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The uptake of cadmium and zinc by mycelia and their accumulation in mycelia and fruiting bodies of edible mushrooms

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Abstract Cd and Zn concentrations were determined by optical emission spectroscopy in various parts of the fruiting body and the mycelium of two wild mushrooms, *Agaricus macrosporus* and *Agaricus silvicola*, and in cultivated *Stropharia rugosoannulata*. Cd was distributed in a characteristic manner within the fruiting body of all three species. The Cd content of the cap was a function of its radius as well as its height. Concentrations of Cd and the chemically related Zn in the investigated mushroom segments were strongly correlated, whereas Al, Cu and Ag correlated poorly with Cd. To our knowledge, this is the first study of Cd contents in wild mycelia. We found similar concentrations of Cd and Zn in isolated mycelia and stems of the corresponding fruiting bodies. In addition, substrates were analysed to study soil-specific effects on Cd accumulation. The extent of Cd and Zn transfer from soil to mushroom was species-specific and influenced by the availability of these two heavy metals, as well as the age of the mushroom. Interestingly, the typical Cd and Zn distributions described here were not affected by the extent of accumulation, indicating that uptake and distribution of Cd and Zn are actually two separate mechanisms.

Key words Cadmium · Zinc · Mushroom · Mycelium · Soil

Introduction

Heavy-metal contamination of fungi caused either by natural processes or human activities is regarded as a possible source for the long-term accumulation of heavy metals in human beings due to the widespread consumption of wild fungi [1–4]. Elevated concentrations of toxic metals have been found in mushrooms growing in urban and industrial environments [5, 6]. Some species of the popular *Agaricus* family, in particular, showed high contents of Cd even in rural sites [7–10], indicating a biological phenomenon rather than environmental pollution. In other families, only a few species were found to accumulate Cd to a similar extent [9]. Cd speciation was investigated in *Agaricus macrosporus* as well as in the commercially important *Agaricus bisporus* [11, 12]. Fungal interactions with toxic metals have been reviewed by Gadd [13]. Relationships between the heavy metal content of mushrooms and different soil parameters have been investigated [14–17].

So far, the mechanism by which Cd-accumulating mushroom species absorb this metal is unknown. One possible hypothesis is a substitution of essential Zn by Cd. The interaction of Cd and chemically related metals during their uptake and accumulation into the fruiting body is still poorly understood. However, Brunnert [18] showed that Zn competes with Cd during uptake into the fruiting bodies of cultivated *Agrocybe aegerita*. Unfortunately, such experiments could not be performed with the *Agaricus* species of interest, because when cultivated, these mushrooms did not develop fruiting bodies. Therefore, we investigated the accumulation of Cd and Zn in more detail under natural growing conditions.

In the Jura mountains of Switzerland we found specimens of *Agaricus macrosporus* and *Agaricus silvicola*. These two Cd-accumulating wild mushrooms were investigated together with another edible saprotrophic basidiomycete, *Stropharia rugosoannulata*, which was cultivated on hay substrates. The aim of this work was to investigate the influence of substrate-specific factors on Cd accumulation. We determined total concentrations of Cd and the chemically related Zn in the substrates, and also elucidated the availability of these metals to the mycelia. Some information on variable Cd

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concentrations in fruiting bodies is available [1, 8, 10, 19], but a detailed description of the distribution of Cd within fruiting bodies, especially within the cap, is lacking. Cd concentrations of wild mycelia have not been determined up until now. Therefore, we also analysed the accumulation of Cd and its homologue Zn in mycelia, stems, caps and lamellae. Quantities of both heavy metals in the investigated mushrooms were compared with those in the substrates (total and available) to study the relationship between Cd and Zn during uptake and accumulation.

Materials and methods

Sampling of wild mushrooms. From September to October 1995, we collected wild *Agaricus macrosporus* and *A. silvicola* specimens together with accessory soil samples from surface horizons at the localities indicated in Table 1. All the sites are situated in relatively unpolluted regions at an elevation of about 1000 m. Both *Agaricus* species were classified according to their macroscopic characteristics as well as the size of their spores. Old and young fruiting bodies were collected. Colour, especially that of the gills, an open and to some extent, the size, were used as criteria to determine an old mushroom. Sampling was carried out using plastic containers.

Cultivation of Stropharia rugosoannulata and *Agaricus macrosporus*. In the same year a mycelium of *S. rugosoannulata* was bought and then cultivated on hay substrates in the presence and absence of added Cd. During the development of the mycelium a total of 40 l of 0.5 mg/l $CdCl_2$ was applied to one hay substrate. The other substrate was moistened with double-distilled water. After 6 weeks the hay substrates were overgrown with mycelia and henceforth Cd-free water was used to keep both cultures moist. Two weeks later, mushrooms were collected from both cultures. As a reduction in the Cd content of the mushrooms from one harvest to the other was expected [16], we only compared mushrooms of the first harvest. All fruiting bodies were stored at –20 °C until they were analysed. According to the method of Meisch [20], we also cultivated *A. macrosporus* mycelia on agar plates containing 0.1, 0.2, 0.5, 0.9 and 5 mg/l available Cd, isolation of these mycelia prior to sample preparation did not pose any problems and therefore is not discussed below.

Isolation of mycelia. As it was difficult to define this mushroom's substrate, we collected soil or hay substrate of the *Stropharia* cultures directly from under the fruiting body at a depth of 10–20 cm, where most of the mycelium clumps were found. The mycelium was closely interlaced with the corresponding soil (Fig. 1A). We separated the mycelium from its substrate mechanically using plastic tweezers and gloves. Electron micrographs were taken of all samples to demonstrate that the mycelia were completely separated from roots and that contamination by soil particles was not apparent (Fig. 1B). There were never any other fungi present at the sampling sites. We only isolated macroscopic

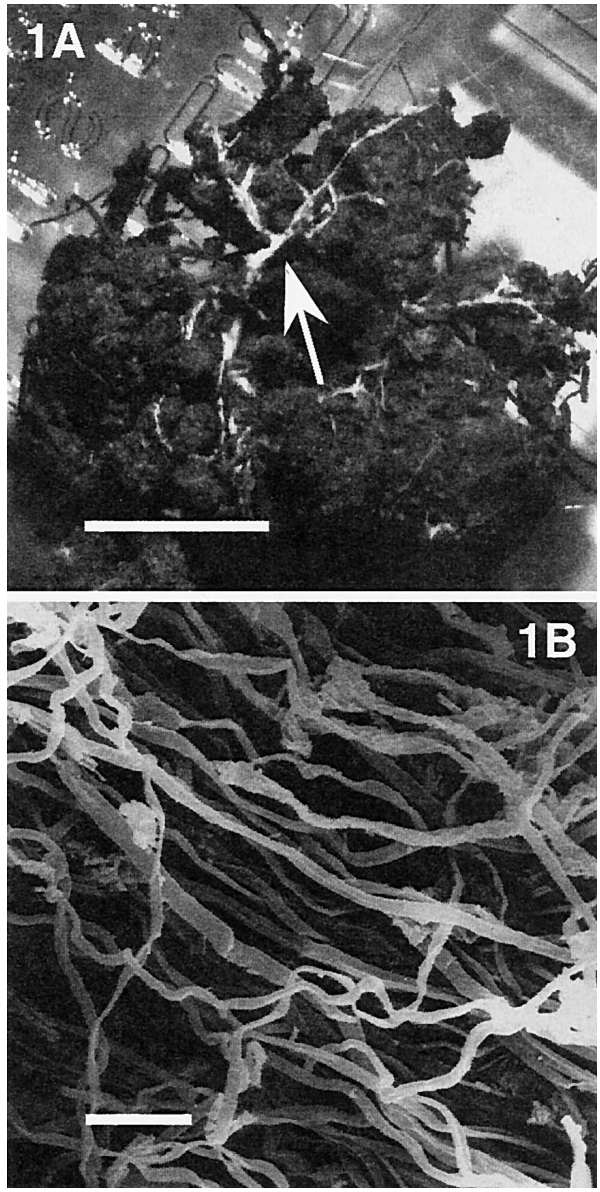


Fig. 1 A, B Mycelium of *Agaricus silvicola* (A.S.), found in Tavannes (Switzerland). **A** Before purification, *scale* 1 cm. The *arrow* points to a clump of mycelium. **B** After purification, *scale* 10 μm

Table 1 Mushrooms, substrates (*S*) and mycelia (*M*) investigated. The letters following S and M refer to the site; *A.M. Agaricus macrosporus, A.S. Agaricus silvicola, S.R. Stropharia rugosoannulata, SC1* 0–3 cm depth, *SC2* 3–6 cm depth, *SC3* 6–12 cm depth

Site	Village	Origin[a]	Mushroom	Substrate	Mycelium
A	Saulcy	Cambisol	A.M.6[b]	SA	MA
B	Saulcy	Cambisol	A.M.1, A.M.2	SB	MB
C	Saulcy	Cambisol	A.M.3	SC_1, SC_2, SC_3	–
D	Montfaucon	Cambisol	A.M.4, A.M.5	SD	MD
E	Tavannes	Rendzina	A.S.1, A.S.2, A.S.3	SE	ME
–	(Culture)	Hay	S.R.	S	M
–	(Culture)	Hay	S.R. + CD	S + Cd	M + Cd

[a] Geological classification according to FAO
[b] The numbers identify individual specimen

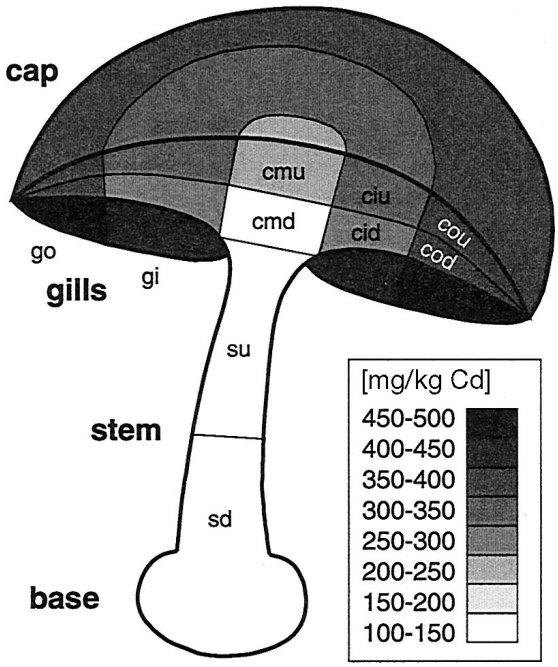


Fig. 2 Distribution of Cd in a sample of an older fruiting body of *Agaricus macrosporus* (A.M.3). Because of the different sizes and shapes of the mushroom samples, it was not always possible to dissect all segments shown (*thin lines*). *S* Stem, *g* gills, *c* cap, *m* middle, *i* inner part, *o* outer part, *u* upper layer, *d* lower layer

mycelial clumps with the typical colour of the mushroom concerned and which were also clearly connected to the base. Thus, any contamination by mycelia of other fungi could be excluded. After separation, all substrates and mycelia were stored at –20 °C. Site E (Table 1) was exceptional in that we were unable to find enough mycelia for each individual mushroom. Therefore, we pooled the mycelia of all specimens at this sample site and analysed this material. For a better comparison, we also bulked all individual substrates as well as segments (Fig. 2) of the fruiting bodies (samples A.S. 2 and A.S. 3 in Table 1 are mixtures of three and two specimens, respectively).

Sample preparation. All mushrooms and mycelia were freeze-dried, cleaned mechanically, cut with a scalpel according to Fig. 2 and then powdered in a ZrO_2 ball mill. A 50-mg subsample of each powder was digested in a mixture of HNO_3 and $HClO_4$ in a Teflon pressure bomb by microwave excitation. Soil samples were dried at 40 °C and sieved to obtain particles of <1.6 mm in diameter. One part of the sieved substrates was ground in the same way as the mushrooms. Subsamples (ca. 0.5 g) of the ground substrates were treated with a mixture of HNO_3 and H_2O_2 in a microwave oven to determine total Cd and Zn contents. The other fraction of the sieved substrates was extracted with $CaCl_2$ solutions (0.05 M/24 h and 0.1 M/1 h, 2 g soil:20 g solution) to evaluate available quantities of Cd and Zn. The pH of the substrates was determined as described by Courchesne [21]. Blank values for the investigated elements were mostly below or near the detection limit.

Analysis. Determinations of heavy metal concentrations were performed by optical emission spectroscopy (OES) using horizontal inductive coupled plasma excitation (ICP). This method was compared to differential pulse anodic stripping voltametry using a homogenized sample of *Amanita muscaria* containing 10.1 mg/kg Cd. The results were within the error margin of the Cd concentration determined from six replicaters by ICP-OES (10.4 ± 0.4 mg/kg^{-1} Cd). The Cd content of this mushroom was comparable to published data (15.5 mg/kg Cd; [9]). Duplicates of mushroom and soil samples were prepared independently. The SD between mushroom duplicates was in the range of 1–5%. Unreplicated determinations were controlled from time to time by analysing three replicates. With this accuracy (4%) it was possible to compare the Cd contents of different parts of the fruiting body.

Results

Cd distribution within fruiting bodies

In general, Cd concentrations in the range of 21–501 and 2.7–46 mg/kg were measured in fruiting bodies of wild *Agaricus macrosporus* (A.M.) and *A. silvicola* (A.S.), respectively (Figs. 2-5, Table 2). Specimen old S.R. + Cd of the *Stropharia* culture, which was supplied with Cd, contained 0.6–18.6 mg/kg Cd. In contrast, low

Table 2 Cd and Zn concentrations in fruiting bodies of A.M. and A.S. are presented in mg/kg dry weight. *r* values of Cd and Zn distribution are given. SDs were typically <5%. For segments, see Fig. 2. For abbreviations, see Table 1

Segment	Old A.M.3		Old A.M.4		Old A.M.5		Old A.S.1	
	Cd (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Zn (mg/kg)
sd	145	98	113	114	109	116	5.5	75
su	120	101	99	106	56	95	6.2	82
cmd	138	112	109	110	53	95	5.6	90
cmu	243	149	201	134	134	127	11.6	93
cid	254	138	187	141	106	127	13.7[a]	138[a]
ciu	315	156	285	169	158	155	–	–
cod	359	181	250	186	148	173	15.1[b]	146[b]
cou	362	210	282	183	180	194	–	–
gi	420	217	306	264	183	261	19.9	190
go	489	239	349	285	264	289	19.2	179
r	0.977		0.909		0.929		0.964	

[a] Segments cid and ciu were not separated here. Data corresponds to section ci (cap, inner part)
[b] Segments cod and cou were not separated here. Data corresponds to section co (cap, outer part)

Cd concentrations of 0.1–1.1 mg/kg were measured in sample old S.R. of the negative control. Interestingly, Cd was typically distributed within all three examined species (Figs. 2–5, Table 2). The highest Cd contents were found in the cap, mostly in the gills at the brim, whereas the lowest contents were within the stem. The ring and the gills had similar Cd contents. The *A. macrosporus* mushrooms allowed a detailed analysis of the Cd distribution within the cap. Cd concentrations in the segment cid (see Fig. 2) were normalized in these specimens to 100% and compared to the Cd contents of the sections ciu, cod and cou respectively (see Fig. 2, Table 3). The Cd content in the cap was mainly a function of its radius. Relative Cd contents of the sections cod and cou at the brim of the cap amounted to 149±15% and 164±19%, respectively. In the corresponding inner segments, cid and ciu, Cd contents were markedly reduced. Interestingly, the sections cid and cod of the lower cap layer, which are directly connected to the Cd-rich gills, contained lower quantities of Cd than the segments above of the same cap radius (inner or outer). Whereas this difference was significant for the inner sections, cid and ciu, it was not for the sections cou and cod at the brim.

Age-specific effects were analysed at sites B and E. Specimens young A.M.1 and old A.M.2 grew close to each other and therefore probably competed for the same substrate (site B). Cd contents in young A.M.1 were approximately double those of old A.M.2 (Fig. 3).

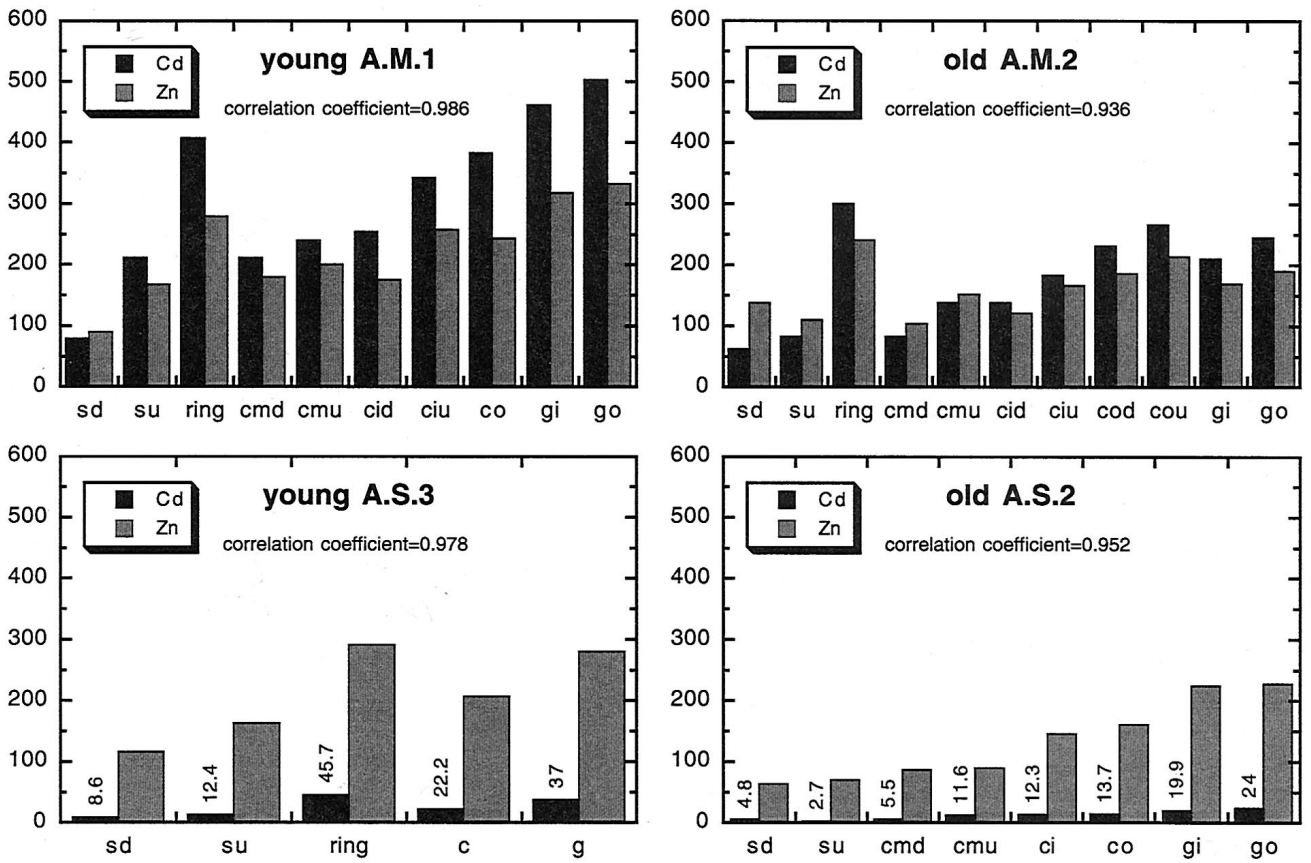
Table 3 Distribution of Cd and Zn in the cap of A.M. specimens. Metal concentrations of the cid segments were normalized to 100% and compared with the concentrations in ciu, cod and cou, respectively. For segments, see Fig. 2. For abbreviations, see Table 1

Segment	Cd (%)	Zn (%)	n[a]
cid	100	100	–
ciu	138±11	124±16	6
cod	149±15	141±10	5
cou	164±19	150±18	5

[a] Number of mushrooms

Also, young A.S. 3 contained about twice as much Cd as old A.S.1 and old A.S.2, which were collected at the same site (site E, Fig. 3, Table 2). Interestingly, Zn concentrations were affected similarly to those of Cd (Fig. 3, Table 2), indicating that Cd and Zn accumulation were influenced by the age of the fruiting bodies.

Fig. 3 Concentrations of Cd and Zn in various parts of the fruiting bodies of A.M. and A.S.. Contents are presented in mg/kg (dry weight). To express the relationship between Cd and Zn, we determined the correlation coefficients by comparing Cd and Zn contents in the corresponding segments. SDs were typically <5%. For abbreviations, see Table 1 and Fig. 2



Correlation between Cd and Zn

The distribution of Zn in the fruiting bodies of all the species was very similar to that of Cd (see also Table 3). Correlation coefficients (r) between 0.899 and 0.986 were found (Figs. 3-5). In sample A.M.6 we also determined concentrations of Cu, Al and Ag. Interestingly, r values of correlations between these elements and Cd were lower than that of the correlation between Cd and Zn. (Cu, $r=0.817$; Al, $r=0.417$; Ag, $r=0.704$; Fig. 5B). In contrast to the observed similarity in distribution, the ratio between Cd and Zn in the mushrooms varied greatly in the three different species. In A.M. fruiting bodies, similar concentrations of both metals were found, whereas specimens of A.S. contained about 10 times more Zn than Cd (Fig. 3, Table 2). In mushrooms from the S.R. culture, which was supplied with Cd, Zn concentrations were about 3 times those of Cd (Fig. 4). S.R. specimens from the negative control contained about 100 times more Zn than Cd.

Cd and Zn concentrations in mycelia

Analysis of the mycelia of all investigated species revealed similar or lower quantities of Cd as compared to the stem of the corresponding fruiting body (Table 4). Highest Cd concentrations of 17–42 mg/kg were found in A.M. mycelia (MA, MB, MD) amounting to 10–20 times that in A.S. mycelia (ME). Cd contents in *Stropharia* mycelia of the culture supplied with Cd were clearly higher than those of the negative control (Table 4). Zn concentrations in mycelia were similar to or higher than those of the corresponding stem. In agreement with data of A.S. fruiting bodies, the Zn content of ME was also markedly increased as compared to the concentration of Cd. Furthermore, we compared wild and cultivated mycelia of A.M.. At comparable available Cd concentrations in the substrate, we found similar Cd quantities in wild and cultivated A.M. mycelia (Tables 5, 6). Meisch [20] showed that Cd acts as a growth factor in *Agaricus abruptibulbus* mycelia. However, we did not observe any growth stimulation by Cd in A.M. mycelia. This is in line with findings for *Agaricus perrarus* mycelia [1].

Table 4 Cd and Zn concentrations in wild A.M., A.S. and cultivated S.R. mycelia. Data are presented in mg/kg (dry weight). SDs were typically <5%. For abbreviations, see Table 1

Mycelia	Cd (mg/kg)	Zn (mg/kg)
MB	42	68
MA	27	86
MD	17	136
ME	1.7	227
M	0.3	34
M+Cd	4.6	41

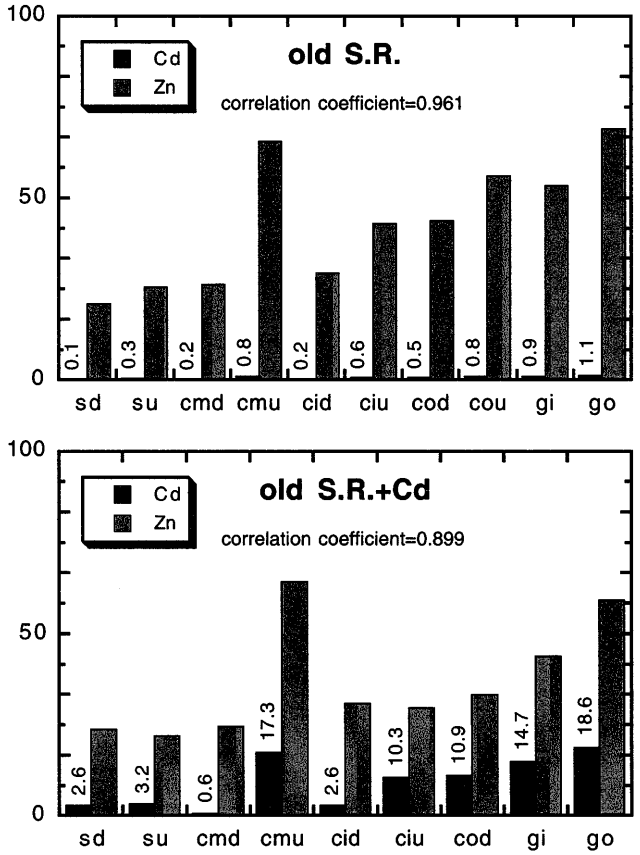


Fig. 4 Concentrations of Cd and Zn in segments of *Stropharia rugosoannulata* (*S.R.*). Data are shown within a range of 0–100 mg/kg. Correlation coefficients indicate the relationships between the distributions of Cd and Zn. SDs were typically <5%. For abbreviations, see Fig. 2

Table 5 Comparison of Cd accumulation in cultivated and wild A.M. mycelia. Data are presented in mg/kg (dry weight). SDs were typically <5%. Data of wild mycelia are shown in *boldface* and were taken from Table 4 and Table 6. For abbreviations, see Table 1

Available Cd	Cultivated mycelia	Wild mycelia
0.1	10	–
0.2	–	**42** (MB)
0.2	–	**17** (MD)
0.2	–	**27** (MA)
0.5	34	–
0.9	78	–
5.0	284	–

Substrate characteristics

The substrates allowed a preliminary analysis of factors influencing the Cd and Zn contents in the investigated mushroom species. Mycelium-available concentrations of Cd and Zn were determined according to a procedure by Fleckenstein [16]. In this study the authors found a good relationship between 0.05 M $CaCl_2$-extractable quantities of Cd and Cd uptake by *Agaricus*

Table 6 Soil characteristics. Concentrations of Cd and Zn are shown in mg/kg (dry weight). M-available (*M.avail.*) quantities of Cd and Zn are compared with calculated plant-available concentrations (*calc.*). SDs of Cd and Zn concentrations were typically <20%. Data in *boldface* indicate results for site C which was exceptionally rich in Cd and Zn. For other abbreviations, see Table 1

S	pH	Total Cd	Total Zn	M.avail. Cd	M.avail. Zn	Cd calc.	Zn calc.
SA	4.92	7.8	119	0.2	1.0	1.4	2.3
SB	5.02	7.1	114	0.2	0.9	1.2	1.8
SC1	**5.15**	**14.1**	**371**	**1.7**	**1.4**	**1.8**	**3.4**
SC2	**5.46**	**12.1**	**395**	**1.5**	**1.0**	**1.2 (2.7)**[a]	**1.9 (1.6)**[a]
SC3	**5.26**	**12.5**	**345**	**2.4**	**1.3**	**1.5 (3.4)**[a]	**2.6 (1.9)**[a]
SD	4.94	6.2	105	0.2	1.4	1.1	2.0
SE	5.55	7.5	177	0.1	2.8	0.8	0.9
S	–	0.2	26.6	–	–	–	–
S + Cd	–	10.8	27.4	–	–	–	–

[a] Values in parentheses were determined by extraction with 0.1 M $CaCl_2$

bisporus. Hornburg and Brümmer [22, 23] devised an equation showing the relationship between plant-available heavy metals, total concentrations in the soil and the pH (Eqs. 1 and 2 for Cd and Zn, respectively). In addition, we compared the mycelium-available soil contents of Cd and Zn to these estimates of plant-available Cd and Zn calculated as follows:

$$\log \text{Cd plant} = 0.813 \times \log \text{Cd total} - 0.394 \times \text{pH} + 1.354 \quad (1)$$

$$\log \text{Zn plant} = 0.753 \times \log \text{Zn total} - 0.867 \times \text{pH} + 3.064 \quad (2)$$

Levels of plant-available metals in soil can be determined experimentally by extraction with 0.1 M $CaCl_2$ solution for 1 h. For two samples we estimated these concentrations (Table 6) in order to check the calculated data. These values differed by a factor of about 2 and 1–2 for Cd and Zn, respectively. In comparison with Hornburg's method some differences in our sample preparation (grinding, digestion) may have been partly responsible for this dissimilarity. The soil pHs of the samples from the Jura mountains revealed values in the range of 4.92 to 5.55 (Table 6). In the majority of cases, calculated plant-available metal contents of these soils were slightly higher than extracted mycelium-available quantities of Cd and Zn. Total soil concentrations of Zn varied from 105 to 395 mg/kg, amounting to about 20–30 times those of Cd. In contrast, available quantities of Cd and Zn were similar, ranging from 0.1 to 2.4 and 0.9 to 3.4 mg/kg, respectively. The hay substrate from the S.R. culture supplied with Cd displayed total Cd concentrations which were about 50 times higher (10.8 mg/kg) than those of the hay substrate from the negative control (0.2 mg/kg). Both substrates contained about 27 mg/kg total Zn.

Discussion

Cd distribution in fruiting bodies

As previously mentioned in the introduction, there are scarce data describing the distribution of Cd in mushrooms. Kojo [19] investigated the Cd and Hg distribution in the stem, cap and gills of two species of mushroom. In a mushroom of *Agaricus arvensis* the gills proved to be richest in Cd, whereas the stem contained a low level of Cd. Similar Cd contents were found in the stem as well as in the gills of *Agaricus campestris* specimens. In contrast, Seeger [9] found the lowest Cd contents in the gills of *Agaricus aestiralis* specimens. In mushrooms of *A. silvicola* and *A. macrosporus* Cd contents in the range of 2–113 and 11–120 mg/kg were measured, respectively [9, 10]. In these latter studies Cd concentrations of the gills amounted to about 5–10 times those of the stems. However, all these studies were based, in most cases, on the analysis of only three different segments, namely the stem, the cap and the gills. Taking into account the small number of segments analysed, our results and those given above [9, 10] are quite comparable. In addition, we found significant differences in the Cd distribution within the cap of A.M. mushrooms. The Cd content of the cap was a function of its radius as well as its height (Table 3). In the caps of A.S. and S.R. Cd was distributed similarly, but there were insufficient numbers of samples to examine the statistical significance of the results (Figs. 3–5, Table 2). Interestingly, the typical distribution pattern described here was not influenced by species-specific factors. Whether or not this distribution of Cd is typical for basidiomycetes is not clear from our data. At least in *A. aestiralis* specimens [9], the distribution of Cd was different from that observed in this study.

Schmitt [24] found higher Cd contents in young A.M. mushrooms than in older ones, whereas Seeger [25] could find only a weak relationship between the age of mushrooms and their Hg content. The influence of mushroom age on Cd accumulation was studied at sites B and E (Table 1), where we found old as well as young specimens. Cd and Zn concentrations in the fruiting bodies of A.M. (site B) and A.S. (site E) were about double those of the corresponding old fruiting bodies (Fig. 3). An additional, geological factor influencing this result could be clearly excluded here, as we compared only mushrooms of the same sample site. Therefore, these findings clearly demonstrated that Cd

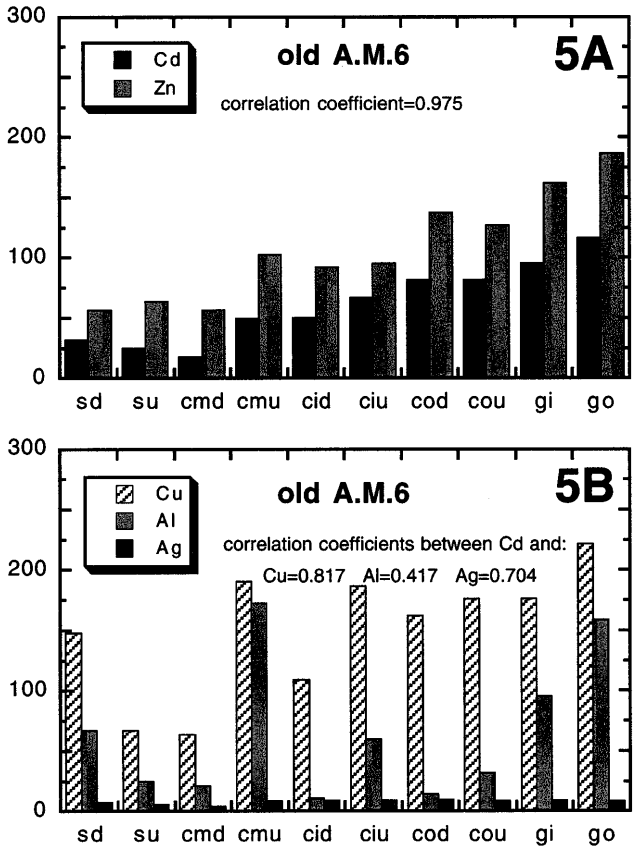


Fig. 5 A Concentrations of Cd and Zn in the fruiting body of old A.M.6, **B** concentrations of Cu, Al and Ag in the same sample. Concentrations within a range of 0–300 mg/kg are shown. Correlation coefficients indicate relationships between Cd and the heavy metals Zn, Cu, Al, Ag. SDs were typically <5%. For abbreviations, see Fig. 2

and Zn accumulation can be influenced by the age of mushrooms.

Cd concentration in mycelia

Up until now, heavy metal concentrations in wild mycelia could not be determined due to isolation problems. Using a separation procedure controlled by electron microscopy, sufficient quantities of mycelia for each mushroom could be collected in the majority of cases. Mycelia of all investigated species contained similar or lower quantities of Cd than the stems of the fruiting bodies (Figs. 2–5, Tables 2, 4). In cultivated mycelia of A.M. we found similar Cd contents to wild mycelia (Table 5). On the basis of levels of available Cd in the substrate (Table 6), Cd was accumulated by a factor of ca. 100 and 20 in wild A.M. and A.S. mycelia, respectively. After absorption, Cd is believed to be transported to the fruiting body via the mycelium [18]. Obviously, the Cd concentration in the mycelium is a function of Cd uptake and transport. However, a detailed description of the uptake mechanism as well as the transport of Cd in the mycelium needs further investigation.

Cd and Zn accumulation

A finding which has to be emphasized is the strong correlation between the distribution of Cd and the chemically related Zn in all analysed mushrooms (Tables 2, 3, Figs. 2–5). Interestingly, Al, Cu and Ag were quite differently distributed in A.M.6 (Fig. 5). This similar behaviour of the essential Zn and its homologue Cd during distribution may be due to the fact that there is no, or only limited, differentiation between these metals in terms of their accumulation by mushrooms. The fact that the chemically related Hg showed a similar distribution pattern to that of Cd and Zn [19] in mushrooms supports this assumption. In contrast to the observed Cd distribution, the extent of Cd accumulation was species-specific. Mushrooms as well as mycelia of A.S. contained 10–20 times less Cd than those of A.M. (Figs. 3–5, Table 2). Corresponding substrates displayed comparable quantities of either mycelium-available or total Cd, which indicated that a soil-dependent factor could be excluded (Table 6). In samples of both S.R. cultures we determined strongly reduced Cd concentrations as compared to those in A.M. (Fig. 4). Zn accumulation appeared to be species-specific as well, but not as pronounced as that of Cd (Figs. 3–5, Table 2). Thus, Cd and Zn appeared to be transferred from soil to mycelia in different quantities, depending on either species-specific factors or the age of the mushroom. But once Cd and Zn (and probably also Hg) were taken up into the mycelium, these elements were distributed identically within the fruiting body. Therefore, we hypothetised that the accumulation of Cd and its distribution within the fruiting body, may be due to two separate mechanisms.

Cd and Zn transfer from soil to mycelia

Up until now, total concentrations of Cd in substrates have been determined in many studies to characterize geological influences. We additionally were interested in the quantities of Cd and its homologue Zn which were available to the mycelia.

In general, total Zn concentrations of all the substrates amounted to about 20–30 times those of Cd. Normally, the Zn/Cd ratio of the continental crust is about 100 [26]. Furthermore, total as well as available quantities of Cd in the examined substrates were unusually high as compared to typical background values in Europe. Similar total Cd concentrations were found only in municipal parks or in polluted regions [17, 26]. Therefore, we wondered whether contamination during the analysis could have been the reason for our unexpected data. To check this, we collected a second, random, substrate sample at site B two months after the initial sampling. There was no statistically significant difference between this sample and the one collected two months previously. The fact that we also determined low total Cd concentrations in the hay substrate

without any added Cd from the S.R. culture (Table 6) supported the accuracy of the soil analysis. Thus, the soil data appeared to be correct. However, the reason for these unexpected data remains unknown, and this should be borne in mind with respect to the rest of this discussion.

Neglecting small differencies between plant- and mycelium-available metal concentrations in the substrates, Cd and Zn seemed to be accessible in similar quantities. In contrast, total concentrations of Zn were much higher than those of Cd. Under oxidizing conditions, adsorption and desorption processes dominate the behaviour of Cd in soil [26]. At neutral or higher pH, the majority of Cd is specifically adsorbed. A decrease in soil pH enhances the unspecifically adsorbed fraction. Already at pH 5, over 30% of total Cd is mobilized. Only a small fraction of Zn is available at this pH. Hence, the observed similarity between available quantities of Cd and Zn is in line with reduced mobilization of Zn as compared to that of Cd. As mentioned previously, specimens of A.M. contained similar quantities of Cd and Zn. On the basis of mycelium-available Cd and Zn, these specimens therefore appeared to take up both metals to the same extent, indicating that this species does not accumulate Cd specifically as reported recently [10]. Whether or not Cd accumulation in A.M. can be ascribed to an uncontrolled absorption of the essential element Zn is not clear from our data. Further investigations are needed to reveal the extent of the relationship between Cd and Zn uptake.

Specimens of A.M. could be collected at different sites (Table 1), and we examined the influence of the soils on Cd accumulation in the mushrooms. In the substrates of site C more available, as well as total, Cd and Zn was found than in all the other substrate samples (Table 6). This finding fitted well with the observation that old mushroom A.M.3, which grew at site C (Table 1), contained more Cd and Zn than other old A.M. specimens, i.e. A.M.4, A.M.5 and A.M.6. This demonstrated that Cd accumulation is also influenced by soil characteristics. Because the pH did not vary greatly at sites A–D, total concentrations of Cd and Zn alone appeared to determine available quantities of these heavy metals here (Eqs 1 and 2).

In summary, the observed uptake of Cd and Zn by mushrooms from soil was dominated by species-specific factors, the age of mushrooms, as well as the availability of the two elements, which was determined by the prevailing pH and the extraction potential of the 0.05 M $CaCl_2$ solution. After absorption by mycelia, Cd and Zn were distributed identically within the fruiting bodies of the investigated species. Interestingly, the distribution described here was not affected by the extent of accumulation of these two heavy metals. This indicated that the uptake of Cd and Zn and their distribution in fruiting bodies are actually controlled by two separate mechanisms.

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