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

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SUPPORTING INFORMATION

Food Chemistry (2017)

Content:

Supplementary Table T1. Analyzed *Cyanoboletus* collections with data on sampling sites, associated host plants, herbarium vouchers and molecular data (EMBL-Bank, GenBank).

ID	date	country	locality	host plants at site	herbarium	molecular data
CBP-01	3.6.2016	Czech Rep.	Prague, Petřín Hill	*Betula, Fagus*	PRM 944069	-
CBP-02	9.6.2016	Czech Rep.	Prague, Cibulka Park	*Quercus, Carpinus*	PRM 944014	LT714705
CBP-03	8.6.2016	Czech Rep.	Hluboká n. Vlt.	*Fagus*	-	-
CBP-04	14.6.2016	Czech Rep.	Poříčko n. Sáz.	*Carpinus, Picea*	PRM 857481	-
CBP-05	11.6.2016	France	Corrèze, region Limousin	*Tilia, Picea*	PRM 944020	-
CBP-06	20.6.2016	Czech Rep.	Vlašim	*Tilia, Quercus, Carpinus*	PRM 944011	-
CBP-07	10.6.2016	Czech Rep.	Čerčany (viaduct)	*Tilia, Quercus*	PRM 944001	LT714706
CBP-08	20.6.2016	Czech Rep.	Čerčany	*Tilia*	-	-
CBP-09	21.6.2016	Czech Rep.	Prague-Klánovice, Úvaly	*Tilia, Quercus*	PRM 944015	-
CBP-10	16.6.2016	Czech Rep.	Žebračka (Přerov)	*Tilia*	PRM 944006	-
CBP-11	16.6.2016	Czech Rep.	Žebračka (Přerov)	*Tilia*	-	-
CBP-12	22.6.2016	Czech Rep.	Jablonné v Podještědí	*Tilia, Quercus, Carpinus*	PRM 944019	-
CBP-13	22.6.2016	Czech Rep.	Jablonné v Podještědí	*Tilia, Picea*	PRM 944018	-
CBP-14	25.6.2016	Czech Rep.	Rančice	*Tilia, Picea*	-	-
CBP-15	17.6.2016	Czech Rep.	Jesenice (Rakovník)	*Quercus, Corylus*	PRM 944013	LT714707
CBP-16	25.6.2016	Czech Rep.	Heřmanův Městec	*Carpinus*	PRM 944021	-
CBP-17	19.6.2016	Czech Rep.	Úsobí (Humpolec)	*Tilia*	PRM 944022	LT714708
CBP-18	8.6.2016	Czech Rep.	Jindřichův Hradec (JH1)	*Tilia*	PRM 935997	LT714709
CBP-19	8.6.2016	Czech Rep.	Jindřichův Hradec (JH2)	*Tilia*	-	-
CBP-20	15.6.2016	Czech Rep.	Borovany	*Tilia, Quercus, Fagus*	PRM 944029	-
CBP-21	8.6.2016	Czech Rep.	Drachkov (Strakonice)	*Quercus*	-	-
CBP-22	3.7.2016	Czech Rep.	Rychnov na Moravě	*Quercus, Corylus, Tilia*	HR 102057	-
CBP-23	10.8.2016	Czech Rep.	Lichnice-Kaňkovy hory	*Picea, Ulmus, Fagus, Carpinus*	HR 99971	-
CBP-24	8.7.2016	Czech Rep.	Rožmitál (Broumov)	*Picea, Fagus, Corylus*	HR 101207	-
CBP-25	7.6.2016	Czech Rep.	Vrchovnice	*Corylus*	HR 102048	-
CBP-26	24.6.2016	Czech Rep.	Bojiště (Ledeč n. Sázavou)	*Tilia, Quercus, Picea*	HR 102059	-
CBP-27	17.8.2016	Czech Rep.	Ústí n. Labem, Vaňov	*Betula*	-	-
CBP-28	24.7.2016	Czech Rep.	Stvolínky	*Tilia*	-	-
CBP-29	6.8.2016	Czech Rep.	Kersko	*Tilia, Quercus*	-	-
CBP-30	7.8.2016	Czech Rep.	Prague, Homolka Pond	*Quercus*	-	-
CBP-31	25.6.2016	Czech Rep.	Svitavy	*Tilia*	-	-
CBP-32	15.8.2016	Czech Rep.	Lipník n. Bečvou	*Quercus, Carpinus, Fagus*	-	-
CBP-33	2005	Czech Rep.	Žofínský prales (Pohorská Ves)	*Fagus*	-	-
CBP-34	26.9.2005	Czech Rep.	Nadějov Hill (Staňkov)	*Fagus*	-	-
CBP-35	27.9.2005	Czech Rep.	Chlum u Třeboně, Bukové kopce	*Fagus*	-	-
CBP-36	30.7.2016	Czech Rep.	Jihlava, Heulos Park	*Tilia, Quercus, Fagus*	-	-
ASP-26	26.9.2015	Portugal	Madeira, Ribeiro Frio	*Quercus*	PRM 935923	LT714704
ASP-36	6.9.2015	Czech Rep.	Lázně Bohdaneč	*Tilia*	HR 90200	-
ASP-82	23.7.2016	USA	NY, Oneida County	*Fagus, Betula*	PRM 944518	LT714710, MF373585

Supplementary Table T2. (A) Instrument settings and performance of ICPQQQMS as used for total arsenic analysis. Settings for speciation analysis were similar; any deviating settings are given in brackets. **(B)** Instrument settings and performance of ICPSFMS as used for analysis of bioavailable soil arsenic.

A. ICPQQQMS settings: total arsenic analysis (speciation analysis)		
Scan Type	MS/MS	
RF Power	1600	W
RF Matching	1.8	V
Smpl Depth	8	mm
Carrier Gas	1.1 (0.85)	L/min
Option Gas	0 (15)	%
Nebulizer Pump	0.1 (0.5)	rps
S/C Temp	2	°C
Extract 1	0	V
Extract 2	-160	V
Omega Bias	-90	V
Omega Lens	6.6	V
Q1 Entrance	-1	V
Q1 Exit	-1	V
Cell Focus	2	V
Cell Entrance	-50	V
Cell Exit	-60	V
Deflect	3.2	V
Plate Bias	-60	V
Q1 Bias	-2	V
Q1 Prefilter Bias	-44	V
Q1 Postfilter Bias	-180	V
4th cell gas flow	25	%
OcP Bias	-5	V
OcP RF	200	V
intensity (1 µg/L As, m/z 75 -> 91)	~12000	CPS

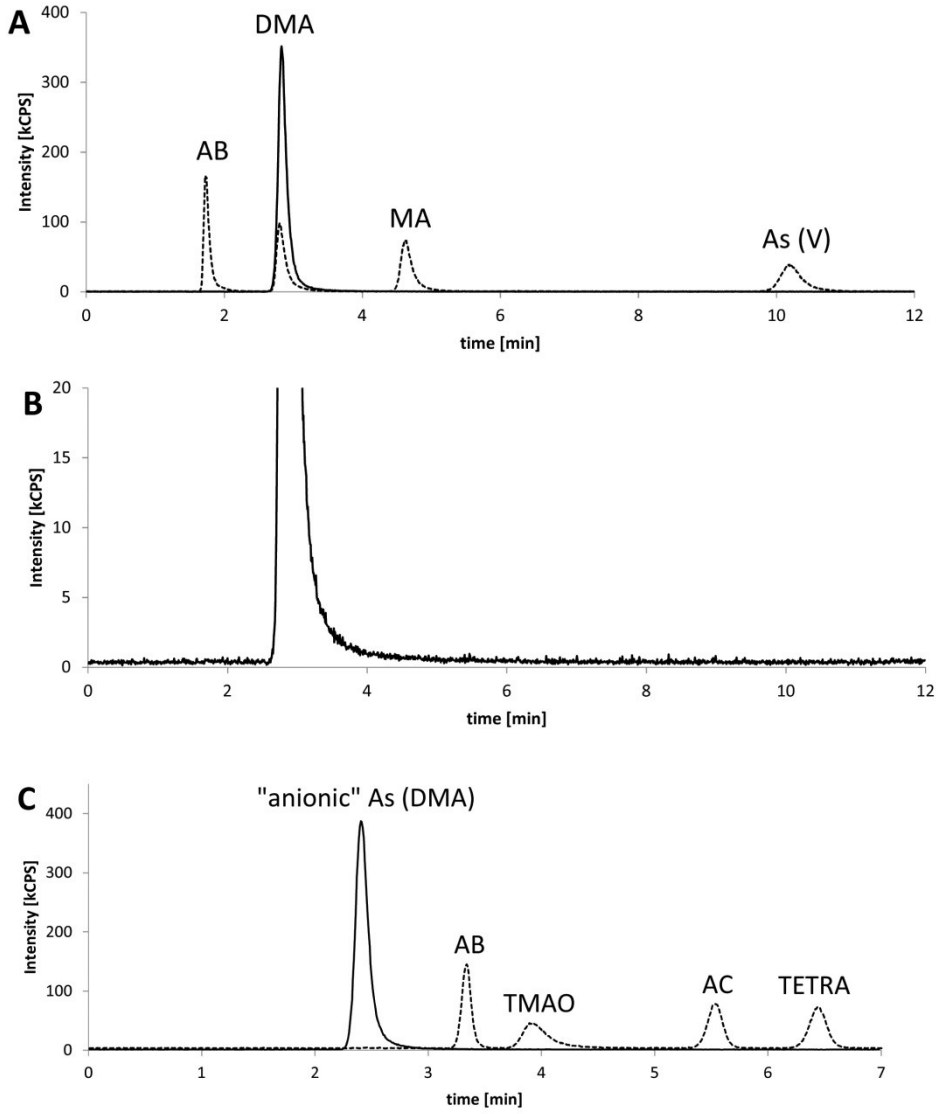
B. ICPSFMS Element 2 (analysis of bioavailable soil arsenic)		
Resolution mode	LR/HR	
RF Power	1200	W
Cool Gas	16	L/min
Sample Gas	0.955	L/min
Auxiliary Gas	0.96	L/min
Peristaltic Pump	4.5	rpm
Spray Chamber Type	Quartz Double-Pass	
Lenses (V)		
Extraction	-2000	V
Focus	-1165	V
X-Deflection	6.9	V
Y-Deflection	7.05	V

Shape	120	V
High Resolution Lenses (V)		
Quad 1	2.41	V
Quad 2	-2.51	V
Focus Quad	-7.15	V
SEM Deflection	-15	V
SEM Voltage (V)	1910	V
Sensitivity (1 ng/g As) Low Mass Resolution	~140000	CPS
Sensitivity (1 ng/g As) High Mass Resolution	~1400	CPS

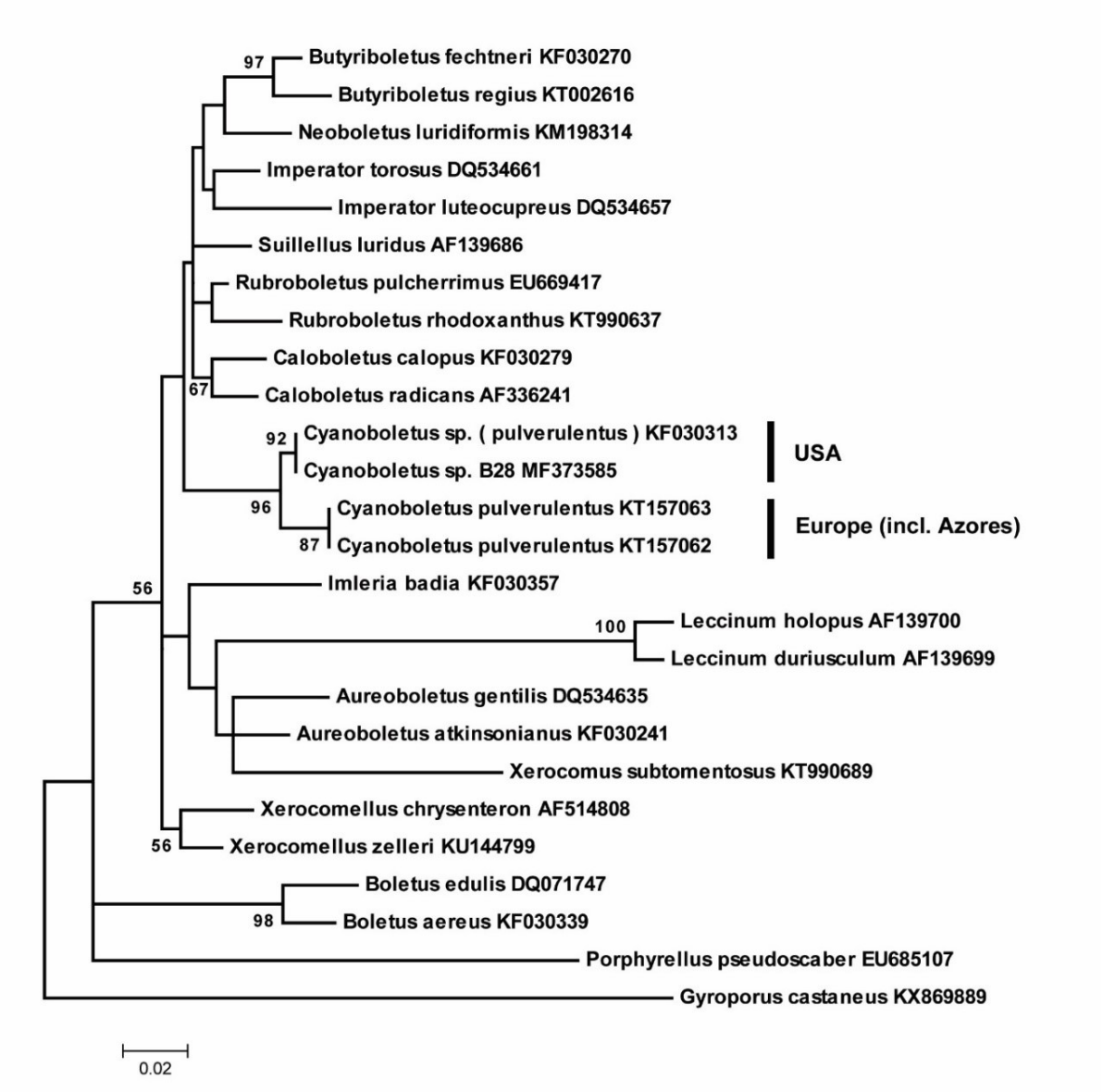
Supplementary Table T3. Total arsenic and arsenic species concentrations (in dry mass) of the individual fruit-bodies of *Cyanoboletus* collections and total/bioavailable arsenic concentrations (in dry mass) in corresponding soils.

sample	total As	extracted As	column recovery	DMA	MA	total soil As	biovailable soil As
	[mg kg^{-1}]	[mg kg^{-1}]	[%]	[mg As kg^{-1}]	[mg As kg^{-1}]	[mg kg^{-1}]	[µg kg^{-1}]
ASP-26	3.2 ± 0.1	2.8	82	2.3	< 0.02	21.9	1266
ASP-36	314 ± 5	311	112	349	0.3	n.a.	n.a.
ASP-82	2.4 ± 0.1	1	100	1.0	< 0.02	n.a.	n.a.
CBP-01	270 ± 30	220	86	190	< 0.02	25.6	1501
CBP-02	400 ± 20	340	88	300	0.29	26.6	1846
CBP-03	120 ± 10	76	86	65	< 0.02	12.6	653
CBP-04	250 ± 30	210	86	180	< 0.02	17.5	985
CBP-05c	45 ± 4	43	86	37	0.08	13.5	699
CBP-06	109 ± 8	91	84	76	< 0.02	36.2	1214
CBP-07	150 ± 10	130	85	110	< 0.02	15.9	1230
CBP-08	81 ± 4	70	84	59	< 0.02	8.45	331
CBP-09f	76 ± 9	65	83	54	< 0.02	21.3	1961
CBP-10a	160 ± 3	140	84	118	< 0.02	9.78	389
CBP-11a	78 ± 4	63	84	53	< 0.02	12.6	505
CBP-12	130 ± 10	100	90	90	< 0.02	8.24	558
CBP-13	270 ± 20	220	82	180	< 0.02	12.1	783
CBP-14	105 ± 4	80	84	67	< 0.02	18.1	2138
CBP-15e	149 ± 3	130	85	110	< 0.02	8.16	561
CBP-16b	220 ± 10	190	84	160	< 0.02	12.3	711
CBP-17	660 ± 50	560	82	460	< 0.02	18.3	1345
CBP-18	49 ± 5	43	81	35	< 0.02	7.96	630
CBP-19	27 ± 2	23	87	20	< 0.02	6.83	307
CBP-20a	250 ± 30	200	85	170	< 0.02	11.9	883
CBP-21	350 ± 20	270	85	230	< 0.02	20.7	882
CBP-22	500 ± 40	430	84	360	< 0.02	9.36	510
CBP-23	520 ± 30	450	82	370	< 0.02	10.8	812
CBP-24	140 ± 4	110	91	100	< 0.02	n.a.	n.a.
CBP-25a	74 ± 7	66	82	54	< 0.02	10.1	352
CBP-26	262 ± 9	220	82	180	< 0.02	15.0	850
CBP-27	610 ± 100	470	83	390	0.52	7.88	606
CBP-28	1300 ± 60	1070	80	860	0.4	13.9	1318
CBP-29	810 ± 30	650	82	530	< 0.02	7.86	735
CBP-30	390 ± 20	350	80	280	0.34	8.80	457
CBP-31	230 ± 4	190	79	150	< 0.02	8.94	418
CBP-32	208 ± 7	180	78	140	< 0.02	n.a.	n.a.
CBP-33	71 ± 7	63	79	50	< 0.02	19.1	1225
CBP-34	71 ± 7	59	80	47	< 0.02	6.05	434
CBP-35	51 ± 4	44	86	38	0.04	9.64	643
CBP-36	160 ± 2	140	86	120	0.09	n.a.	n.a.

Supplementary Figure F1. **(A)** Anion-exchange chromatogram of and a calibration standard containing 10 µg As L-1 of AB, DMA, MA and As (V). **(B)** Detail of the extract's anion-exchange chromatogram. **(C)** Cation-exchange chromatogram of an extract (CBP-21) a calibration standard containing 10 µg As L-1 of AB, TMAO, AC, and TETRA.



Supplementary Figure F2. Evolutionary analysis (LSU rRNA) by Maximum Likelihood method showing the phylogenetic placement of the unidentified American collection of *Cyanoboletus* (ASP-82, B-28, MF373585) among selected members of related boletoid genera.



The tree with the highest log likelihood (-4486.9848) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.2633)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 26 nucleotide sequences. There were a total of 1027 positions in the final dataset. Bootstrap support values >50 are shown along the branches.