

Supplementary Information

Selenium and organic- and inorganic-Hg in stir-fried mushrooms

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Analytical methods for the instrumental determination of Se, Hg and o-Hg

Aliquots of the freeze-dried and powdered mushroom samples (both raw and stir-fried) were analysed for Se using an inductively-coupled plasma - mass spectrometer (ICP-MS) on an ELAN DRC II instrument (PerkinElmer, SCIEX, Canada). The instrument was fitted with a Meinhard concentric nebulizer, a cyclonic spray chamber, dynamic reaction cell, Pt cones and a quadrupole mass analyzer. It was operated using the dynamic reaction cell (DRC) which consists of an enclosed quadrupole analyser which is positioned between the ion lens system and the main analyzing quadrupole. The advantage of this technology minimises most polyatomic interferences to near background levels resulting in a significant improvement in instrument detection limits. The instrument may also be operated in multi-elemental mode which provides data for a number of other elements in addition to Se. The measurements were calibrated using standard solutions ($0.1 \mu\text{g L}^{-1}$ to $100 \mu\text{g L}^{-1}$) of Se.

Hg analysis has been described in detail in an earlier publication (A, B). The Hg content was determined on a Cold Vapor Atomic Absorption Spectrometry (CV-AAS) instrument, the MA-2000 mercury analyzer (Nippon Instruments Corporation) using direct sample thermal decomposition combined with gold wool trapping, amalgamation of Hg vapor and Hg desorption. Mercury was quantitatively determined at a wavelength of 253.7 nm with instrument operation in both, high (25 to 150 ng Hg per sample) and low (3 to 20 ng Hg per sample) modes as required. Calibration curves were prepared with the measurement of standard solutions of 3, 5, 10, 15, 20, 25, 50, 100, 150 and 200 μL .

For MeHg/o-H determinations, the freeze-dried and powdered samples ($\sim 250 \text{ mg dw}$) were hydrolysed in a falcon tube, with 4 mL of 48 percent HBr, using a shaker (5 min/4.8 g). The hydrolysate was extracted with 10 mL of toluene by shaking (20 min/4.8 g) and then centrifuging (10 min/3000 g). The centrifuged supernatant was transferred quantitatively and the extraction repeated and supernatants combined. The final combination yielded 15 mL of extract. MeHg was back-extracted successively with 3 mL and 2 mL portions of 1 percent(v/v) L-Cys chloride in 12.5 percent Na_2SO_4 and 0.775 percent CH_3COONa solution by shaking for 20 min/4.8 and centrifuging for 10 min at 3000 g. The L-Cys extracts were combined in a glass measurement vial and 200 μL were thermally decomposed in the hg analyser, followed by measurement with CV-AAS over a measuring range of $0.0031\text{--}0.110 \text{ mg}\cdot\text{kg}^{-1}$. The MQL achieved by this method was $0.0031 \text{ mg}\cdot\text{kg}^{-1} \text{ dw}$.

Table S1. Species, sample size and moisture content

Species, site and sample size (number of fruiting bodies)	Total raw biomass	Control (reference) raw biomass			Raw biomass for processing	Stir fried product
	Gram (ww)	Gram (ww)	Gram (dw)	Moisture (%)	Gram (ww)	Moisture (%)
<i>Boletus bainiugan</i> (n=22)	456.3	220.5	24.0	89.7	234.0	59.7
<i>Butyriboletus regius</i> Huang Caoba, Hongta District	804.5	405.3	58.6	85.2	395.2	58.3

(n=45)						
<i>Cantharellus cibarius</i> (n=131)	787.0	412.2	59.9	83.9	371.2	54.0
<i>Laccaria vinaceoavellanea</i> (n=515)	459.4	211.5	28.7	88.4	209.1	58.8
<i>Russula virescens</i> Huoning county, Longshu village (n=33)	513.5	259.7	28.6	88.5	248.6	52.8
<i>Scleroderma yunnanense</i> (n=47)	702.2	351.1	57.0	83.7	350.3	57.8
<i>Termitomyces eurrhizus</i> Longshu village, Huaning county (n=36)	787.0	412.1	59.9	83.9	371.2	49.8
<i>Tricholoma matsutake</i> Yuxi city, Yunnan province (n=15)	633.4	314.5	32.0	89.9	315.9	53.9

Mushrooms of the following species were purchased from the central food market in the city of Yuxi (Figs S1-S6). Ready prepared selections for diners are also shown along with stir-frying process (Figs S7-S8).



Fig S1. *Boletus bainiugan* (photo by Jerzy Falandysz)



Fig S2. *Butyriboletus roseoflavus* (photo by Jerzy Falandysz)



Fig S3. *Cantharellus cibarius*, *Laccaria vinaceoavellanea* and *Russula virescens* (local market in Yuxi; photo by Jerzy Falandysz)



Fig S4. *Laccaria vinaceoavellanea* (Yunnan; photo by Jerzy Falandysz)



Fig S5. *Termitomyces eurrhizus* (Yunnan; photo by Jerzy Falandysz)



Fig S6. *Trich* (photo by Jerzy Falandysz)



Fig S7. Mushrooms for selection by diners (Yuxi, restaurant; photo by Jerzy Falandysz)



Fig S8. Wok stir-frying of mushrooms in deep oil at a street kiosk (photo by Jerzy Falandysz)
Table S2. Se, THg and o-Hg concentrations in raw and processed mushrooms: effect of processing (%), and mass fraction of o-Hg in THg (%)

Species, product and effect	Dry weight data (mg kg ⁻¹)			o-Hg in THg (%)	Wet (whole) weight data (mg kg ⁻¹)		
	Se	THg	o-Hg		Se	THg	o-Hg

<i>Boletus bainiugan</i>							
Fresh - raw	28 ± 1	4.1 ± 0.10	0.047±0.041	1.2	2.9	0.42	0.0048
Stir-fried	15 ± 1	1.0±0.1	0.005±0.001	0.5	6.0	0.40	0.0020
Effect (%)	-46	-76	-89		+110	-4.8	-58
<i>Butyriboletus roseoflavus</i>							
Fresh - raw	13 ± 0	2.8 ± 0.00	0.038±0.034	1.4	1.9	0.41	0.0056
Stir-fried	6.9 ± 0.7	1.2 ± 0.14	0.008±0.002	0.67	2.9	0.50	0.0033
Effect (%)	-47	-57	-79		+53	+22	-41
<i>Cantharellus cibarius</i>							
Fresh - raw	0.37 ± 0.00	0.068 ± 0.001	0.012±0.015	18	0.060	0.011	0.0019
Stir-fried	WD	0.034 ± 0.002	0.003±0.000	8.8	WD	0.016	0.0014
Effect (%)	WD	-50	-75		WD	+46	-26
<i>Laccaria vinaceoavellanea</i>							
Fresh - raw	0.59 ± 0.05	0.093 ± 0.009	0.014±0.010	15	0.068	0.011	0.0016
Stir-fried	0.36 ± 0.06	0.032 ± 0.002	0.007±0.004	22	0.15	0.013	0.0029
Effect (%)	-39	-66	-50		+120	+18	+81
<i>Russula virescens</i>							
Fresh - raw	0.70 ± 0.03	0.17± 0.002	0.017±0.006	10	0.081	0.020	0.0020
Stir-fried	0.36 ± 0.03	0.14± 0.01	0.015±0.001	11	0.17	0.066	0.0071
Effect (%)	-49	-18	-12		+110	+230	+250
<i>Scleroderma yunnanense</i>							
Fresh - raw	0.27 ± 0.02	0.036 ± 0.0007	0.003±0.001	8.3	0.044	0.0059	0.00049
Stir-fried	0.14 ± 0.01	0.023 ± 0.0010	0.001±0.001	4.4	0.059	0.0097	0.00042
Effect (%)	-48	-36	-67		+34	+64	-14
<i>Termitomyces eurhizus</i>							
Fresh - raw	1.5±0.0	0.34 ± 0.002	0.006±0.002	1.8	0.24	0.055	0.00097
Stir-fried	0.89±0.11	0.18 ± 0.002	0.004±0.001	2.2	0.45	0.090	0.0020
Effect (%)	-41	-47	-33		+88	+64	+106
<i>Tricholoma matsutake</i>							
Fresh - raw	1.5±0.1	0.59 ± 0.03	0.017±0.012	2.9	0.15	0.060	0.0017
Stir-fried	1.1±0.1	0.19 ± 0.01	0.010±0.007	5.8	0.50	0.088	0.0046
Effect (%)	-27	-68	-41		+230	+47	+170

Table S3. Selenium content in 100 g portions of stir-fried mushroom dishes (μg in wet product) and estimated daily and weekly intakes

Species	Se (μg in 100 g dish)	Estimated daily Se intake ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g /or/ 300 g dish ^a	Estimated weekly Se intake ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g x 7 dishes ^b	Estimated daily Se intake corrected for 25% absorption rate ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g /or/ 300 g dish ^a	Estimated weekly Se intake corrected for 25% absorption rate ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g x 7 dishes ^b
<i>Bo. bainiugan</i>	600	10//30	70	2.5//7.5	18
<i>Bt. roseoflavus</i>	290	4.8//15	34	1.2//3.8	8.5
<i>C. cibarius</i>	WD (90)	(1.5)//(4.5)	(11)	(0.38)//(1.1)	(2.8)
<i>L. vinaceoavellanea</i>	15	0.25//0.75	1.8	0.063//0.19	0.45
<i>R. virescens</i>	17	0.28//0.85	2.0	0.070//0.21	0.50
<i>S. yunnanense</i>	5.9	0.098//0.30	0.69	0.025//0.075	0.17
<i>T. eurrhizus</i>	45	0.75//2.3	5.3	0.19//0.58	1.3
<i>T. matsutake</i>	50	0.83//2.5	5.8	0.21//0.63	1.5
Mean $\pm$ SD	139 $\pm$ 209	2.3 $\pm$ 3.5//7.0 $\pm$ 10.4	16 $\pm$ 26	0.58 $\pm$ 0.87//1.8 $\pm$ 2.6	4.2 $\pm$ 6.2
Median ^c	48	0.80//2.4	6.0	0.20//0.60	1.4

Notes:

^a (for an adult weighing 60 kg; one mushroom dish of 100 g or of 300 g per day.

^b (for an adult weighing 60 kg; seven mushroom dishes - each 100 g - per week.

^c The European dietary reference value for Se considered to be an adequate intake for adults (70 kg body weight) is 70 μg per day ($1 \mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) with 85 μg daily for breastfeeding women. The tolerable upper level dietary intake is 255 μg day (including pregnant and lactating women) ^(C,D). In the USA, the recommended dietary allowance (RDA) for Se at 14+ years of age is 55 μg daily (0.7 μmol daily) with 70 μg daily for breastfeeding women. The tolerable upper level dietary intake is 400 μg daily ^(E).

Table S4. THg content in 100 g portions of stir-fried mushroom dishes (μg in wet product) and estimated daily and weekly intakes

Species	THg (μg in 100 g dish)	Estimated daily THg intake ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g /or/ 300 g dish ^a	Estimated weekly THg intake ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g x 7 dishes ^b	Estimated daily THg intake corrected for 25% absorption rate ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g /or/ 300 g x 1 dish ^a	Estimated weekly THg intake corrected for 25% absorption rate ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g x 7 dishes ^b
<i>Bo. bainiugan</i>	40	0.67//2.0	4.7	0.17//0.50	1.2
<i>Bt. roseoflavus</i>	50	0.83//2.5	5.8	0.21//0.63	1.5
<i>C. cibarius</i>	1.6	0.027//0.080	0.19	0.0067//0.020	0.047
<i>L. vinaceoavellanea</i>	1.3	0.0022//0.065	0.15	0.0054//0.016	0.038
<i>R. virescens</i>	6.6	0.11//0.33	0.77	0.028//0.083	0.19
<i>S. yunnanense</i>	0.97	0.016//0.049	0.11	0.0040//0.012	0.028
<i>T. eurrhizus</i>	9.0	0.15//0.45	1.1	0.038//0.11	0.26
<i>T. matsutake</i>	8.8	0.15//0.44	1.0	0.037//0.11	0.26
Mean $\pm$ SD	15 $\pm$ 19	0.24 $\pm$ 0.32//0.74 $\pm$ 0.96	1.7 $\pm$ 2.2	0.062 $\pm$ 0.081//0.19 $\pm$ 0.24	0.44 $\pm$ 0.58
Median	8.0	0.13//0.39	0.89	0.030//0.097	0.23

Notes:

^a (for an adult weighing 60 kg; one mushroom dish of 100 g or of 300 g per day.

^b (for an adult weighing 60 kg; seven mushroom dishes - each 100 g - per week.

Table S5. o-Hg content in 100 g portions of stir-fried mushroom dishes (μg in wet product) and estimated daily and weekly intakes

Species	o-Hg (μg in 100 g dish)	Estimated daily o-Hg intake ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g /or/ 300 g dish ^a	Estimated weekly o-Hg intake ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g x 7 dishes ^b	Estimated daily o-Hg intake corrected for 25% absorption rate ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g /or/ 300 g x 1 dish ^a	Estimated weekly o-Hg intake corrected for 25% absorption rate ($\mu\text{g} \cdot \text{kg}^{-1} \text{bw}$) 100 g x 7 dishes ^b
<i>Bo. bainiugan</i>	0.20	0.0033//0.010	0.023	0.00083//0.0025	0.0058
<i>Bt. roseoflavus</i>	0.33	0.0055//0.017	0.039	0.0014//0.0041	0.0096
<i>C. cibarius</i>	0.14	0.0023//0.0070	0.016	0.00058//0.0018	0.0041
<i>L. vinaceoavellanea</i>	0.29	0.0048//0.015	0.034	0.0012//0.0038	0.0085
<i>R. virescens</i>	0.71	0.012//0.036	0.084	0.0030//0.0090	0.021
<i>S. yunnanense</i>	0.042	0.0007//0.0021	0.0049	0.00018//0.00053	0.0012
<i>T. eurhizus</i>	0.20	0.0033//0.010	0.023	0.00083//0.0025	0.0058
<i>T. matsutake</i>	0.46	0.0077//0.023	0.054	0.0019//0.0058	0.013
Mean $\pm$ SD	0.30 $\pm$ 0.21	0.0050 $\pm$ 0.0036//0.015 $\pm$ 0.011	0.035 $\pm$ 0.025	0.0012 $\pm$ 0.0009//0.0038 $\pm$ 0.0027	0.0086 $\pm$ 0.0062
Median	0.25	0.0041//0.013	0.029	0.0010//0.0032	0.0072

Notes:

^a (for an adult weighing 60 kg; one mushroom dish of 100 g or of 300 g per day.

^b (for an adult weighing 60 kg; seven mushroom dishes - each 100 g - per week.

Table S6. Total-Hg (THg) and o-Hg contents in mushroom dishes ($\mu\text{g}\cdot\text{kg}^{-1}$ wet [whole] product) for the studied fungi and estimated oral exposure after a single ingestion of 500 g portion

Species	Content ($\mu\text{g}\cdot\text{kg}^{-1}$ ww)		Portion (size) ingested			
	i-Hg	o-Hg	500 g			
			Estimated intake ($\mu\text{g}\cdot\text{kg}^{-1}$ bw)		Estimated intake corrected for 25% absorption rate ($\mu\text{g}\cdot\text{kg}^{-1}$ bw)	
			i-Hg	o-Hg	i-Hg	o-Hg
<i>Bo. bainiugan</i>	398	2.0	3.3	0.017	0.83	0.0042
<i>Bt. roseoflavus</i>	497	3.3	4.2	0.028	1.0	0.0069
<i>C. cibarius</i>	14.6	1.4	0.24	0.012	0.030	0.0029
<i>L. vinaceoavellanea</i>	10.1	2.9	0.17	0.024	0.021	0.0060
<i>R. virescens</i>	58.9	7.1	0.98	0.060	0.12	0.015
<i>S. yunnanense</i>	9.28	0.42	0.16	0.0035	0.019	0.00088
<i>T. eurrhizus</i>	88	2.0	1.5	0.017	0.18	0.0042
<i>T. matsutake</i>	83.4	4.6	1.4	0.039	0.17	0.0096
Mean $\pm$ SD	145 $\pm$ 191	3.0 $\pm$ 2.1	2.4 $\pm$ 3.2	0.025 $\pm$ 0.018	0.30 $\pm$ 0.40	0.0063 $\pm$ 0.0044
Median	71	2.5	1.2	0.021	0.15	0.0052

Notes: Basic information in Tables S1 and S2; Body weight (60 kg); WD (without data)

Table S7. Hazard indices of iHg and o-Hg through the ingestion of studied mushroom dishes

Culinary treatment and species	Ingestion rate ($\text{g}\cdot\text{day}^{-1}$)	Concentration ($\mu\text{g}\cdot\text{g}^{-1}$ ww)		Hazard index (H)	
		i-Hg	o-Hg	i-Hg	o-Hg

<i>Bo. bainiugan</i>	16.5	0.40	0.0020	0.37	0.0055
<i>Bt. roseoflavus</i>	16.5	0.50	0.0033	0.46	0.0091
<i>C. cibarius</i>	16.5	0.016	0.0014	0.015	0.0039
<i>L. vinaceoavellanea</i>	16.5	0.013	0.0029	0.012	0.0080
<i>R. virescens</i>	16.5	0.066	0.0071	0.061	0.020
<i>S. yunnanense</i>	16.5	0.0097	0.00042	0.0089	0.0012
<i>T. eurrhizus</i>	16.5	0.090	0.0020	0.083	0.0055
<i>T. matsutake</i>	16.5	0.088	0.0046	0.081	0.013
Mean $\pm$ SD	16.5	0.15 $\pm$ 0.19	0.0030 $\pm$ 0.0021	0.14 $\pm$ 0.18	0.0082 $\pm$ 0.0058
Median	16.5	0.077	0.0025	0.0071	0.0069
<i>Bo. bainiugan</i>	33	0.40	0.0020	0.73	0.011
<i>Bt. roseoflavus</i>	33	0.50	0.0033	0.92	0.018
<i>C. cibarius</i>	33	0.016	0.0014	0.029	0.0077
<i>L. vinaceoavellanea</i>	33	0.013	0.0029	0.024	0.016
<i>R. virescens</i>	33	0.066	0.0071	0.12	0.039
<i>S. yunnanense</i>	33	0.0097	0.00042	0.018	0.0023
<i>T. eurrhizus</i>	33	0.090	0.0020	0.17	0.011
<i>T. matsutake</i>	33	0.088	0.0046	0.16	0.025
Mean $\pm$ SD	33	0.15 $\pm$ 0.19	0.0030 $\pm$ 0.0021	0.27 $\pm$ 0.35	0.017 $\pm$ 0.012
Median	33	0.077	0.0025	0.14	0.014
<i>Bo. bainiugan</i>	66	0.40	0.0020	1.5	0.022
<i>Bt. roseoflavus</i>	66	0.50	0.0033	1.8	0.012
<i>C. cibarius</i>	66	0.016	0.0014	0.059	0.036
<i>L. vinaceoavellanea</i>	66	0.013	0.0029	0.048	0.032
<i>R. virescens</i>	66	0.066	0.0071	0.24	0.078
<i>S. yunnanense</i>	66	0.0097	0.00042	0.036	0.0046
<i>T. eurrhizus</i>	66	0.090	0.0020	0.33	0.022
<i>T. matsutake</i>	66	0.088	0.0046	0.32	0.051
Mean $\pm$ SD	66	0.15 $\pm$ 0.19	0.0030 $\pm$ 0.0021	0.54 $\pm$ 0.70	0.033 $\pm$ 0.023
Median	66	0.077	0.0025	0.28	0.028

References

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