Effects of Cadmium on Aquatic Hyphomycetes

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Two kinds of experiments, sporulation and growth experiments, were carried out to demonstrate the effect of cadmium on aquatic hyphomycetes. Oak (*Quercus petraea* L.) leaves were exposed in a hard-water stream (Lüssel, Swiss Jura) and a soft-water stream (Ibach, Black Forest) for 2 months. In the laboratory, fungal sporulation on the leaves in stream water enriched with cadmium (as $CdCl_2$) was studied. A measurable effect was found when the cadmium concentration exceeded 0.1 ppm (0.1 mg/liter). Concentrations higher than 100 ppm inhibited conidium production completely. This toxic effect of cadmium was species dependent and much higher in soft water (water with low concentrations of calcium and magnesium) than in hard water. Growth experiments with *Alatospora acuminata* Ingold, *Clavariopsis aquatica* De Wildeman, *Flagellospora curvula* Ingold, *Heliscus lugdunensis* Saccardo and Therry, and *Tetracladium marchalianum* De Wildeman showed the same pattern of cadmium sensitivity as that seen in the sporulation experiments. Mycelial growth was less sensitive to cadmium than was fungal sporulation. High concentrations of competing cations (e.g., calcium and zinc) or potential ligands could reduce cadmium toxicity. Calcium content seems to be the most important factor responsible for the different sensitivity of aquatic hyphomycetes in hard and soft water.

Among various pollutants, cadmium has recently attracted worldwide interest. It is a trace metal without any known biological function and is toxic even at very low doses. Industrial output of products containing cadmium (e.g., plastic pigments, nickel-cadmium batteries, PVC stabilizers), as well as the mining of zinc ores containing 0.05 to 0.35% cadmium, have accelerated the mobilization of this metal beyond any natural transport levels. The dry and wet deposition of cadmium dust resulting from these industrial activities has raised its concentration in soils, water, and sediments and subsequent transport to the biota (12).

A great number of papers on the toxicity of cadmium for plants, animals, and humans have appeared. Most studies have dealt with the interaction between cadmium and either the biota or the environment. Only a few investigations have been directed towards the multiple interaction among the environment and the biota and cadmium (14, 19). The physicochemical characteristics of the environment may either lessen or magnify the toxicity of a pollutant (3). Studies with microorganisms have shown that zinc and magnesium (13, 16), clay minerals (3), pH (2, 4), and organic ligands (18, 20) can influence the toxicity of cadmium.

The purpose of this study was to evaluate the effects of cadmium on aquatic hyphomycetes in stream waters of different chemical characteristics and to identify factors responsible for different sensitivities to cadmium. In fresh water, these fungi play an important role as food source and food degraders for several invertebrates (6, 7). Although cadmium levels in most European streams and rivers do not reach acutely toxic levels, cadmium uptake and accumulation by fungi and subsequent transport to higher trophic levels could be of ecological significance (9).

MATERIALS AND METHODS

Water chemistry. A hard-water stream in the Swiss Jura, the Lüssel, and a soft-water stream in the German Black Forest, the Ibach, were chosen as objects of this study.

Detailed descriptions of the study areas have been given by Bärlocher and Rosset (8). Over a period of 2 years, the pH, temperature, alkalinity, and levels of cations and anions in these streams were measured regularly. Cations were measured by atomic absorption spectrophotometry on a Unicam SP 90, and anions were measured by visual spectrophotometry on a Unicam SP 1700, both by standard methods (1). Alkalinity was determined by potentiometric titration with 0.01 M hydrochloric acid on a Metrohm E 536 potentiograph.

Sporulation experiments. Oak leaf disks (4.5 cm^2) were placed in mesh bags (mesh size, 1 mm) and exposed in both rivers for 2 months. In the laboratory, the leaves were washed in tap water and aerated separately for 48 h in stream water enriched with increasing amounts of cadmium. A control group of leaf disks was aerated without added cadmium. Samples were then filtered through membrane filters (pore size, 5 μm), and the numbers of conidia produced by aquatic hyphomycetes on leaves were counted. This experiment was carried out six times in the course of 1 year. Cadmium was added as $CdCl_2 \cdot H_2O$. Concentrations of Cd were 0.01, 0.1, 1, 10, 33, and 100 ppm (mg/ml).

Growth experiments. Five species of aquatic hyphomycetes were isolated from single spores and grown on 0.1% malt extract agar at 11°C.

Growth inhibition by cadmium. Growth of five selected fungi at 20°C was measured after 16 to 44 days on buffered agar plates (3 g of malt extract, 15 g of agar, 0.69 g of peptone, 1.01 g of KNO_3, 0.34 g of KH_2PO_4, 0.57 g of $K_2HPO_4 \cdot 3H_2O$, 0.175 g of NaCl, and 0.25 g of $MgSO_4 \cdot 7H_2O$ per 1 liter of water). The influence of cadmium on the growth of aquatic hyphomycetes was measured at the pH levels of the two streams, 7.1 and 8.3. Cadmium concentrations were 0, 0.1, 1, 10, and 100 ppm.

Experiments with Ca, Mg, and Zn. Calcium, magnesium, and zinc were added to medium amended with cadmium to determine the possible antagonistic or synergistic effects of these metals. The pH was adjusted to 7.0. The concentrations of cadmium were 0, 1, 10, and 33, or 100 ppm. The concentrations (in parts per million) of the other metals were: Ca (as $CaCl_2$); 0, 1, 10, and 100; Mg (as

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TABLE 1. Temperature, pH, and chemical characteristics of the two streams from summer 1981 to summer 1982

Stream	Mean (range) temp (°C)	Mean (range) pH	Mean (range) concn[a] of:						
			Ca	Mg	K	Alkali (meq/liter)	Si	Phenols	Humic acids
Ibach	6.4 (0.9–11.8)	7.2 (6.8–7.6)	4.4 (3.0–5.7)	0.6 (0.3–1.0)	2.9 (2.0–3.7)	0.27 (0.20–0.38)	3.0 (2.3–3.4)	0.18 (0.16–0.19)	1.8 (1.2–2.4)
Lüssel	9.1 (3.6–14.5)	8.3 (8.1–8.4)	86.9 (83.4–90.6)	5.6 (4.1–6.5)	16.2 (14.1–18.3)	4.04 (3.70–4.47)	1.7 (1.6–2.0)	0.02 (0.021–0.023)	0.3 (0.2–0.5)
Ratio	13:1		1:20	1:10	1:6	1:15	2:1	9:1	6:1

[a] Concentrations are in parts per million except where indicated otherwise.

$MgSO_4 \cdot 7H_2O$), 0, 3, 10, and 100; Zn (as $ZnCl_2$), 0, 1, 10, and 100.

Experiments with EDTA. EDTA was added as EDTA-Na_4 at concentrations of 0, 15, 45, and 135 ppm to growth medium containing cadmium at 0, 1, 10, or 100 ppm. The pH was adjusted to 7.0.

RESULTS

Water chemistry. The mean values of the chemical characteristics of the two streams are shown in Table 1. The Lüssel had a higher pH value, a higher alkalinity, and more calcium, magnesium, and potassium, whereas the Ibach had a higher content of silicate and organic substances such as humic acids and phenolic compounds. This result corresponds to the different geological substrata of the areas: limestone in the Jura and crystalline rocks in the Black Forest.

Sporulation experiments. Concentrations of up to 0.1 ppm had little influence on spore production. Between 0.1 and 33 ppm, the number of conidia decreased by a factor of 2 to 230. When the concentration reached 100 ppm, fewer than 100 spores were found for the Ibach, indicating that spore production in soft water was almost completely suppressed at concentrations higher than 33 ppm (Fig. 1). Another striking observation was that cadmium toxicity was much higher in soft-water hyphomycete communities. Figure 2 shows the numbers of spores produced at different cadmium concentrations as a percentage of the control group on a probit scale. The concentration which led to a 50% reduction in the number of conidia (LC_{50}) could be computed by linear regression. These LC_{50}s were 0.4 ppm for the Ibach and 12.1 ppm for the Lüssel. Toxicity in the soft water was thus 30 times higher.

Sporulation experiments in which distilled water instead of stream water was used showed a small increase in toxicity for the Ibach community (LC_{50}, 0.2 ppm) and a greatly increased Cd toxicity for the Lüssel community (LC_{50}, 0.4 ppm).

Cadmium not only influenced the total spore production but also the relative abundance of individual species, as is demonstrated in Fig. 3. The number of conidia produced by each species in pure stream water was defined as 100%. Conidium production by *Heliscus lugdunensis* Saccardo and Therry, *Tetracladium marchalianum* De Wildeman, and *Flagellospora curvula* Ingold was stimulated at low cadmium concentrations, whereas *Clavariopsis aquatica* De Wildeman and *Alatospora acuminata* Ingold produced fewer spores even at low concentrations of cadmium. To estimate the degree of sensitivity, it is best to compare inhibition of sporulation at LC_{50}s. *H. lugdunensis* and *T. marchalianum* were the least sensitive fungi in both streams. *A. acuminata*

was the most sensitive species, and *C. aquatica* and *F. curvula* showed average sensitivities.

Growth experiments. Fungal growth was inhibited at concentrations of >1 ppm (Fig. 4). There was a large range of sensitivity to Cd. The LC_{50}s were 3.3 ppm for *A. acuminata*, 7.2 ppm for *F. curvula*, 15.1 ppm for *C. aquatica*, 20.4 ppm for *T. marchalianum*, and 46.8 ppm for *H. lugdunensis*. There was a good correlation between sensitivity patterns in growth and sporulation experiments. Mycelial growth, however, was less sensitive to Cd than was sporulation between pH 7.1 and 8.3. A pH of 7.1 to 8.3 did not seem to have any major effect on cadmium toxicity. Reduction in growth area and LC_{50} did not show any significant difference at the pH levels of the streams.

Experiments with Ca, Zn, and Mg. Table 2 shows the influence of calcium on cadmium toxicity. At concentrations of 10 and 100 ppm, calcium reduced the toxic effect of the heavy metal for all five hyphomycete species. A similar experiment with zinc showed almost the same result as the calcium experiment (Table 3). Zinc may also have reduced Cd toxicity; zinc itself, however, is toxic at concentrations of > 100 ppm. In contrast, magnesium seems to have had only a slight or no effect at all on the toxicity of cadmium.

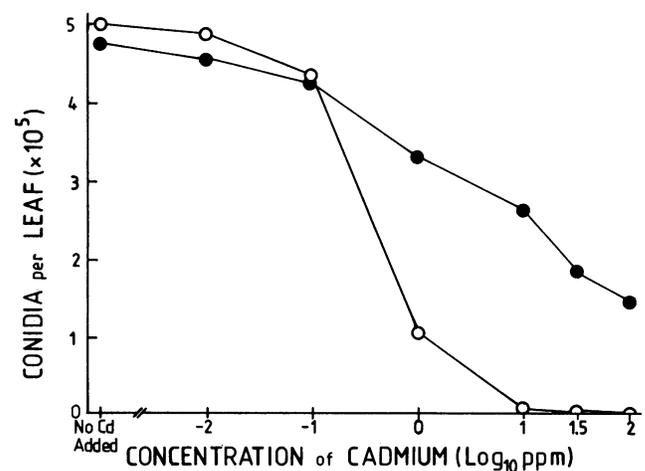**EDTA experiments.** EDTA was toxic at concentrations of

FIG. 1. Effect of cadmium on sporulation by aquatic hyphomycetes in Lüssel (●) and Ibach (○) water. Conidia produced in 48 h in the laboratory by oak leaf disks (4.5 cm²) after 2 months of stream exposure was measured at cadmium concentrations of 0, 0.01, 0.1, 1, 10, 33, and 100 ppm.

≥135 ppm (Table 4). EDTA may nevertheless have reduced sensitivity to Cd, but only when EDTA and Cd concentrations were of the same order of magnitude.

DISCUSSION

There are two feasible explanations for the different behaviors of the Lüssel and the Ibach fungi in stream water amended with cadmium. (i) Chemical characteristics of the stream water could influence the toxicity of Cd for hyphomycetes. If this hypothesis is correct, we should be able to identify chemical factors in the stream water that control cadmium toxicity. (ii) The fungal community in the hardwater stream could consist of more cadmium-resistant hyphomycetes. Our experiments support the first hypothesis. Thus, sporulation experiments in distilled water revealed a greatly increased Cd sensitivity in the Lüssel fungi (the LC_{50} decreased from 12.1 to 0.4 ppm) but not in the Ibach fungi (the LC_{50} decreased from 0.4 to 0.2 ppm) when compared with their behavior in native stream water. This observation suggests that water chemistry, not an innately greater resistance of the Lüssel fungal community, is the primary reason for the different sensitivity to Cd. The results of growth experiments show that there was a good correlation between the effect of Cd on sporulation and growth (for Ibach fungi, r = 0.884; $P < 0.05$).

Calcium and zinc ions act as antagonists of cadmium. They are of the same size and have chemical properties similar to those of cadmium ions (21). It is generally assumed that the toxic effect of the heavy metal is caused by an erroneous binding of Cd to proteins. For example, metallothionein proteins are known to irreversibly bind cadmium instead of zinc (17). The enzyme thereby loses its catalytic

FIG. 3. Influence of cadmium on the sporulation of five selected hyphomycetes for the Ibach (A) and Lüssel (B) communities. The number of spores produced by the control group (no Cd added) was defined as 100% for each species. Symbols: ●, *T. marchalianum*; ■, *H. Tugdunensis*; ◆, *F. curvula*; ◇, *C. aquatica*; ○, *A. acuminata*.

function. Thus, zinc and cadmium compete for an active site of the enzyme, and high Zn concentrations increase the chance of this ion being bound, which reduces the toxic effect of cadmium. Nothing is known as yet about the
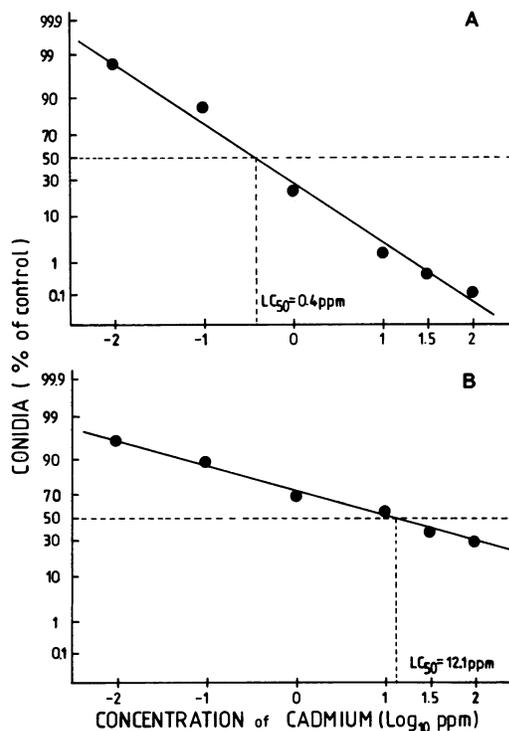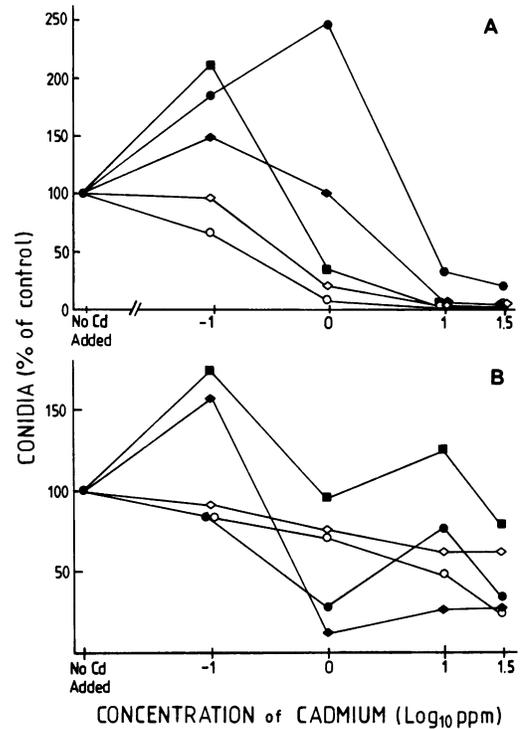
FIG. 2. Effect of cadmium on sporulation by aquatic hyphomycetes in Ibach (A) and Lüssel (B) water. Conidia numbers as a percentage of the control (no Cd added) on a probit scale allowed computation of LC_{50}s by linear regression.
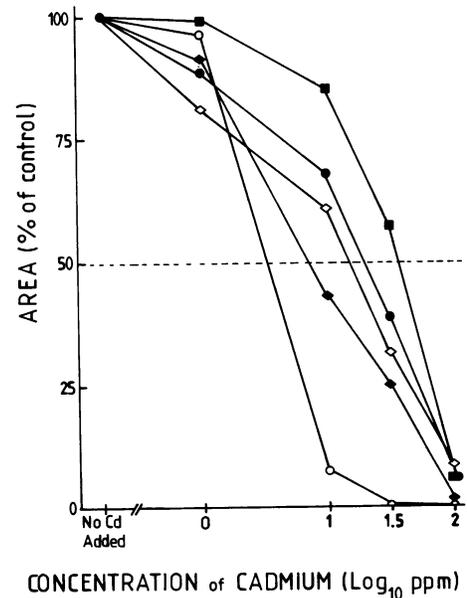
FIG. 4. Influence of cadmium on growth of five selected hyphomycetes on agar. Growth area of control group (no Cd added) was defined as 100% for each species. Symbols: ■, *H. lugdunensis*; ●, *T. marchalianum*; ◇, *C. aquatica*; ◆, *F. curvula*; ○, *A. acuminata*.

TABLE 2. Influence of cadmium on growth of aquatic hyphomycetes on agar with various calcium concentrations

Fungus	Ca concn (ppm)	Mean ± SEM % of control (growth area [cm^2]) with Cd concn (ppm) of[a]:			
		0	1	10	100
H. lugdunensis	0	100 ± 0.9 (50.6 ± 0.44)	99 ± 0.8 (49.9 ± 0.43)	87 ± 0.8 (43.7 ± 0.41)	6 ± 0.4 (3.1 ± 0.18)
	1	100 ± 1.5 (50.3 ± 0.73)	97 ± 0.9 (48.6 ± 0.43)	89 ± 0.8 (44.9 ± 0.41)	31 ± 1.2 (15.4 ± 0.60)
	10	100 ± 1.5 (50.3 ± 0.73)	99 ± 0.9 (49.9 ± 0.43)	94 ± 1.7 (47.3 ± 0.84)	40 ± 2.6 (19.9 ± 1.32)
	100	100 ± 1.7 (51.9 ± 0.88)	94 ± 1.4 (49.0 ± 0.72)	87 ± 2.0 (45.0 ± 1.03)	58 ± 3.1 (29.9 ± 1.62)
T. marchalianum	0	100 ± 2.1 (23.8 ± 0.50)	86 ± 1.9 (20.4 ± 0.46)	68 ± 2.6 (16.1 ± 0.62)	13 ± 0.8 (3.1 ± 0.18)
	1	100 ± 3.0 (26.1 ± 0.78)	90 ± 2.8 (23.5 ± 0.74)	67 ± 2.0 (27.6 ± 0.52)	20 ± 1.3 (5.2 ± 0.35)
	10	100 ± 1.2 (26.7 ± 0.53)	94 ± 1.2 (25.2 ± 0.31)	69 ± 3.4 (18.4 ± 0.92)	32 ± 1.6 (8.4 ± 0.44)
	100	100 ± 2.0 (26.4 ± 0.53)	99 ± 3.0 (26.1 ± 0.78)	80 ± 2.1 (21.0 ± 0.56)	51 ± 2.3 (13.4 ± 0.60)
C. aquatica	0	100 ± 5.3 (25.5 ± 1.34)	85 ± 2.2 (21.8 ± 0.57)	55 ± 2.2 (14.1 ± 0.57)	9 ± 3.0 (2.3 ± 0.77)
	1	100 ± 3.5 (25.5 ± 0.88)	92 ± 6.0 (23.5 ± 1.54)	65 ± 2.8 (16.6 ± 0.71)	32 ± 2.9 (8.2 ± 0.73)
	10	100 ± 1.2 (25.2 ± 0.31)	95 ± 3.0 (24.1 ± 0.75)	67 ± 4.2 (16.9 ± 1.05)	37 ± 1.9 (9.3 ± 0.47)
	100	100 ± 2.0 (27.3 ± 0.54)	92 ± 3.0 (25.2 ± 0.82)	67 ± 4.0 (18.3 ± 1.10)	66 ± 4.1 (18.1 ± 1.13)
F. curvula	0	100 ± 1.2 (24.3 ± 0.30)	86 ± 2.3 (21.0 ± 0.56)	37 ± 1.5 (8.9 ± 0.37)	9 ± 0.7 (2.2 ± 0.18)
	1	100 ± 4.2 (26.4 ± 1.10)	85 ± 1.1 (22.3 ± 0.29)	36 ± 0.7 (9.4 ± 0.19)	24 ± 1.0 (6.3 ± 0.26)
	10	100 ± 1.2 (26.7 ± 0.32)	92 ± 1.9 (24.6 ± 0.51)	39 ± 0.7 (10.4 ± 0.20)	29 ± 1.1 (7.6 ± 0.28)
	100	100 ± 2.3 (28.6 ± 0.66)	87 ± 1.1 (24.9 ± 0.31)	43 ± 3.5 (12.4 ± 1.00)	36 ± 1.7 (10.4 ± 0.49)
A. acuminata	0	100 ± 2.3 (20.4 ± 0.46)	97 ± 3.3 (19.9 ± 0.68)	14 ± 1.3 (2.8 ± 0.27)	0 (0)
	1	100 ± 1.3 (22.3 ± 0.29)	98 ± 2.5 (21.8 ± 0.55)	11 ± 1.6 (2.5 ± 0.35)	1 ± 0.1 (0.3 ± 0.03)
	10	100 ± 1.2 (24.9 ± 0.31)	100 ± 1.2 (24.9 ± 0.31)	17 ± 0.9 (4.2 ± 0.23)	2 ± 0.2 (0.6 ± 0.05)
	100	100 ± 1.2 (25.2 ± 0.31)	100 ± 2.0 (25.2 ± 0.51)	49 ± 2.1 (12.4 ± 0.54)	4 ± 0.2 (1.0 ± 0.06)

[a] Values for mean percentage of the control ± standard error of the mean are based on control plates which contained no Cd. Values for mean area of growth ± standard error of the mean were calculated after 20 days (*H. lugdunensis*), 21 days (*T. marchalianum*), 36 days (*C. aquatica* and *F. curvula*), and 43 days (*A. acuminata*) of incubation.

TABLE 3. Influence of cadmium on growth of aquatic hyphomycetes on agar with various zinc concentrations

Fungus	Zn concn (ppm)	Mean ± SEM % of control (growth area [cm²]) with Cd concn (ppm) of[a]:			
		0	1	10	33
H. lugdunensis	0	100 ± 1.6 (43.0 ± 0.67)	99 ± 0.9 (42.7 ± 0.40)	85 ± 3.0 (36.6 ± 1.30)	57 ± 2.0 (24.6 ± 0.86)
	1	100 ± 0.9 (44.5 ± 0.41)	98 ± 0.9 (43.4 ± 0.40)	84 ± 0.9 (37.5 ± 0.38)	80 ± 2.1 (35.6 ± 0.92)
	10	100 ± 1.2 (45.8 ± 0.42)	99 ± 1.5 (45.4 ± 0.69)	93 ± 0.9 (42.5 ± 0.40)	81 ± 0.8 (37.1 ± 0.37)
	100	100 ± 1.6 (43.0 ± 0.67)	99 ± 2.3 (42.5 ± 1.00)	102 ± 0.9 (43.8 ± 0.40)	93 ± 0.9 (39.9 ± 0.39)
T. marchalianum	0	100 ± 1.1 (30.8 ± 0.34)	89 ± 1.8 (27.3 ± 0.54)	68 ± 1.8 (21.0 ± 0.56)	39 ± 1.1 (12.0 ± 0.35)
	1	100 ± 1.1 (30.8 ± 0.34)	90 ± 1.0 (27.7 ± 0.32)	89 ± 4.5 (27.3 ± 1.39)	54 ± 1.4 (16.6 ± 0.42)
	10	100 ± 1.2 (27.7 ± 0.32)	93 ± 1.1 (25.8 ± 0.31)	96 ± 1.2 (26.7 ± 0.32)	65 ± 2.7 (18.1 ± 0.74)
	100	100 ± 4.6 (22.3 ± 1.02)	92 ± 2.1 (20.4 ± 0.46)	95 ± 2.1 (21.2 ± 0.47)	86 ± 3.0 (19.1 ± 0.67)
C. aquatica	0	100 ± 4.0 (23.8 ± 0.99)	84 ± 2.3 (19.9 ± 0.54)	58 ± 1.6 (13.9 ± 0.38)	30 ± 2.9 (7.2 ± 0.69)
	1	100 ± 3.2 (30.2 ± 0.96)	96 ± 2.5 (28.9 ± 0.76)	60 ± 2.9 (18.1 ± 0.87)	29 ± 0.6 (8.7 ± 0.18)
	10	100 ± 1.9 (30.2 ± 0.56)	93 ± 1.1 (28.0 ± 0.32)	69 ± 0.9 (20.7 ± 0.28)	37 ± 2.0 (11.2 ± 0.99)
	100	100 ± 4.9 (29.2 ± 1.44	101 ± 2.8 (29.5 ± 0.83)	88 ± 1.1 (25.8 ± 0.31)	66 ± 3.9 (19.4 ± 1.13)
F. curvula	0	100 ± 3.0 (26.1 ± 0.78)	91 ± 1.9 (23.8 ± 0.50)	43 ± 0.8 (11.1 ± 0.21)	25 ± 1.0 (6.6 ± 0.26)
	1	100 ± 0.8 (25.5 ± 0.21)	99 ± 1.2 (25.3 ± 0.31)	57 ± 1.5 (14.5 ± 0.39)	37 ± 2.8 (9.3 ± 0.72)
	10	100 ± 2.6 (22.3 ± 0.58)	100 ± 1.3 (22.3 ± 0.29)	80 ± 2.9 (17.9 ± 0.65)	57 ± 0.9 (11.5 ± 0.21)
	100	100 ± 3.3 (21.0 ± 0.70)	101 ± 2.2 (21.2 ± 0.47)	101 ± 2.2 (21.2 ± 0.47)	81 ± 1.2 (17.1 ± 0.25)
A. acuminata	0	100 ± 1.3 (21.5 ± 0.28)	96 ± 1.3 (20.7 ± 0.28)	8 ± 0.4 (1.6 ± 0.08)	0 (0)
	1	100 ± 2.2 (21.2 ± 0.47)	95 ± 1.3 (20.2 ± 0.28)	8 ± 0.7 (1.8 ± 0.14)	0 (0)
	10	100 ± 1.4 (20.2 ± 0.28)	100 ± 1.4 (20.2 ± 0.28)	30 ± 0.7 (6.0 ± 0.15)	3 ± 0.3 (0.50 ± 0.07)
	100	100 ± 2.3 (19.4 ± 0.68)	101 ± 0.5 (19.6 ± 0.10)	81 ± 3.1 (15.7 ± 0.61)	12 ± 1.2 (2.4 ± 0.24)

[a] Values for mean percentage of the control ± standard error of the mean are based on control plates which contained no Cd. Values for mean area of growth ± standard error of the mean were calculated after 18 days (*H. lugdunensis*), 27 days (*T. marchalianum*), 36 days (*C. aquatica*), 41 days (*F. curvula*), and 44 days (*A. acuminata*) of incubation.

TABLE 4. Influence of cadmium on growth of aquatic hyphomycetes on agar with various EDTA concentrations

Fungus	EDTA concn (ppm)	Mean ± SEM % of control (growth area [cm^2]) with Cd concn (ppm) of[a]:			
		0	1	20	100
H. lugdunensis	0	100 ± 1.2 (28.6 ± 0.33)	101 ± 1.2 (28.9 ± 0.33)	70 ± 1.9 (19.9 ± 0.55)	16 ± 1.2 (4.7 ± 0.33)
	15	100 ± 1.1 (29.5 ± 0.33)	103 ± 1.2 (30.5 ± 0.34)	78 ± 1.7 (22.9 ± 0.49)	30 ± 1.3 (8.9 ± 0.37)
	45	100 ± 3.7 (29.5 ± 0.83)	103 ± 1.2 (30.6 ± 0.34)	84 ± 1.1 (24.9 ± 0.31)	30 ± 0.6 (8.9 ± 0.18)
	135	100 ± 0.8 (28.0 ± 0.22)	113 ± 1.2 (31.5 ± 0.34)	114 ± 1.2 (31.8 ± 0.35)	36 ± 1.2 (10.2 ± 0.33)
T. marchalianum	0	100 ± 5.5 (23.8 ± 1.30)	85 ± 1.2 (20.2 ± 0.28)	64 ± 3.4 (15.2 ± 0.80)	12. ± 1.7 (2.9 ± 0.40)
	15	100 ± 5.3 (22.9 ± 1.27)	90 ± 1.2 (21.5 ± 0.28)	65 ± 1.2 (15.4 ± 0.28)	13 ± 2.2 (3.0 ± 0.52)
	45	100 ± 8.3 (21.8 ± 1.82)	104 ± 1.3 (22.6 ± 0.29)	89 ± 3.1 (19.4 ± 0.68)	22 ± 1.5 (4.7 ± 0.33)
	135	100 ± 3.4 (9.1 ± 0.31)	90 ± 2.0 (8.2 ± 0.18)	255 ± 3.3 (23.2 ± 0.30)	81 ± 4.6 (7.4 ± 0.42)
C. aquatica	0	100 ± 2.9 (28.0 ± 0.81)	88 ± 3.1 (24.6 ± 0.86)	59 ± 0.9 (16.4 ± 0.25)	19 ± 1.0 (5.4 ± 0.27)
	15	100 ± 2.4 (27.0 ± 0.64)	88 ± 1.9 (23.8 ± 0.50)	75 ± 1.0 (20.2 ± 0.28)	38 ± 1.2 (10.2 ± 0.33)
	45	100 ± 2.0 (27.3 ± 0.54)	98 ± 4.5 (26.7 ± 1.22)	78 ± 1.7 (21.2 ± 0.47)	39 ± 1.8 (10.6 ± 0.50)
	135	100 ± 4.2 (23.8 ± 0.99)	84 ± 5.9 (19.9 ± 1.41)	118 ± 1.3 (28.0 ± 0.32)	47 ± 4.6 (11.1 ± 1.09)
F. curvula	0	100 ± 5.1 (9.3 ± 0.47)	100 ± 7.0 (9.3 ± 0.65)	26 ± 1.1 (2.5 ± 0.10)	7 ± 0.5 (0.7 ± 0.05)
	15	100 ± 3.5 (8.6 ± 0.30)	123 ± 4.7 (10.6 ± 0.40)	41 ± 3.7 (3.5 ± 0.32)	15 ± 0.8 (1.3 ± 0.07)
	45	100 ± 2.5 (5.9 ± 0.15)	112 ± 8.8 (6.6 ± 0.52)	90 ± 4.1 (5.3 ± 0.24)	22 ± 1.2 (1.3 ± 0.07)
	135	100 ± 8.1 (2.4 ± 0.19)	86 ± 10.6 (2.0 ± 0.25)	238 ± 15.3 (5.6 ± 0.36)	68 ± 3.4 (1.6 ± 0.08)
A. acuminata	0	100 ± 3.4 (12.8 ± 0.44)	94 ± 2.7 (12.0 ± 0.35)	10 ± 1.4 (1.4 ± 0.18)	0 (0)
	15	100 ± 6.4 (13.4 ± 0.86)	100 ± 1.6 (12.8 ± 0.22)	20 ± 1.5 (2.7 ± 0.20)	9 ± 2.7 (1.2 ± 0.36)
	45	100 ± 1.8 (12.4 ± 0.22)	103 ± 1.8 (12.8 ± 0.22)	29 ± 3.9 (3.6 ± 0.48)	11 ± 1.5 (1.4 ± 0.18)
	135	100 ± 3.8 (2.9 ± 0.11)	86 ± 5.5 (2.5 ± 0.16)	403 ± 18.3 (11.7 ± 0.53)	66 ± 6.2 (1.9 ± 0.18)

[a] Values for mean percentage of the control ± standard error of the mean are based on control plates which contained no Cd. Values for mean area of growth ± standard error of the mean were calculated after 16 days (*H. lugdunensis*), 21 days (*T. marchalianum*), 30 days (*F. curvula*), 38 days (*A. acuminata*), and 44 days (*C. aquatica*) of incubation.

interactions between Cd and Ca ions, although it seems likely that they are based on mechanisms similar to that between Zn and Cd.

In stream water, naturally occurring organic ligands (e.g., humic acids and phenols) might also interact with cadmium (11). EDTA has high complex formation constants with several cations. It is not a ligand specific for Cd, and it forms complexes with ions essential for fungal growth. This explains its toxicity towards fungi observed in this study.

Baccini and Suter (5) have shown that the toxicity of heavy metals depends on their chemical speciation. The main toxic CD species in freshwater are $Cd(aq)^{2+}$ ions. Cadmium-EDTA complexes are biologically unavailable for the fungi and are therefore nontoxic. Cadmium toxicity is caused by remaining free $Cd(aq)^{2+}$ ions (15, 18). At very high Cd concentrations, the formation of cadmium carbonate may also reduce the effect of the heavy metal.

In summary, the lower sensitivity of fungi sporulating in hard Lüssel water was almost certainly due to its higher Ca content. Calcium ions presumably compete with Cd for binding sites at enzymes or other proteins (e.g., carriers). In this way, they may reduce the irreversible damage due to Cd-protein complexing. Although the content of organic ligands was higher in the Ibach fungi, concentrations were too low to have any effect at high Cd concentrations.

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