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Cadmium and Mercury in Macrofungi — Mechanisms of Transport and Accumulation

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Summary

The accumulation of metals in fruiting bodies was studied from samples collected from different environments. A broad variation was found within the cadmium and mercury contents of *Agaricus* fungi. This variation was considerable both between and within species and even between fruiting bodies of the same individual. A positive correlation between the catalase activity and mercury contents was found.

The contents of total (TSH) and protein bound (PSH) sulfhydryl groups were also studied. In species growing on lawns the TSH content was higher (mean 12 μmol/g) than in forest (mycorrhizal) species (mean 3.9 μmol/g). In mean 83 % of the sulfhydryl groups were protein bound. Free sulfhydryl groups (NPSH) were found from 43 % of the samples. There was a strong correlation between the sulfhydryl and mercury contents in mycorrhizal fungi but only a week correlation between cadmium and SH contents and almost no correlations for other metals (Al, Cu, Mn, Fe, Zn).

1. Introduction

Many investigations have dealt with the metal contents of fungi, especially edible ones. The strong accumulation of cadmium and mercury in some edible mushrooms is of special interest when considering human health. The accumulation of these metals is extraordinarily strong in mushrooms belonging to the genus *Agaricus* (Psalliota).

The factors governing the accumulation of metals in fungi are poorly known. It has been assumed (Byrne et al., 1976; Stijve and Besson, 1976) that the accumulation of mercury could depend on the sulfhydryl, disulfide and methionine groups of the proteins. The contents of proteins and amino acids have been investigated primarily for evaluating the nutritional value of macrofungi. Cadmium has been found to be bound to low-molecular weight proteins in fungi. However, no sulfhydryl groups have been found from proteins rich in cadmium (Esser and Brunnert, 1986; Schmitt and Meisch, 1985).

The purpose of the present study was to study the occurence of seven metals of some common macrofungi in relation to some physiological parameters. We were especially interested in the interaction of the metal contents and the sulfhydryl groups and the transport and accumulation mechanisms of cadmium and mercury.

2. Material and methods

2.1 Variation of Hg and Cd in *Agaricus*

The samples were collected from the Helsinki area in 1979—1984. The determination of species belonging to the genus *Agaricus* were performed by W. *Jakowlev* (Tampere, Finland) on the basis of spore characteristics and colour (Schaeffer) reaction.

For the determination of intraindividual variation in fruiting bodies of *Agaricus* arvensis forty nine samples were collected during three years (1980—1982) from a suburb of Helsinki. As the fruiting bodies were growing on a very limited site they were considered belonging to the same individual. The size (dry weight) and developmental stage (young/fully developed) of the fruiting bodies were recorded. The distribution of Cd and Hg within the cap was studied from three specimens of *Agaricus.*

2.2 Chemical form of mercury

The methyl mercury content was determined by B. Ohlin (National Food Administration, Uppsala, Sweden) by gas chromatography from four dried samples of *Agaricus* after dilution in HCl, extraction in toluene, binding to cysteine and a new toluene extraction.

The binding of mercury to *Lagermannia gigantea* was studied by gel filtration. One fruiting body was homogenized with an Ultra Turrax homogenizer. 1 μg of Hg (as $HgCl_2$) was added to 20 g of homogenated sample, which was filtrated through a 60 cm long Sephadex G-100 column (ø = 25 mm, flow rate approximately 1 ml/min).

2.3 Catalase activity

The catalase activity was analysed from 21 separately collected samples. For the determination 3 % H_2O_2 was added to 5 ml of fresh, homogenized samples (Ultra-Turrax) in test tubes. The flotation time for small pieces (ø = 6 mm) of paper (Whatman 1), which is a measure of the enzyme activity, was measured (Gagnon et al., 1959). The catalase content was estimated by measuring catalase dilutions with the same method.

2.4 Sulfhydryl content

Sixty five samples of 36 species were collected in the autumns of 1985 and 1986 from the Helsinki area. Eighteen of the fungi were collected from lawns (mainly lawn decomposer species), 43 from forest areas (mainly mycorrhizal species) and four were growing on trees (wood decomposing species). Before analysis the fruiting bodies were cleaned and rinsed with distilled water. If the species had a separate cap only the cap was used. The fruiting bodies were cut using a polyethylene knife. The analyses were usually performed from one specimen.

The content of sulfhydryl groups was determined by using a modification of the method of Sedlak and Lindsay (1968). By using this method it was possible to determine both the total amount of sulfhydryl groups (TSH) and the amount of free (non-protein bound) groups (NPSH). The amount of protein bound SH-groups (PSH) was calculated from the above mentioned values.

Approximately 3 g of a fresh sample was homogenized (Ultra-Turrax) in 8 ml of 0.02 M EDTA. For the determination of TSH a complex forming agent 5,5-ditiobisnitrobenzoic acid (DNTB) was added to 0.5 ml of homogenized sample. Tris-buffer (1,5 ml, 0.2 M, pH 8.2) and methanol were added to 10 ml. The colour was allowed to form in 15 minutes while shaking. The absorbances at 412 nm (slit 2 nm) were measured after centrifugation (15 min, 3500 rpm, ∅ 15 cm) by a double beam spectrophotometer (Shimadzu UV-260). The NPSH content was determined by adding 1 ml of 50 % CCl_3COOH and 4 ml distilled water to 5 ml of homogenated sample. After centrifugation 4 ml of 0.4 M Tris-buffer and 0.1 ml of DTNB were added to 2 ml of the supernatant liquid. The colour was measured within 5 min. The absorbance was analysed as described earlier.

Sedlak and Lindsay (1968) stated that no changes in SH-groups should occur after deep-freezing for one night. However, we found a slight decrease in the contents of sulfhydryl groups after deep-freezing. Consequently all SH-determinations in the present study have been performed on fruiting bodies collected the same day. It was not possible to keep the samples on ice during the homogenization but before and after this step they were stored cold (+ 4 °C). Before and during the centrifugation the samples were kept at room temperature. The absorbance caused by other components was eliminated by subtracting the absorbance value of samples without addition of DNTB.

2.5 Metal analyses

Before analysis of the metal contents the samples were dried at + 40 °C. The mercury contents were measured from a HNO_3 — H_2SO_4 digest by cold vapour atomic absorption (Perkin-Elmer/Coleman MAS-50). The other metals were analysed after dry ashing and digestion in HCl by flame atomic absorption spectrometry (Perkin Elmer 360). NaCl was added before analysing Al.

Results

3.1 Variation of Cd and Hg contents in *Agaricus* fungi.

There was a very broad variation within the contents of cadmium and mercury in the genus *Agaricus* (Table 1). The mean mercury content in *Agaricus edulis* was 3.5 mg/kg and that of *A. comtulus* 95 mg/kg. The mean cadmium content in *A. arvensis* and *A.*

Table 1: Variation in Hg and Cd contents of fruiting bodies of *Agaricus* species.

	Hg				Cd			
	N	mean	SD	range	N	mean	SD	range
A. arvensis	126	6.5	8.1	0.69—50	106	30	27	1.0—110
A. lutosus	1	0.51			1	1.7		
A. silvicola	7	16	12	2.4—40	7	42	41	1.7—110
A. comtulus	3	95	15	96—110	3	4.9	1.9	2.8—6.4
A. augustus	1	30						
A. nivescens	2	17	11	9.0—25	2	4.6	0.92	3.9—5.2
A. campestris	11	7.9	6.0	1.7—26	9	30	32	1.7—97
A. edulis	11	3.5			1	4.2		

Table 2: Variation in mercury and cadmium content in fruiting bodies of different size (dry weight) and developmental stage of one individual of *Agaricus arvensis.*

	n	mean	SD	range
Hg: all specimens	49	1.8	1.4	0.69—9.8
Cd: all specimens	49	44	17	9.6—100
Hg: dry weight 0—2 g	10	1.7	0.65	0.8—3.1
2—4 g	3	1.4	0.38	1.0—1.7
4—8 g	4	1.5	0.28	1.2—1.8
Cd: dry weight 0—2 g	9	56	24	27—100
2—4 g	3	32	5.6	26—37
4—8 g	4	49	9.5	39—62
Hg: young specimens	12	1.7	0.63	0.8—3.1
fully developed	3	1.6	0.32	1.2—1.8
Cd: young specimens	11	52	24	26—100
fully developed	3	48	15	33—62

Table 3: Distribution of mercury and cadmium (mg/kg dw) within the fruiting bodies. The fruiting bodies of *A. campestris* were collected from the same site at same time.

		Hg	Cd
A. campestris			
fully developed	stalk	7.0 (100 %)	32 (100 %)
	cap*	8.4 (120 %)	97 (303 %)
	lamellae	13 (186 %)	43 (134 %)
A. campestris			
young	stalk	2.9 (100 %)	26 (100 %)
	cap*	3.2 (110 %)	49 (188 %)
	lamellae	4.7 (162 %)	23 (88 %)
A. arvensis			
fully developed	stalk	0.56 (100 %)	12 (100 %)
	ring	3.3 (589 %)	
	cap*	0.94 (168 %)	33 (275 %)
	gleba	1.1 (196 %)	54 (450 %)
	lamellae	1.7 (304 %)	73 (608 %)

* except lamellae

campestris was 30 mg/kg while it was essentially lower in the small-sized species (*A. lutosus, A. comtulus, A. nivescens;* mean 4.3 mg/kg).

The variations in mercury and cadmium contents of one individual of *Agaricus arvensis* may be considerable (Table 2). There were significant differences for both cadmi-

um and mercury and no correlation between the metal contents and the size (dry weight) of the fruiting body could be detected.

Both cadmium and mercury are quite unevenly distributed within the fruiting bodies (Table 3). In *A. campestris* the lamellae contain significantly more mercury than the rest of the cap and the stalk in both the young and the fully developed specimen. The highest concentration was found in the ring of *A. arvensis*. The distribution of cadmium was more irregular but also here the lowest values were found from the stalk.

3.2 Chemical form of mercury in the fruiting bodies of *Agaricus*

Only small contents of methyl mercury (max. 0.42 mg/kg = 5 % of Hg-tot) could be detected from the four ***Agaricus*** samples analysed (Table 4). The binding of mercury in ***Lagermannia gigantea*** seems to concentrate to big molecular compounds without any peak for low-molecular weight proteins (Fig. 1).

Table 4: Total Hg, methyl Hg, and Cd content of four *Agaricus* fruiting bodies (mg/kg dw).

	Hg-tot	CH_3-Hg	CH_3Hg %	Cd
A. campestris	4.7	⩽0.3	⩽6 %	20
A. arvensis	6.0	⩽0.3	⩽5 %	110
A. arvensis	2.3	⩽0.3	⩽13 %	44
A. nivescens	9.0	0.42	5 %	5.2

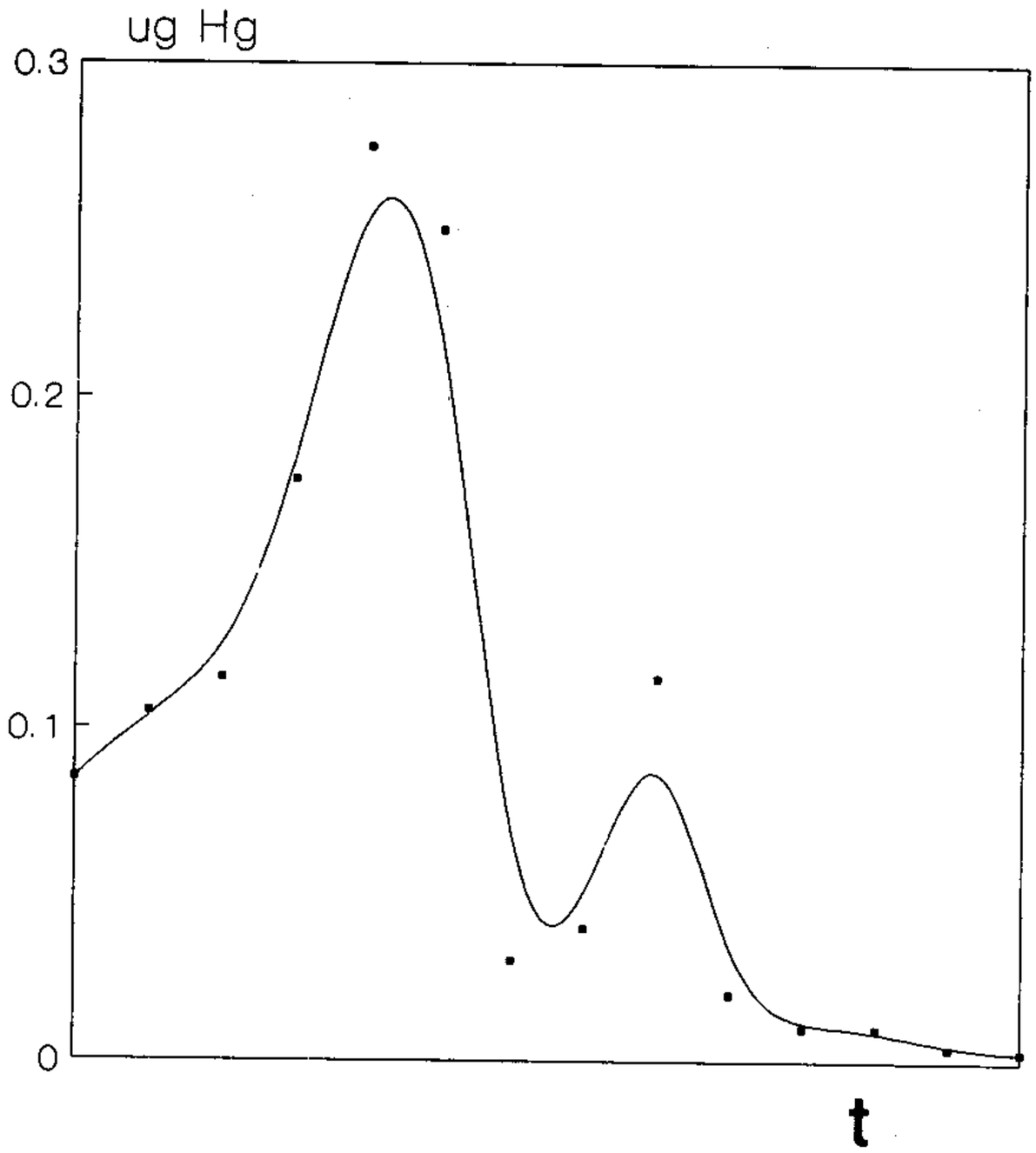

Fig. 1: Distribution of mercury in fractions of *Lagermannia gigantea* after gel filtration (molecule size decreases with increasing time).

3.3 Catalase activity and Hg

There were significant differences in the catalase content (measured as enzyme activity) of the different species studied (Table 5). There was a positive correlation ($r = 0.44$, $p < 0.05$) between the catalase content and the mercury content of fungi. The fruiting bodies growing on lawns contained significantly more catalase than those growing in forests (means 4900 and 180 mg/kg respectively). The fruiting bodies growing in forests (mainly mycorrhizal fungi) have with few exceptions low catalase activity and low mercury content. The correlation between catalase and mercury contents was higher ($r = 0.58$; $p < 0.05$) in this group than in the whole material.

Table 5: Catalase content (estimated on the basis of its activity) and mercury content of fungi.

	catalase mg/kg	Hg mg/kg
Fruiting bodies growing on lawns (N = 5):		
mean ± SD	4900 ± 4400	2.5±0.76
range	300 – 10 000	1.9—3.7
Agaricus sp.	9300	2.7
Agaricus sp.	10000	1.8
Agaricus sp.	2800	1.9
Agaricus sp.	2100	2.5
Coprinus comatus	300	3.7
Fruiting bodies growing in forests (N = 15):		
mean + SD	180±170	0.58±0.86
range	< 5 – 440	0.02–3.5
Catharellus tubaeformis	100	0.27
Cantharellula umbonata	240	1.0
Cantharellula umbonata	< 5	1.0
Hygrophorus hypothejus	430	0.30
Hygrophorus hypothejus	550	3.5
Hygrophoropsis aurantiaca	440	0.53
Lactarius rufus	140	0.10
Lactarius rufus	93	0.23
Lactarius sp.	99	0.35
Albatrellus ovinus	68	0.22
Russula aeruginea	220	0.02
Russula vinosa	32	0.15
Leccinum versipelle	53	0.17
Suillus luteus	20	0.40
Amanita muscaria	280	0.50
Fruiting bodies growing on trees (N = 1):		
Climecoidon septentrionalis	68	0.17

Table 6: Sulfhydryl contents of fungi (mmol/kg dry wt).

	N		TSH	NPSH	NPSH %
Fruiting bodies growing on lawns:					
	18	mean	12	2.4	16
		range	0—51	0—9.3	0—72
Agaricus sp.	10	mean	13	1.3	5
		range	0—51	0—5.1	0—19
Coprinus comatus	2	range	11—15	1.8—2.1	14—16
C. atramentarius	1		14	5.5	39
Lyophyllum connatum	1		8.9	5.7	64
Marasmius oreades	1		13	9.3	72
Calvatia excipuliformis	2	range	5.3—8.4	0	0
Bovista nigrescens	1		13	4.8	37
Fruiting bodies growing in forests:					
	43	mean	3.9	1.5	19
		range	0—26	0—10	0—128
Paxillus involutus	1		0	0	0
Cortinarius sp.	1		0	0	0
Dermocybe cinnamomeolutea	1		9.2	0.55	6.0
Clitocybe sp.	2	range	0—0.92	0	0
Lepista nebularis	1		1.8	0	0
Cantharellula umbonata	1		0	0	0
Tricholoma album	2	range	4.1—4.6	1.5—1.7	33—41
Lactarius sp.[1]	8	mean	1.1	0	0
		range	0.69—1.6	0	0
Russula sp.[2]	8	mean	1.6	0.35	13
		range	0—3.3	0—1.8	0—64
Amanita muscaria	3	mean	6.6	3.6	55
		range	5.9—7.5	3.4—3.7	45—63
Chroogomphus rutilus	2	range	0—2.4	0—0.69	0—29
Hygrophorus pustulatus	1		8.1	3.8	47
H. hypothejus	1		6.4	2.9	45
Cantharellus cibarius	2	range	0.88—2.2	0	0
C. tubaeformis	1		1.4	0	0
Leccinum scabrum	2	range	4.9—5.7	0	0
L. versipelle	1		0.95	0	0
Suillus granulatus	2	range	6.8—11	8.7—10	91—128
Boletus edulis	3	mean	19	7.4	39
		range	13—26	5.0—10	38—42

Table 6 continued

	N		TSH	NPSH	NPSH %
Fruiting bodies growing on trees:					
	4	mean	3.6	0.6	8.5
		range	0—9.8	0—2.1	0—21
Pholiota squarrosa	1		0	0	0
Mycena sp.	1		9.8	2.1	21
M. galericulata	1		2.1	0	0
Hohenbuehelia serotina	1		2.4	0.30	13
Whole material:	65	mean	6.2	1.7	17
		range	0—51	0—10	0—128

[1] *L. necator* (5), *L. torminosus* (2) and *L. rufus* (1)
[2] *R. aeruginea* (3), *R. paludosa* (1), *R. emetica* (1) and *Russula* sp. (3).

3.4 Sulfhydryl groups

The mean difference between parallel total sulfhydryl determinations was 23 % within the genus *Agaricus* and 4 % for the other species. The latter value indicates a good analytical accuracy. The bigger variation between the parallel determinations in *Agaricus* samples was possibly caused by an uneven distribution within the cap.

The mean TSH-value of all samples was 6.2 μmol/g. In mean 83 % of the sulfhydryl groups were protein bound. The intraspecific differences were small while there were considerable differences between different species and groups of species (Table 6). The amount of TSH was highest in the lawn decomposing species (mean 12 μmol/g). In the mycorrhizal fungi the TSH-value was considerably lower (mean 3.9 μmol/g) and the proportion of non-protein bound SH-groups was higher than in the lawn decomposers. Free SH-groups (NPSH) were found from 43 % of the samples.

The highest SH-concentrations were found in specimens of *Boletus edulis* and *Agaricus sp.* In the group of mycorrhizal fungi higher SH-values were recorded mainly within *Boletaceae.* In two samples of *Suillus granulatus* almost all of the SH-groups was non-protein bound. In the few samples of wood decomposers the sulfhydryl contents were usually low.

Table 7: Contents of sulfhydryl groups in the cap of three *Agaricus*-samples (A—C).

	Centre		Brim	
	TSH μmol/g	NPSH %	TSH μmol/g	NPSH %
A	34	18	64	9
B	7.7	21	10	6
C	9.6	0	4.1	0

The distribution of SH-groups within the cap was analysed from three *Agaricus*-samples. There seem to be some differences between the SH-contents in the centre and in the brim of the cap (Table 7). This would, however, require a more detailed investigation.

3.5 Metal contents of fungi

There were significant differences between the contents of heavy metals in the different ecological groups of fungi (Table 8). For the most metals the mean content was highest in fungi growing on lawns and lower in species collected from forests (mycorrhizal species) and fungi growing on wood. Within genus **Agaricus** the highest contents of cadmium and mercury were found in specimens with very low contents of sulfhydryl groups.

Low contents of cadmium were found from most of the samples: 71 % contained less than 5 mg/kg. The mean content of lawn decomposers (11 mg/kg) was higher than that of mycorrhizal fungi (4.9 mg/kg) or wood decomposer fungi (2.8 mg/kg). High concentrations were recorded in *Agaricus* (max 84 mg/kg), in *Amanita muscaria* (max 25 mg/kg) and in *Boletaceae* (max 15 mg/kg).

In 88 % of the samples the mercury content was less than 5 mg/kg (Table 8). The mean content in lawn decomposers (4.5 mg/kg) was significantly higher than in mycorrhizal fungi (0.90 mg/kg) or wood decomposing fungi (0.44 mg/kg). The highest concentrations (max 14 mg/kg) were found from *Agaricus* fungi.

The manganese and aluminium concentrations (means 12—15 and 43—65 mg/kg respectively) were quite evenly distributed between different ecological groups. The highest Al concentrations were found in *Russula paludosa* and *Amanita muscaria*. The distribution of copper contents resembled that of cadmium and mercury with significantly higher values in lawn decomposer fungi (200 mg/kg) than in mycorrhizal (35 mg/kg) or wood decomposing (34 mg/kg) species. The maximum concentration (860 mg/kg) was found in a sample of *Agaricus*.

The iron concentrations were slightly higher in lawn and wood decomposers (100 and 97 mg/kg respectively) than in mycorrhizal species (56 mg/kg). High values were found from *Tricholoma album* and *Agaricus sp.* The mean zinc content was almost the same for lawn decomposers (150 mg/kg) and mycorrhizal fungi (140 mg/kg) while the wood decomposers contained less of this metal (58 mg/kg).

Table 8: Metal contents of fungi μg/g dw) collected for SH-analyses.

	N		Hg	Cd	Al	Cu	Mn	Fe	Zn
Fruiting bodies growing on lawns:									
	21	mean	4.5	11	45	200	15	100	150
		range	0.54—14	1.0—84	0—100	52—860	7.5—36	56—220	62—280
Agaricus sp.	13	mean	4.4	19	45	270	14	110	160
		range	1.6—14	1.0—84	0—100	52—860	9.8—18	56—220	100—260
Coprinus comatus	1		6.7	2.2	55	78	10	110	91
C. atramentarius	1		0.54	1.3	39	68	7.5	92	130
Lyophyllum connatum	1		7.7	5.5	61	140	26	110	88
Marasmius oreades	1		6.4	1.3	58	120	19	69	99
Calvatia excipuliformis	2	range	1.4—3.6	2.3—2.6	20—31	67—80	23—36	73—100	210—280
Bovista nigrescens	1		6.8	2.8	39	100	11	67	110
Lycoperdon pyriforme	1		0.91	3.2	68	73	14	120	62

Table 8 continued

	N		Hg	Cd	Al	Cu	Mn	Fe	Zn
Fruiting bodies growing in forests:									
	39	mean	0.90	4.9	43	35	12	56	140
		range	0.03—5.3	0.09—25	0—150	0—100	5.2—34	9.6—250	23—280
Paxillus involutus	1		0.03	0.89	61	60	8.5	41	170
Cortinarius sp.	2	range	0.26—0.66	—	—	—	—	—	—
Tricholoma album	1		0.41	1.3	48	5.8	20	250	89
Lactarius necator	7	mean	0.56	1.1	45	44	9.7	55	170
		range	0.19—1.4	0.4—2.3	34—72	24—88	6.8—16	27—78	97—280
L. rufus	1		0.08	0.61	20	25	17	30	75
L. torminosus	1		0.98	0.51	30	26	11	42	160
Russula sp.	6	mean	0.23	1.2	54	24	16	39	150
		range	0.07—0.81	0.09—2.5	17—150	7.2—45	12—19	15—100	72—230
Amanita muscaria	4	mean	2.6	22	65	26	7.4	71	170
		range	0.19—5.3	17—25	32—140	13—38	6.2—9.8	28—170	150—200
Chroogomphus rutilus	2	range	0.04—0.15	0.35—0.65	21—46	0	5.2—6.8	9.6—14	23
Clitocybe sp.	1		1.9	1.0	27	93	28	52	66
Lepista nebularis	1		1.1	1.3	12	90	13	77	86
Cantharellula umbonata	1		1.1	3.4	100	34	14	130	76
Hygrophorus pustulatus	1		0.31	2.9	24	38	16	28	95
H. hypothejus	1		2.5	—	—	—	—	—	—
Cantharellus cibarius	1		0.11	0.45	34	13	20	32	59
C. tubaeformis	1		0.39	0.70	66	8.6	34	91	40
Leccinum scabrum	2	range	0.31—0.86	0.94—3.5	31—33	9.4—32	6.6—12	23—52	94—260
L. versipelle	1		1.2	13	8.7	100	9.8	21	280
L. vulpinum	1		0.14	7.5	64	67	7.2	75	140
Suillus granulatus	1		0.82	1.3	30	20	5.4	36	—
Boletus edulis	2	range	2.5—3.2	9.8—15	0—17	33—36	11—13	33—34	180—200
Fruiting bodies growing on trees:									
	3	mean	0.44	2.8	65	34	12	97	58
		range	0.03—0.63	1.1—4.4	40—89	23—45	9.8—14	64—130	46—70
Pholiota squarrosa	1		0.62	4.4	89	45	14	130	46
Mycena galericulata	1		0.47	1.1	40	23	9.8	64	70
Hohenbuehelia serotina	1		0.03	—	—	—	—	—	—
Whole material	63	mean	2.1	7.5	45	96	13	74	140
		range	0.03—14	0.09—84	0—150	0—860	5.2—36	9.6—250	23—280

3.6 Correlations

The correlations between the parameters are calculated as if the material were normally distributed. Even if the distribution was not fully normal the correlation matrix (Table 9) gives some information about the relationships between the sulfhydryl groups and the metal contents and between the different metals.

There was only a week correlation between the cadmium content and the SH-content of mycorrhizal fungi. In decomposer fungi there was no correlation. For mercury there was a strong correlation between the metal content and the SH-content in mycorrhizal species. This correlation was slightly stronger for free SH-groups than for the protein-

Table 9: Risk levels of correlations between SH- and metal contents of fungi. A = whole material, L = fruiting bodies growing on lawns, F = fruiting bodies growing in forests. 0: $p < 0.1$, 1: $p < 0.05$, 2: $p < 0.01$, $p < 0.001$ (calculated as if the values were normally distributed).

	Hg	Cd	Al	Cu	Mn	Fe	Zn
TSH	AF 13	F 2	F −0				
NPSH	AF 13	F 1					L −2
PSH	AF 12	F 1	F −1	A 0			
Hg	—	ALF 323	L 2	A 3	A 0	A 1	
Cd		—	AL 01	AL 31	F −0	A 1	AF 11
Al			—			ALF 333	
Cu				—		AL 31	ALF 111
Mn					—	A 1	F −1
Fe						—	
Zn							—

bound groups. There were no significant correlations between the SH-content and any other metal.

The correlation between cadmium and mercury was strong in all groups. However, if the *Agaricus* samples are omitted, no significant correlation can be found between these two metals. The copper content correlated with Hg, Cd, Fe, and Zn. There was also a strong correlation between the contents of iron and aluminium in all groups of fungi.

Within different groups of fungi there was often a correlation between mercury and manganese: *Lactarius necator* ($r = 0.911$, $n = 7$), *Agaricus sp.* ($r = 505$, $n = 13$), *Boletaceae* ($r = 0.948$, $n = 5$). In *Amanita muscaria* there was negative correlation between mercury and copper ($r = 0.992$, $n = 4$).

4. Discussion

The uptake of metals in fungi is in many respects different from that of plants. Most macrofungi contain significantly more zinc and copper than green plants and the strong accumulation of mercury and cadmium in certain species are examples of these differ-

ences. There are several investigations concerning the contents of amino acids in macrofungi. Among the sulfur-containing amino acids methionine and cystine have usually been analysed (eg. McKellat and Kohrman, 1975; Hayes and Haddad, 1976; Jandaik and Kapoor, 1976). Unfortunately the words "cysteine", "cystine" and "1/2-cystine" are often used without an exact definition, which fact may cause some confusions. In macrofungi the total content of sulfur is 0.5—3.9 mg/g of dry weight (Sihvonen, 1985) and 19—33 mg/g of the protein content (Rautavaara, 1947).

Hattula (1968, 1969) found very low contents of methionine while the amounts of cystine were significantly higher: 7—16 mg/g of dry, ashfree fungus. Most cystine was found from *Agaricus arvensis* and least from *Cantharellus cibarius*. On the contrary Samajpati (1978) detected more methionine (5—13 mg/g) than cystine (1—6.2 mg/g) in the mycelial protein of several macrofungi. Aalto and Kreula (1972) found relatively low contents of methionine and cysteine in *Boletus edulis, Lactarius trivalis*, and *L. torminosus*.

Chang and Chan (1973) observed a change in the protein composition of *Volvariella volvacea* during the growth. The highest protein content was found in young, developing fruiting bodies, which indicated a rapid physiological-biochemical differentiation in this stage of development. The cap of this species contained much more proteins with a much more complicated electrophoretical spectrum in the cap than in the stalk or sheath. Krupa and Bränström (1974) found also a great variation of the composition of free and protein-bound amino acids during the growth of *Boletus variegatus*.

The decomposing of hydrogen peroxide by catalase is obviously related to the decomposing capacity of fungi. In higher plants often more than 95 % of the SH-groups are in glutathione (GSH; Grill et al., 1979). The GSH-cysteine plays an essential role in the oxidation-reduction processes of plants. Considerable catalase activity values have been earlier found in lawn decomposing species (Lamaison et al., 1975). Hence the positive correlation found here between the mercury content and the catalase activity is not surprising. Ogata et al. (1981) found also a positive correlation between the uptake of mercury from air and the catalase activity.

The metal concentrations obtained in this study correspond to values reported earlier for this area (Laaksovirta and Lodenius, 1979; Kuusi et al., 1981). The mean concentrations of all metals were higher in lawn decomposer species than in mycorrhizal species as found in earlier investigations. For aluminium, manganese and zinc these differences were very small. The few samples of wood decomposers contained more aluminium than the lawn decomposers and as much iron but less of other metals.

The correlation between cadmium and mercury was obviously to great extent due to the strong accumulation of these metals in *Agaricus* species. High cadmium concentrations were found in fruiting bodies of *Agaricus* but not in other species growing on lawns, which is in agreement with earlier findings (Kuusi et al., 1981). The correlation between heavy metals and sulfhydryl groups was most evident in the mycorrhizal fungi.

Even as the accumulation of both cadmium and mercury in fungi seems to be related to the physiological and enzymatic activity, there seems to be no competition between the uptake of these two metals. The distribution of sulfhydryl groups between ecological groups resembles much more that of mercury than that of cadmium and it seems obvious that these two metals are accumulated in fungi by different physiological mechanisms. Brunnert and Zadrazil (1985) found that zinc competed with cadmium but not with

mercury in the uptake of metals into the fruiting bodies of *Agrocybe aegerita*. They also assumed that fruiting bodies exert a strict control on the uptake of mercury.

Brunnert and Zadrazil (1981) found that both cadmium and mercury were accumulated more effectively to fungi at low concentrations of these metals in the substrate. This would indicate an uptake and transport of these metals together with a carrier – possibly the same as for essential metals. The correlations between metals in different species indicate a possible uptake of mercury with copper and/or manganese and an uptake of cadmium with zinc and/or iron. Within the cap the metals might be bound and the carrier be liberated for new transport. Cadmium might be bound to mycophosphatine (cf. Schmitt and Meisch, 1985) and mercury to SH-groups. Thus the sulfhydryl groups might be involved both in transport and storage processes.

If the metal binding groups were metallothioneine like compounds three SH-groups would be required for binding of one metal ion (Cherian and Goyer, 1978). Thus 0.21 μmol/g of SH-groups would be needed to bind the maximum content of mercury found in this material (14 μg/g or 0.07 μmol/g). The binding of the maximum cadmium content (84 μg/g or 0.75 μmol/g) would require 2.3 μmol/g of sulfhydryl groups. Even if the SH-content of fungi usually seems to be enough for binding considerable amounts of heavy metals, it could not be responsible for all bindings of heavy metals. In some cases the sulfhydryl content might be an limiting factor.

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Zusammenfassung

Cadmium und Quecksilber in Pilzen — Transport- und Anreicherungsmechanismen

Die Anreicherung von Metallen in Pilzen aus verschiedenen Umgebungen (Wiesen, Wälder, Bäume) wurde untersucht. Die Variation der Cadmium- und Quecksilbergehalte in *Agaricus*-Pilzen war erheblich sowohl zwischen und innerhalb verschiedener Arten als auch im Fruchtkörper eines einzigen Individuums. Es wurde eine positive Korrelation zwischen der Aktivität der Katalase und dem Quecksilbergehalt gefunden.

Die Gehalte von gesamt- (TSH) und in Proteinen gebundenen (PSH) Sulphhydryl-Gruppen wurden auch untersucht. In Arten, die auf Wiesen wachsen (humuszersetzende Arten), waren die TSH-Gehalte höher (Mittelwerte 12 μmol/g) als in Arten, die in Wäldern wachsen (mycorrhizatragende Arten; Mittelwerte 3.9 μmol/g). Die in Proteinen gebundenen Sulphhydryl-Gruppen betrugen 70 % von TSH. Freie Sulphhydryl-Gruppen kamen in 43 % der Pilze vor. Die Korrelation zwischen Sulphhydryl-Gruppen und Quecksilber war stark in mycorrhizatragenden Arten aber nur schwach mit Cadmium. Keine Korrelationen wurden zwischen Sulphhydryl-Gruppen und anderen Metallen gefunden.

References

Aalto, A. and Kreula, M., 1972: Eräiden metsäsienten aminohappo-ja rasvahappokoostumuksesta. Karjantuote 55, 264—265.

Brunnert, H. and Zadrazil, F., 1981: Translocation of cadmium and mercury into the fruiting bodies of *Agrocybe aegerita* in a model system using agar platelets as substrate. Eur. J. Appl. Microbiol. Biotechnol. 12, 179—182.

Brunnert, H. and Zadrazil, F., 1985: The influence of zinc on the translocation of cadmium and mercury in the fungus *Agrocybe aegerita* (a model system). Angew. Bot. 59, 469—477.

Byrne, A. R., Ravnik, V., and Kosta, L., 1976: Trace element concentrations in higher fungi. Sci. Total Environ. 6, 65—78.

Chang, S. T. and Chan, K. Y., 1973: Quantitative and qualitative changes in proteins during morphogenesis of the basidiodcarp of *Volvariella volvacea*. Mycol. 65, 355—364.

Cherian, M. G. and Goyer, R. A., 1978: Metallothioneins and their role in the metabolism and toxicity of metals. Life Sci. 23, 1—10.

Esser, J. and Brunnert, H., 1986: Isolation and partial purification of cadmium-binding components from fruiting bodies of *Agaricus bisporus*. Environ. Pollut. 41, 263—275.

Gagnon, M., Hunting, W. M., and Esselen, W. B., 1959: New method for catalase determination. Anal. Chem. 31, 144—146.

Grill, D., Esterbauer, H., and Klösch, U., 1979: Effect of sulphur dioxide on glutathione in leaves of plexing peptides of higher plants. Science 230, 674—676.

Hattula, M. L., 1968: Liuosviljelymenetelmällä tuotetun sienimyseelin aminohappokoostumus. Unpubl., Univ. Helsinki, Dept. Microbiol. 1—111.

Hattula, M. L., 1969: Sienet valkuaisaineiden lähteenä. Kotitalous 33, 16—19.

Hayes, W. A. and Haddad, N., 1976: The food value of the cultivated mushroom and its importance in the mushroom industry. Mushroom J. 40, 104—110.

Jandaik, D. L. and Kapoor, J. N., 1976: Amino acid composition of mushroom *Pleurotus sajor-caju* (Fr.) Sin. ger. Mushroom J. 41, 154—156.

Krupa, S. and Bränström, G., 1974: Studies on the nitrogen metabolism in ectomycorrhizae II. Free and bound amino acids in the mycorrhizal fungus *Boletus variegatus*, in the root system of *Pinus sylvestris* and during their association. Physiol. Plant. 31, 279—283.

Kuusi, T., Laaksovirta, K., Liukkonen-Lilja, H., Lodenius, M., and Piepponen, S., 1981: Lead, cadmium, and mercury contents of fungi in the Helsinki area and in unpolluted control areas. Z. Lebensm. Unters. Forsch. 173, 261—267.

Laaksovirta, K. and Lodenius, M., 1979: Mercury content of fungi in Helsinki. Ann. Bot. Fenn. 16, 208—212.

Lamaison, J. L., Pourrat, A., and Pourrat, H., 1975: Activité catalasique des macromycetes. Obtention d'une catalase purifiée. Ann. Pharm. Fr. 33, 441—446.

McKellar, R. L. and Kohrman, R. E., 1975: Amino acid composition of the morel mushroom. J. Agric. Food Chem. 23, 464—467.

Ogata, M., Kenmotsu, K., Hirota, N., and Naito, M., 1981: Relationship between uptake of mercury vapor by mushrooms and its catalase activity. Bull. Environ. Contam. Toxicol. 27, 816—820.

Rautavaara, T., 1947: Suomen sienisato. W. Söderström, Porvoo.

Samajpati, N., 1978: Nutritive value of some Indian edible mushrooms. Mushroom Sci. 10, 695—703.

Schmitt, J. A. and Meisch, H. U., 1985: Cadmium in mushrooms — distribution, growth effects, and binding. Trace Elements Med. 2, 163—166.

Sedlak, J. and Lindsay, R. H., 1968: Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192—205.

Seeger, R., Nutzel, R., and Dill, U., 1978: Cadmium in Pilzen. Z. Lebensm. Unters. Forsch. 166, 23—34.

Sihvonen, M. L., 1985: Mineral element composition of two species of Finnish mushrooms. In: T. D. Lekkas (ed.), Heavy metals in the environment, Vol. 1, 512—514. Int. Conf. Athens, Sept. 1985.

Stijve, T. and Besson, R., 1976: Mercury, cadmium, lead, and selenium content of mushroom species belonging to the genus *Agaricus*. Chemosphere 2, 151—158.

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