimethylarsinic acid
Soil
Health risk
HPLC-ICPMS

ABSTRACT

The edible ink stain bolete (*Cyanoboletus pulverulentus*) was found to hyperaccumulate arsenic. We analyzed 39 individual collections determined as *C. pulverulentus*, mostly from the Czech Republic. According to our results, concentrations of arsenic in *C. pulverulentus* fruit-bodies may reach 1300 mg kg^{-1} dry weight. In most collections, data for total and bioavailable arsenic in underlying soils were collected but no significant correlation between the soil arsenic content and arsenic concentrations in the associated fruit-bodies was found. Within the fruit-bodies, we found the majority of arsenic accumulated in the hymenium. Besides occasional traces of methylarsonic acid (MA), the arsenic speciation in all mushroom samples consisted solely of dimethylarsinic acid (DMA) and no inorganic arsenic was detected. Because of the carcinogenic potential of DMA, *C. pulverulentus* should not be recommended as an edible mushroom and its consumption should be restricted.

VÝSLEDKY

1) Vysoké rozpětí koncentrací As v plodnicích:

2.4 – 1300 mg/kg

Průměr a SMODCH: 250 ± 260 mg/kg

Medián: 160 mg/kg

= jedná se skutečně o jeden druh, nebo je ta variace způsobená jinými faktory?

VÝSLEDKY

Jak je to s druhy: existuje v datasetu více modraček?

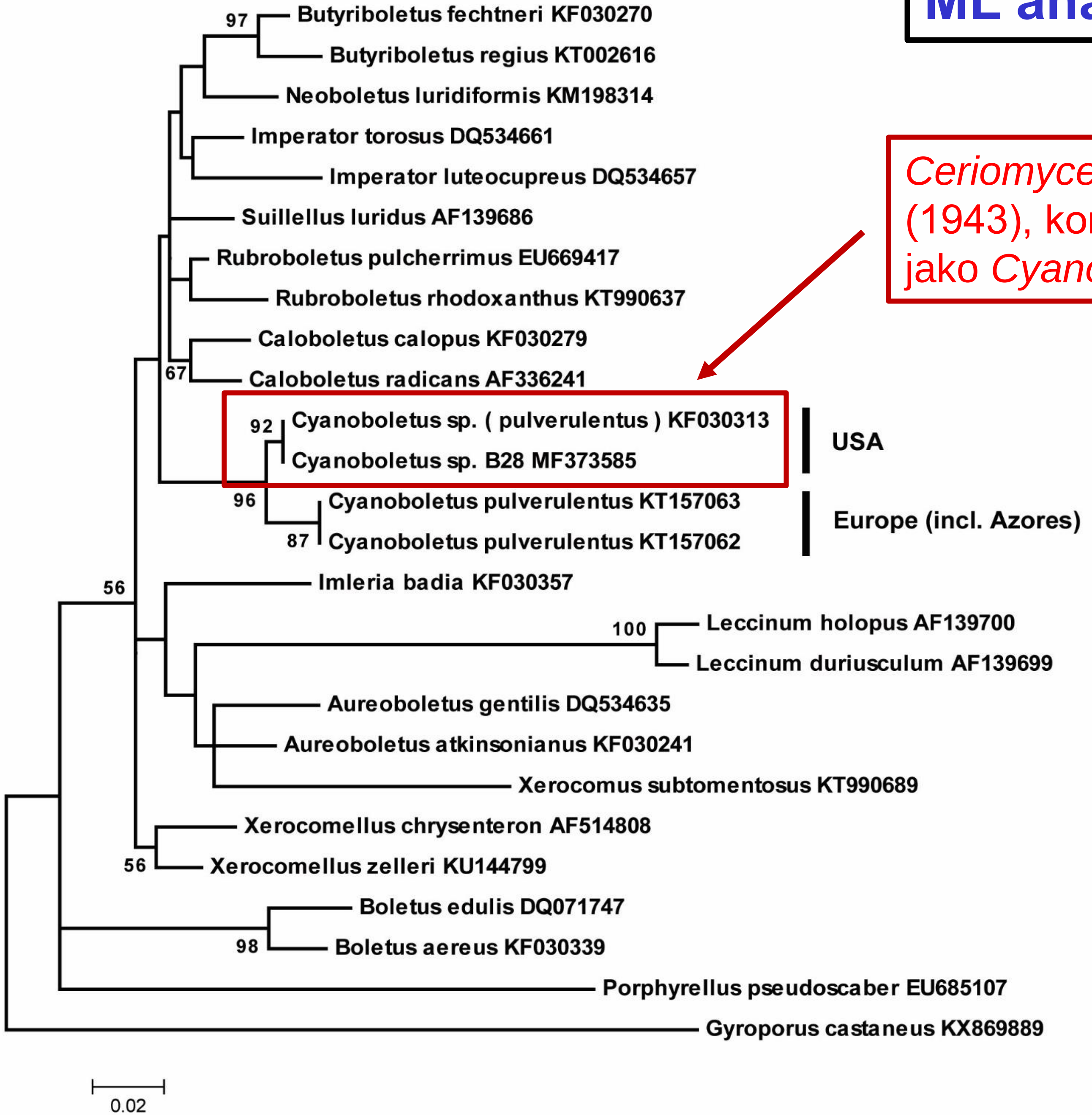
ITS rDNA pro šest evropských kolekcí identická

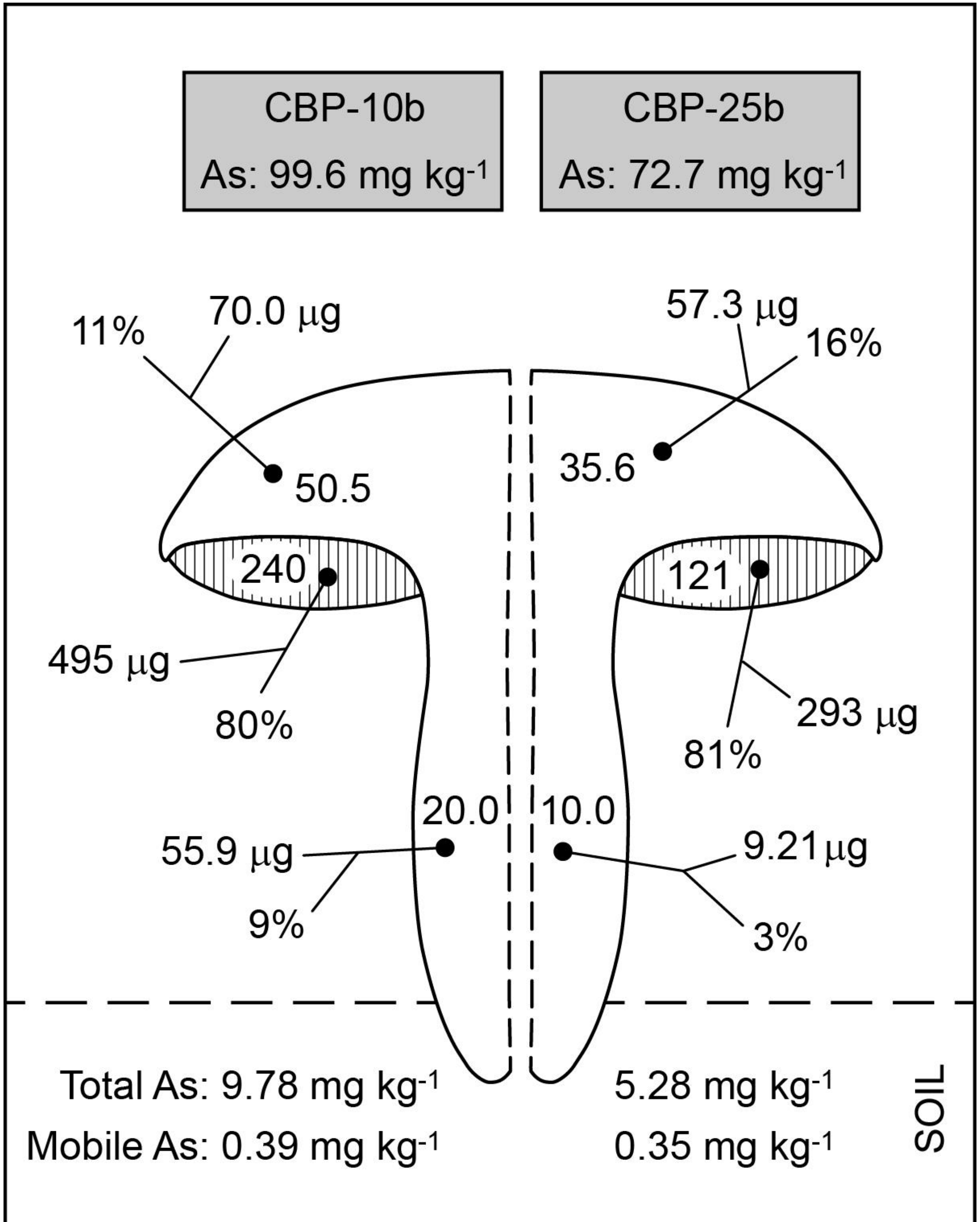
Americký sběr odlišný (sekvence LT714710, 86% podobnost)

Do jakého rodu patří?
Je to *Cyanoboletus*?

ML analýza (LSU rDNA)

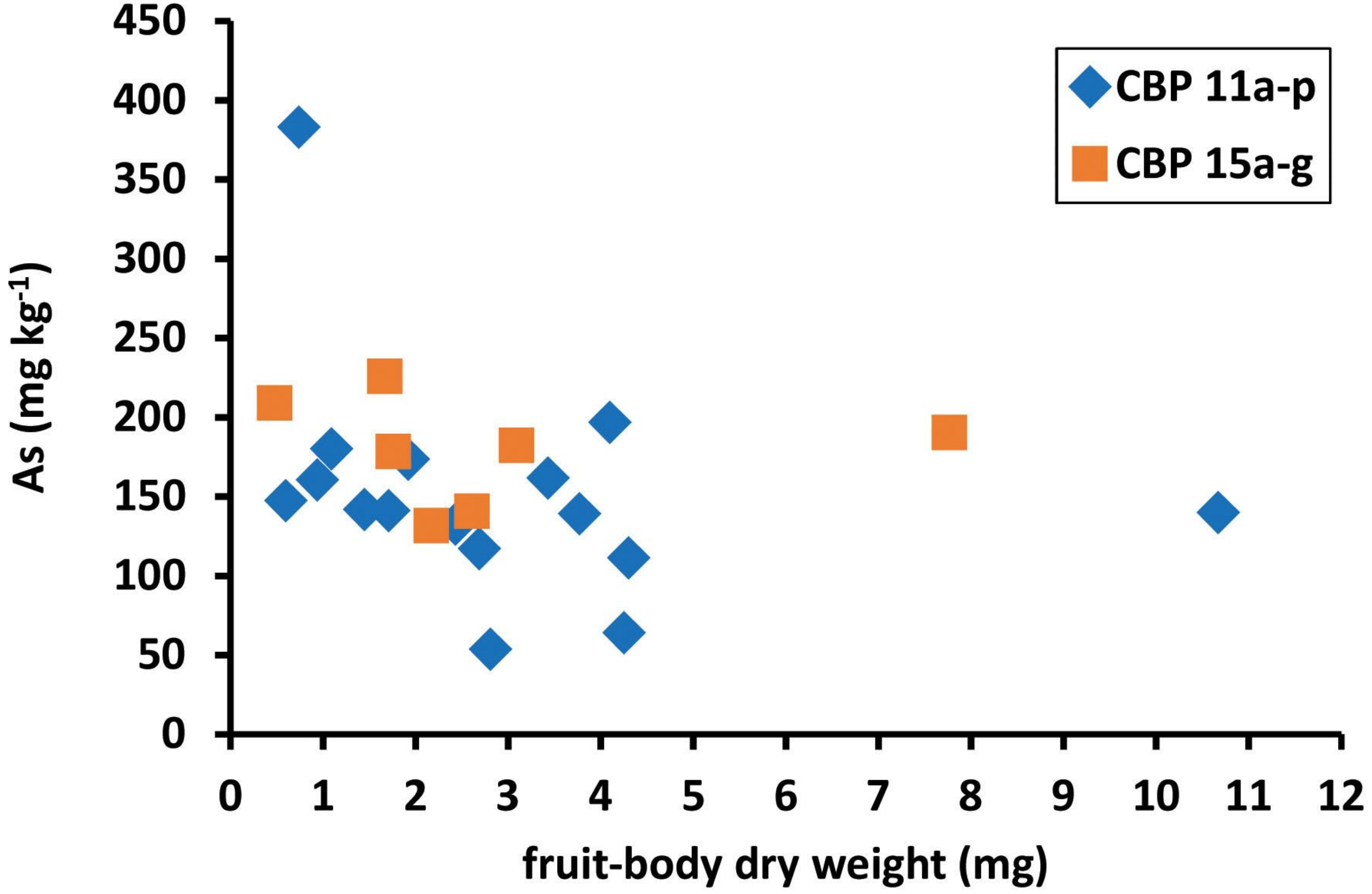
Ceriomyces cyaneitinctus Murrill (1943), kombinován v roce 2021 jako *Cyanoboletus cyaneitinctus*.



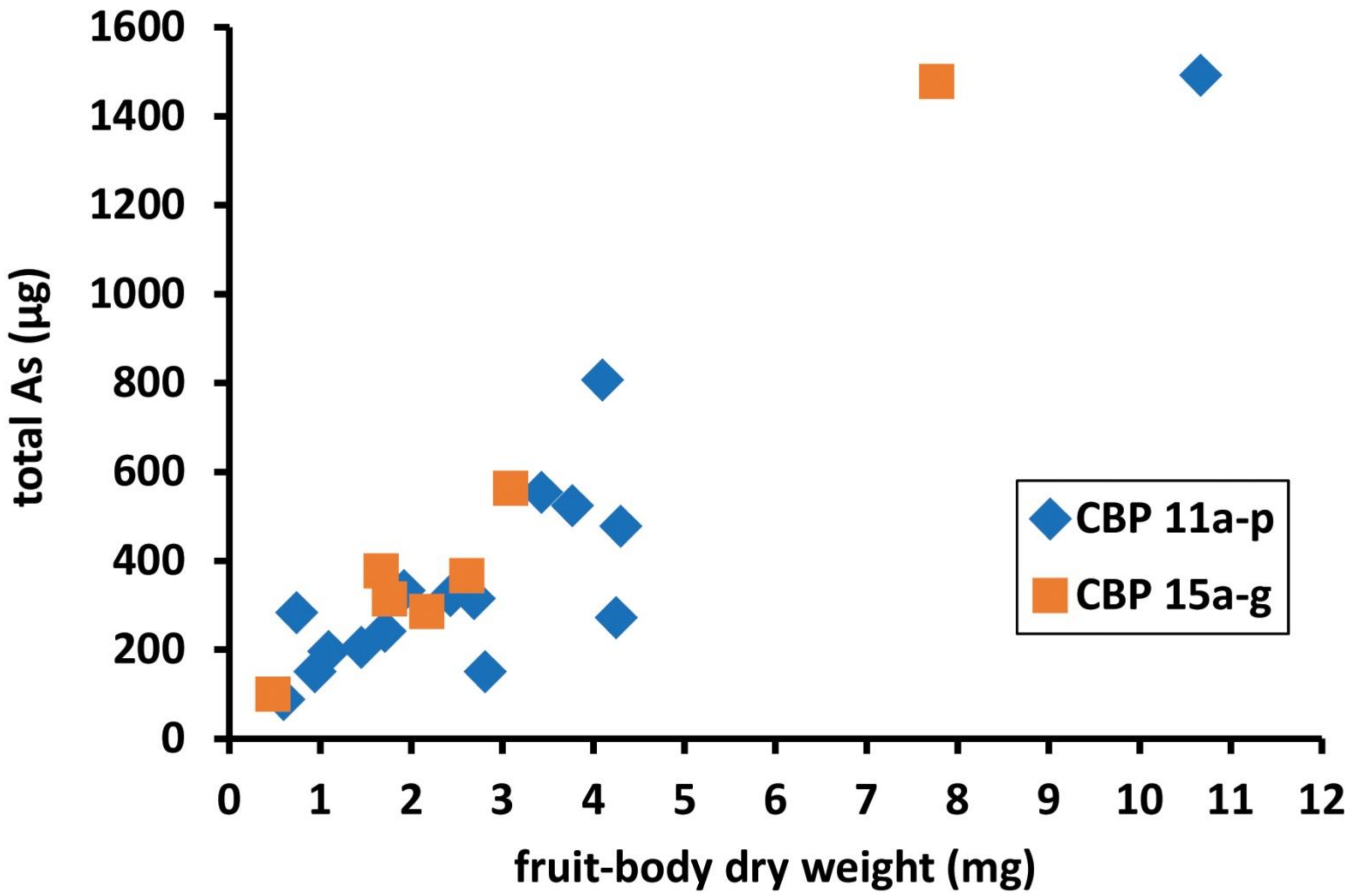


Dvě plodnice
Dvě podobné lokality
Podobné koncentrace
Stejná distribuce

Koncentrace As / hmotnost plodnice



Celkové množství arzénu / hmotnost plodnice



VÝSLEDKY

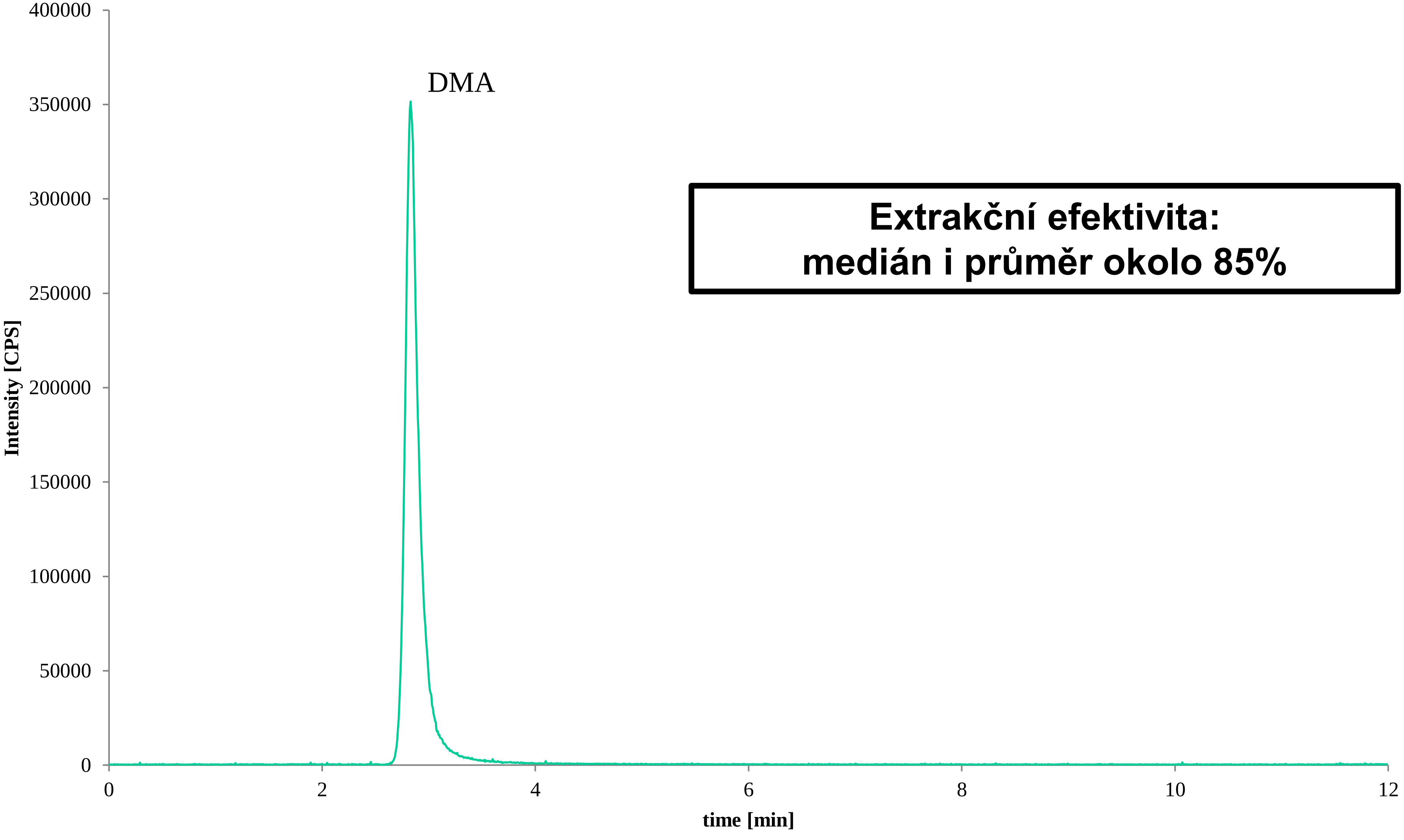
C. pulverulentus preferuje kyselé půdy (Ah horizont), s pH (H_2O) v rozmezí 3.6–7.0 (medián 5.1).

As v půdách (Ah): 6–35 mg/kg (medián 12.2 mg/kg).

Nebyla nalezena významná závislost mezi koncentrací As v půdách (celkový / biodostupný arzén) a koncentrací v plodnicích modraček.

Jen a pouze DMA*

***se stopami MA v několika kolekcích**



Extrakční efektivita:
medián i průměr okolo 85%

VÝSLEDKY

DMA: látka s nízkou akutní toxicitou, ale

→ pravděpodobně karcinogenní (United States Environmental Protection Agency)

→ jednoznačně karcinogenní (International Agency for Research on Cancer)

European Food Safety Agency:

zhodnocení rizika karcinogenních substancí pomocí kalkulace MoE: Margin of Exposure, mezní expozice

VÝSLEDKY

70 kg těžká (lehká) osoba

běžná koncentrace As v plodnici (115 mg/kg)

Konzumace více než 90 g čerstvé houby ročně teoreticky zvyšuje riziko vzniku rakoviny.

Takže je to na vás…

Záhada baňky velkokališné

Is arsenic responsible for the toxicity of the hyperaccumulating mushroom *Sarcosphaera coronaria*?

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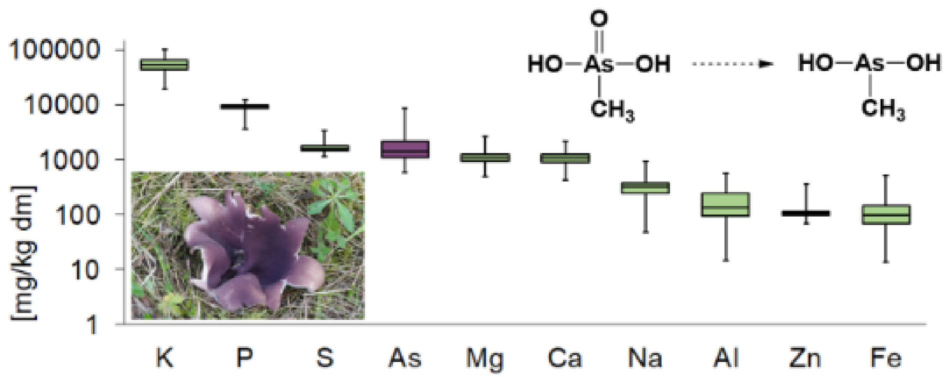
HIGHLIGHTS

- Arsenic accounts for up to record breaking 0.89% of dried *Sarcosphaera coronaria*.
- No correlations between arsenic in soil and fungal fruit-bodies from Czech Republic.
- Methylarsonic acid accounts for >90% of the total arsenic in fruit-bodies.
- Significant concentrations of highly toxic methylarsonous acid in fruit-bodies
- Arsenic could be responsible for the toxicity of *Sarcophaera coronaria*.

GRAPHICAL ABSTRACT



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ABSTRACT

The Violet Crown Cup, *Sarcosphaera coronaria*, is a rather inconspicuous mushroom, but with an interesting and unresolved mystery. In earlier days, the mushroom was considered edible, but several poisonings were reported in the early 20th century. The reason for the seemingly sporadic toxicity of *S. coronaria* is still unknown. One possible explanation is arsenic, since Crown Cups can take up high amounts of this element. We investigated the arsenic concentration and arsenic speciation in *S. coronaria* with inductively coupled plasma mass spectrometry (ICPMS) and HPLC coupled to ICPMS and found up to incredible 0.9% As (dry mass). Most of it was present as methylarsonic acid (MA), a less toxic form of this element. However, low concentrations of the highly toxic methylarsonous acid [MA (III)] were also detected. The amounts were too low to pose an acute risk for consumers, but the concentration of MA (III) significantly increased during simulated gastric digestion. We could not unambiguously identify arsenic as the toxic constituent of *S. coronaria*, but we demonstrated that the extremely toxic MA (III) can be formed under certain circumstances, which should be carefully investigated in future.

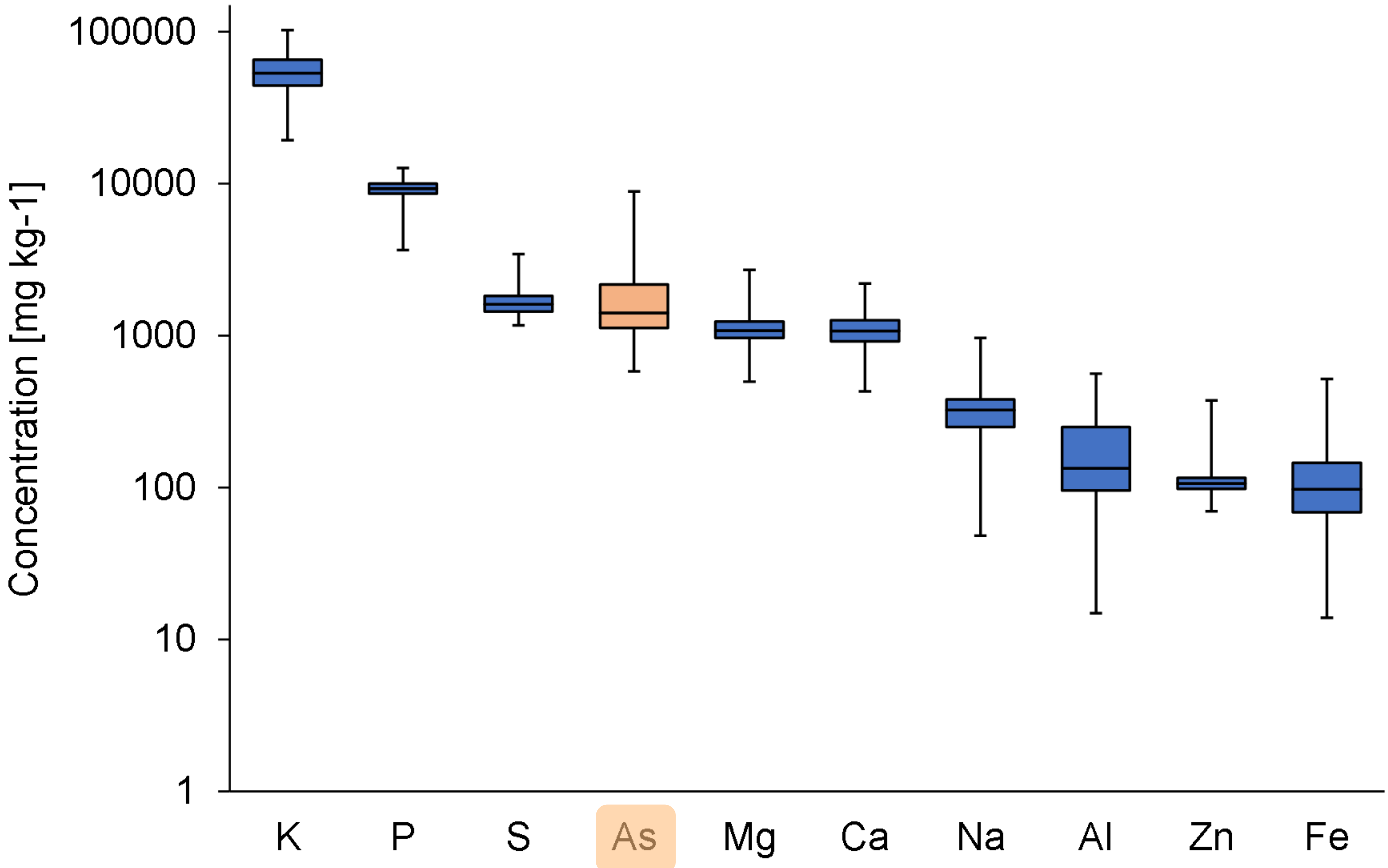
Baňka velkokališná – *Sarcosphaera coronaria*

Table 3. As concentrations (mg kg^{-1}dry mass) in *Sarcosphaera coronaria*, reported by various authors. n = number of samples; n.a. = not available. *Reported as 15 mg kg^{-1} (fresh mass basis).

Year	Origin	n	Mean	range	Source
1990	Switzerland	4	872	360 - 2130	(Stijve et al., 1990)
1995	Switzerland, Slovenia	3	877	150* - 2120	(Byrne et al., 1995)
2001	Switzerland	15	647	248 - 2410	(Stijve, 2001)
1997, 2003, 2006	Italy	17	> 1000	153 - 3160	(Cocchi, 2003; Cocchi et al., 2006; Cocchi and Vescovi, 1997)
2004	Czech Republic	1	7090	-	(Borovička, 2004)
2007	Turkey	1	8.8	-	(Konuk et al., 2007)
2008	USA, CA	n.a.	610	n.a.	(Stijve, 2008)
2020	Czech Republic	62	1800 ± 1500	380 - 8900	This work
2020	Northern America	9	33 ± 36	3.2 - 120	This work

Table 4. As concentrations (in mg kg^{-1} dry mass) in soil (mean ± standard deviation of three samples) and corresponding *Sarcosphaera coronaria* samples (mean ± standard deviation of all samples from the same site), and the calculated bioaccumulation factor (BAF = mushrooms/soil)

Sample	**Soil**	***S. coronaria***	**BAF**
ASP-045	6.8 ± 0.12	8900	1310
ASP-127	22 ± 1.2	1200 ± 300	55 ± 14
ASP-128	15 ± 0.46	2200 ± 800	150 ± 50
ASP-129	17 ± 2.6	1800 ± 320	110 ± 20
ASP-130	16 ± 0.74	1300 ± 240	83 ± 15
ASP-131	16 ± 0.65	1100 ± 480	68 ± 30
ASP-132	48 ± 0.47	3820 & 3510	79 & 72
ASP-133	37 ± 0.98	1600 ± 430	43 ± 12
ASP-134	12 ± 0.46	4100 ± 2400	340 ± 200
ASP-135	13 ± 0.56	2900 ± 530	230 ± 40
ASP-178	22 ± 1.2	670 ± 380	31 ± 17
ASP-179	10 ± 0.24	1400	141
ASP-180	13 ± 0.1	790	59
ASP-142	6.6 ± 0.3	29 ± 5.9	4.4 ± 0.9

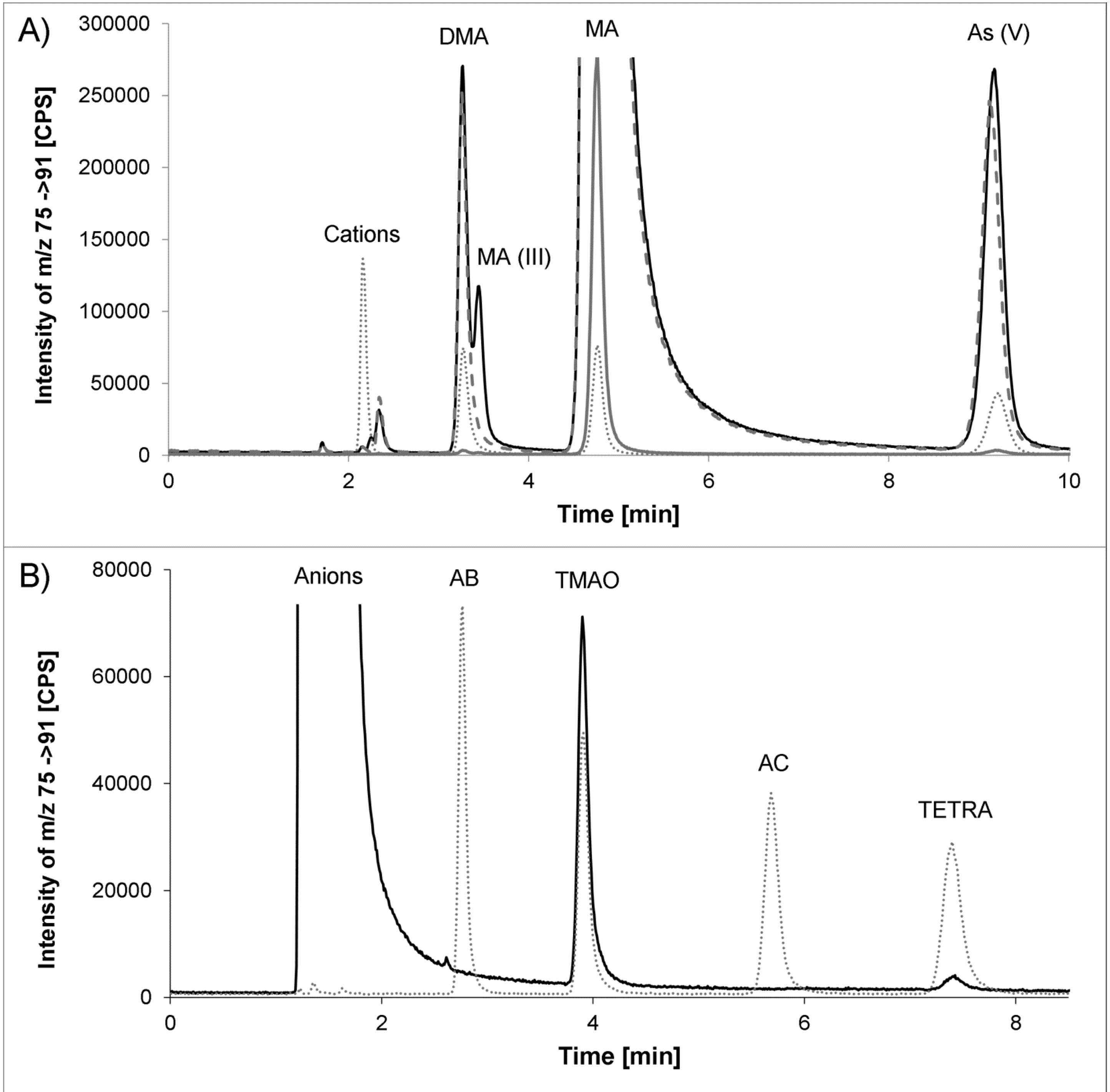


Concentration [mg kg-1]
100000
10000
1000
100
10
1
K
P
S
As
Mg
Ca
Na
Al
Zn
Fe

Table 5. As species in *Sarcosphaera coronaria* samples from the Czech Republic (CZ) and Northern America (NA), in % of total As. Traces = between 0.005 and 0.015 mg As kg^{-1}. n.d. = not detected (LOD: 0.005 mg As kg^{-1}). Not detected in any sample: AB, AC, DMAA, TMAP, AC2. “Not found as species” = Extracted As minus sum of all species. “Not extracted” = Total As concentration minus extracted As.

toxická

Species	Mean ± standard deviation [%]		Median [%]		Range [%]	
	CZ	NA	CZ	NA	CZ	NA
MA	85 ± 13	77 ± 7.5	83	74	51 - 120	65 - 91
MA (III)	0.15 ± 0.19	1.9 ± 2.4	0.1	0.36	0.0002 - 1.1	0.066 - 6.9
DMA	0.25 ± 0.24	0.46 ± 0.23	0.17	0.49	0.027 - 0.98	0.17 - 0.91
iAs	0.52 ± 0.44	0.11 ± 0.10	0.49	0.094	0.001 - 2.1	n.d. (n=3) - 0.23
TMAO	0.06 ± 0.15	traces	0.02	traces	0.0018 - 1.1	n.d. (n=4), traces (n=5)
TETRA	0.006 ± 0.015	n.d.	0.0021	n.d.	traces (n=28) - 0.11	n.d.
Not found as species	12 ± 12	6.8 ± 3.8	16	7.3	≤ 41	≤ 11
Not extracted	1.3 ± 7.6	14 ± 6	1.5	14	≤ 22	8.6 - 27


A)
DMA
MA
As (V)
Cations
MA (III)
Intensity of m/z 75 ->91 [CPS]
Time [min]
300000
250000
200000
150000
100000
50000
0
0
2
4
6
8
10
B)
Anions
AB
TMAO
AC
TETRA
Intensity of m/z 75 ->91 [CPS]
Time [min]
80000
60000
40000
20000
0
0
2
4
6
8

Může trávení zvýšit podíl vysoce toxické MA (III)?

Table 6. Simulation of gastric digestion of *Sarcosphaera coronaria* sample ASP-045, in % of the control sample. The values are the average of two independent replicates, which always differed less than 10 % from each other.

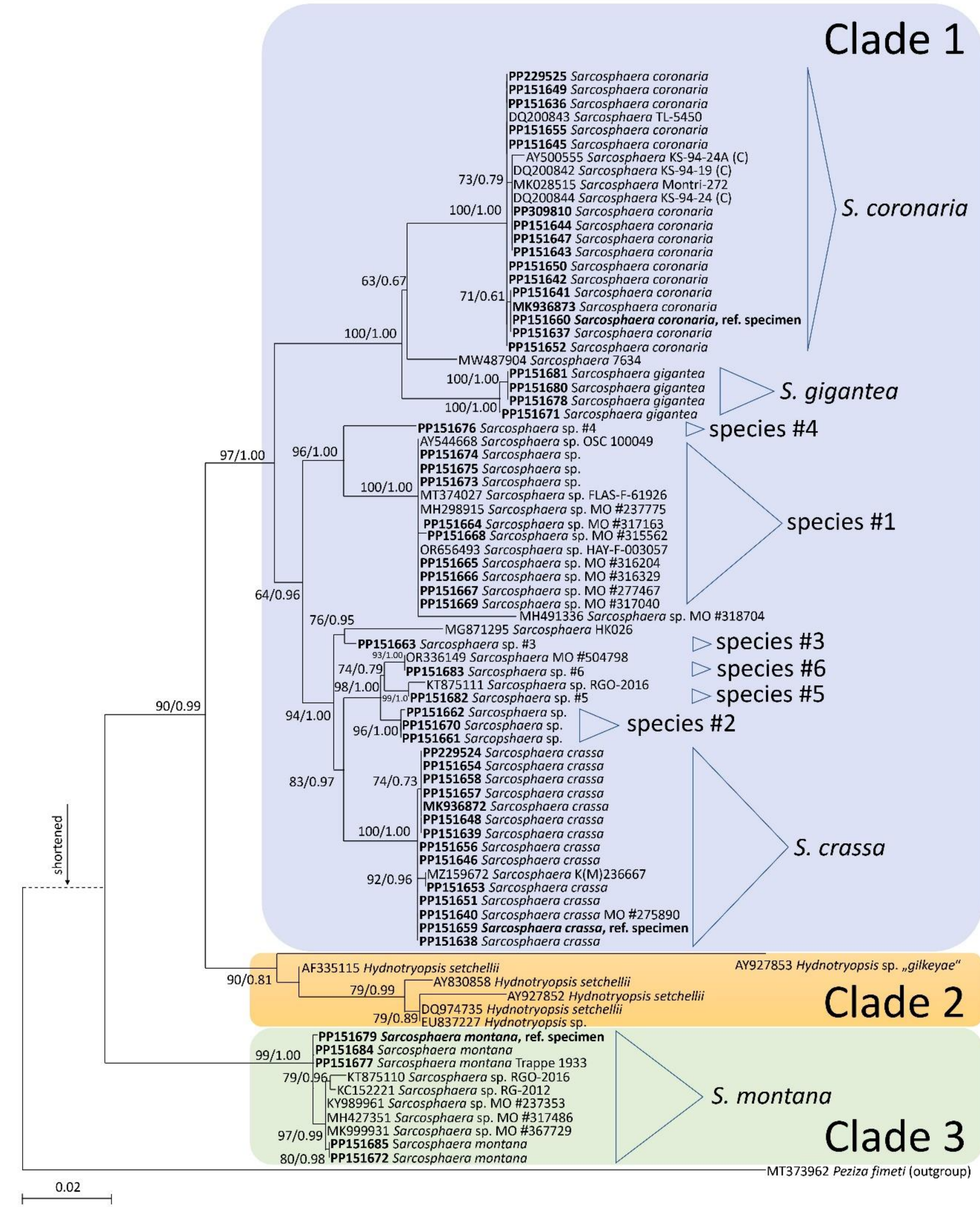
Duration	MA	MA (III)	DMA	As (V)	TMAO	TETRA
1 hour	110	260	115	210	95	100
2 hours	115	290	120	205	110	130
4 hours	125	405	130	210	105	140
12 hours	115	440	110	220	119	132

ZÁVĚR

Koncentrace As v baňce velkokališné mohou dosáhnout až 0,9% v sušině. Plodnice ze Severní Ameriky mají koncentrace výrazně nižší (? jiný biologický druh).

Hlavní sloučeninou As v baňce je MA, byla zjištěna i její vysoce toxická forma MA (III), ale v malém množství.

Toxicitu baňky není možné vysvětlit vysokým obsahem As, nelze však vyloučit vliv kuchyňského zpracování či dalších faktorů.



Baňky – trochu více druhů, než jsme čekali…

Sarcosphaera coronaria (foto Jiří Souček)

Sarcosphaera crassa


"That's all Folks!"