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Toxicity of Zinc to Fungi, Bacteria, and Coliphages: Influence of Chloride Ions

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A 10 mM concentration of Zn^{2+} decreased the survival of *Escherichia coli*; enhanced the survival of *Bacillus cereus*; did not significantly affect the survival of *Pseudomonas aeruginosa, Nocardia corallina,* and T1, T7, P1, and φ80 coliphages; completely inhibited mycelial growth of *Rhizoctonia solani*; and reduced mycelial growth of *Fusarium solani, Cunninghamella echinulata, Aspergillus niger,* and *Trichoderma viride.* The toxicity of zinc to the fungi, bacteria, and coliphages was unaffected, lessened, or increased by the addition of high concentrations of NaCl. The increased toxicity of zinc in the presence of high concentrations of NaCl was not a result of a synergistic interaction between Zn^{2+} and elevated osmotic pressures but of the formation of complex anionic Zn-Cl species that exerted greater toxicities than did cationic Zn^{2+}. Conversely, the decrease in zinc toxicity with increasing concentrations of NaCl probably reflected the decrease in the levels of Zn^{2+} due to the formation of Zn-Cl species, which was less inhibitory to these microbes than was Zn^{2+}. *A. niger* tolerated higher concentrations of zinc in the presence of NaCl at 37 than at 25°C.

Anthropogenic pollutants emitted by industrial and domestic activities and deposited into the environment may have an adverse impact on the activity, ecology, and population dynamics of the indigenous microbiota (e.g., see reference 5). The influence of the physicochemical factors of the environment into which a pollutant is deposited on the toxicity of that pollutant to the microbiota has often been neglected in environmental studies (9). For example, the toxicity of cadmium towards bacteria and fungi was reduced in the presence of the clay mineral montmorillonite (7, 8) but was potentiated at alkaline pH values, presumably as a result of the formation of the hydroxylated species $CdOH^+$ which was more toxic than divalent Cd^{2+} (6, 8, 9).

The studies reported herein further demonstrate that the anionic composition of the environment influences the toxicity of heavy metals (i.e., zinc) to microbes and viruses. As a model system, Zn^{2+} and Cl^- ions were selected as the cationic heavy metal and anion, respectively. Several studies have demonstrated interactions between Zn^{2+} and other divalent cations (e.g., Cd^{2+}, Mg^{2+}, Cu^{2+}) and their combined effects on bacteria (1, 43), fungi (2, 35), and unicellular algae (11). However, there is little information on whether and how the inorganic anionic components of the environment influence the toxicity of divalent cations.

Zinc is a micronutrient and is essential for maximal growth of microbes and other cells. Zinc is a component or activator of some enzymes (e.g., alcohol dehydrogenase, aldolase [55], DNA-dependent RNA polymerase of T7 coliphage [19], RNA-dependent DNA polymerase of avian myeloblastosis virus [4]); maintains the integrity of ribosomes (44), biological membranes, the double helix of DNA (18), and the cell wall of gram-negative bacteria (20); is required for growth and subtilin production by *Bacillus subtilis* (21); is a component of the cell wall of *Escherichia coli* and is involved in the penetration of T2 and T4 coliphages into the host cells (33); and is required for growth of *Helminthosporium sativum* (42) and *Verticillium albo-atrum* (50), for aflatoxin production by *Aspergillus parasiticus* (37), and for sclerotia production by *Whetzelinia sclerotiorum* (53).

However, elevated concentrations of Zn may be inhibitory or toxic to cellular activities and growth, with the specific inhibitory concentration being dependent on the activity or cell being investigated. For example, zinc chloride ($ZnCl_2$) at 0.08 mM stimulated and at 0.5 mM inhibited growth of *E. coli* (57). Zinc has been shown to inhibit respiration (39) and growth (48) of fungi, germination of fungal spores (49), growth of unicellular algae (11) and protozoans (15, 47), bacterial conjugation (40), nitrification in soil (56), and replication of rhinoviruses (32) and to block adsorption of M13 coliphage to its host bacterium (52).

Concentrations of Zn above ambient background levels in the environment result primarily from anthropogenic activities. High concentrations of Zn were present in fresh waters receiving effluents from strip mines (41); in sediments from marine coastal regions receiving discharges from domestic sewers (12); and in soils near highways (36), coal-burning power plants (31), and smelters. For example, some soils within 9.7 km from a smelter in British Columbia contained 1,394 μg of Zn per g (27), and some soils within 1 km of a smelter complex at Palmerton, Pa., contained 135,000 μg of Zn per g (13). Soils surrounding industrial and smelter complexes that emit metal oxides, including those of Zn, exhibited reduced rates of organic matter decomposition (46), soil respiration (17), and phosphorus mineralization (51), as well as reductions in the numbers of bacteria, including actinomycetes, fungi (28), and lichens (38). Deposition of heavy metals, including Zn, on the surfaces of leaves resulted in an alteration in the composition and species diversity of the phylloplane microbiota (23).

Once a heavy metal is deposited into a specific environment, its chemical form, mobility, and availability for uptake by the biota are dependent on the physicochemical factors of that environment. For example, Zn^{2+} complexes with Cl^- and, depending on the Cl^- concentration, forms $ZnCl^+$, $ZnCl_2$, $ZnCl_3^-$, and $ZnCl_4^{2-}$ (25). Divalent Zn^{2+} and these Zn-Cl species may exert differing toxicities on the microbiota.

In soil, Cl^- is mobile, and its concentration is dependent on the porosity of the soil and the extent of leaching. Additional amounts of Cl^- may be introduced into soil by the application of salt (primarily a mixture of 95% NaCl and 5% $CaCl_2$) to remove ice and snow from roadways. Salt, mainly as $CaCl_2$, is also used as a dust palliative on unpaved roads (54). In saline and saline-sodic soils, the Cl^- concentration of the soil solution ranges from 42,600 to 103,000 μg/g, and the Cl^- concentration in seawater is about 20,000 μg/ml (25).

MATERIALS AND METHODS

Source and maintenance of microorganisms and viruses. Microorganisms and coliphages were obtained from the culture collection of the Laboratory of Microbial Ecology at New York University. Bacteria were grown and maintained on nutrient agar (Difco) amended with 1% glucose: *E. coli* and *Pseudomonas aeruginosa* were grown at 37°C, and *Bacillus cereus* and *Nocardia corallina* were grown at 25°C. *Cunninghamella echinulata, Aspergillus niger, Rhizoctonia solani, Fusarium solani,* and *Trichoderma viride* were grown and maintained on Sabouraud dextrose agar (Difco or Scott Laboratories) at 25°C. Purified T1, T7, P1, and φ80 bacteriophages of *E. coli* were prepared as described elsewhere (10).

Description of experiments. (i) Fungi. Fungi were grown on petri dishes containing approximately 15 ml of Sabouraud dextrose agar (pH 5.6). After incubation for several days at 25°C, circular fungal plugs (3 to 5 mm in diameter), made with a sterilized metal cork borer, were transferred, with the fungal growth up, to the center of new dishes containing the same agar unamended or amended with 0.01, 0.1, 1, or 10 mM $ZnSO_4 \cdot 7H_2O$. After at least 1 day of equilibration and after various periods of incubation at 25°C, the diameters of mycelial growth, in four directions, and the radial growth rates (in millimeters per day) were determined (6). Mycelial growth was measured on days 2 and 3 after inoculation for *T. viride* and *C. echinulata,* on days 2, 3, and/or 4 for *A. niger,* and on days 3 and 4 for *R. solani* and *F. solani*; however, longer time intervals were required for measurement of growth on agar containing inhibitory concentrations of Zn^{2+}. Growth rates were also determined for fungi on agar amended with 0, 1, or 10 mM Zn^{2+} and 0, 0.1, 0.5, or 1 M NaCl, to provide different ratios of the Zn-Cl species $ZnCl^+$, $ZnCl_2$, $ZnCl_3^-$, and $ZnCl_4^{2-}$ (25). Similar studies with some Zn-NaCl concentrations were also conducted at 37°C. To differentiate between the effects of a possible synergistic interaction between Zn^{2+} and Cl^- or between Zn^{2+} and elevated osmotic pressures, 0.5 M NaCl or $NaNO_3$ or 1 M glucose or xylose (to give theoretically equivalent osmotic pressures [22]) were added to agars with and without Zn^{2+}. Three petri dishes were used for each concentration of Zn^{2+} and all other permutations, and each experiment was repeated at least once.

(ii) Bacteria. Preliminary studies demonstrated that growth of representative bacteria was unaffected in agar medium supplemented with up to 1 mM Zn^{2+} and was reduced at 10 mM Zn^{2+}. Thus, a concentration of 10 mM Zn^{2+} was employed to study the influence of Zn^{2+} in the absence and presence of NaCl on the survival of bacteria in a non-nutrient medium.

Bacteria were grown for approximately 18 h at 25 or 37°C in nutrient broth (Difco) amended with 1% glucose. The cells were then washed twice with phthalate buffer (hydrogen phthalate-sodium hydroxide, pH 6), resuspended in phthalate buffer, and refrigerated at 4°C. All experiments were performed at pH 6 to avoid the formation of hydroxylated Zn species (25).

Portions (0.2 ml) of the bacterial suspensions were inoculated into tubes (13 by 100 mm) containing 1.8 ml of phthalate buffer amended with 10 mM $ZnSO_4 \cdot 7H_2O$ or $MgSO_4 \cdot 7H_2O$ and with either 0, 0.1, 0.5, 1, or 5 M NaCl to yield a total volume of 2.0 ml. Divalent Mg^{2+} served as the control because it does not form complex Cl^--containing species. The tubes were agitated on a Vortex-Genie for 5 s and placed in a rotating drum (36 rpm) at 25°C for 3 h; portions were removed; 0.1 ml of serial dilutions in tris(hydroxymethyl)aminomethane (Tris) buffer (1 mM Tris–1 mM $MgSO_4 \cdot 7H_2O$–1 mM NaCl, adjusted to pH 7 with HCl) was inoculated into petri dishes; 8 ml of molten nutrient agar (1% glucose–0.8% nutrient broth–0.7% agar [Bacto; Difco]) was added; the dishes were inverted and incubated at 25 or 37°C; and bacterial colonies were counted after 2 to 4 days. The small amount (i.e., 8 ml) of this soft agar was em-

ployed, because preliminary studies showed that greater quantities of medium or a larger percentage of agar delayed colony development. Two tubes were used for each concentration of NaCl in combination with Mg^{2+} or Zn^{2+}, and each experiment was repeated at least twice.

In other experiments, 0.2-ml portions of a suspension of *E. coli* were inoculated into tubes containing 1.8 ml of phthalate buffer amended with 10 mM Zn^{2+} or Mg^{2+} and either 1 M NaCl, 1 M $NaNO_3$, 2 M glucose, or 2 M xylose. Survival of the bacteria was determined as described above. Two tubes were used for each combination, and the experiments were repeated twice.

(iii) Coliphages. Portions (0.2 ml) of the coliphage suspensions were inoculated into tubes containing 1.8 ml of phthalate buffer amended with 10 mM Mg^{2+} or Zn^{2+} and with either 0, 0.1, 0.5, 1, or 5 M NaCl. The tubes were agitated on a Vortex-Genie for 5 s and then placed in a rotating drum (36 rpm), housed in a 25°C incubator, for 3 h. Portions (0.5 ml) were then removed and serial dilutions were performed in Tris buffer (pH 7); 0.1-ml portions were inoculated into 4.5 ml of an overlay agar medium (1% tryptone–0.5% yeast extract–0.5% NaCl–0.7% agar [Bacto]), which was then seeded with 0.4 ml of *E. coli* strain B. The underlay agar (10 ml) consisted of nutrient agar containing 1% glucose. The plates were inverted and incubated at 37°C, and plaques were counted after 18 h. Two tubes were used for each concentration of NaCl with either Zn^{2+} or Mg^{2+}, and each experiment was repeated at least three times.

The data were calculated as the arithmetic mean ± standard error of the mean, and Student's *t* test was performed to determine the significance of the variation between control and experimental mean values; *P* of 0.05 or less were considered significant.

RESULTS

Fungi. Concentrations of Zn^{2+} up to 1 mM did not significantly inhibit the growth of fungi. However, 10 mM Zn^{2+} significantly decreased the mycelial growth rates of *F. solani, C. echinulata, A. niger,* and *T. viride* and completely inhibited growth of *R. solani* (Table 1).

In both the absence and presence of Zn^{2+}, growth rates of *A. niger* were greater at 37 than at 25°C, and more abundant sporulation was noted in cultures incubated at 37°C. At both 25 and 37°C, concentrations of Zn^{2+} up to 1 mM did not significantly decrease growth rates, but, at 10 mM, rates were reduced to approximately 50% of the control (i.e., no Zn^{2+}) at both temperatures (Table 2).

The effect of different concentrations of NaCl on the toxicity of Zn to mycelial growth was variable. In the absence of Zn^{2+}, 0.5 M NaCl greatly reduced and 1 M NaCl completely inhibited growth of *R. solani.* In the absence of NaCl or with 0.1 M NaCl, a concentration of 1 mM Zn^{2+} did not significantly influence growth. However, with 0.5 M NaCl, growth of *R. solani* was greater in the presence of 1 mM Zn^{2+} than

TABLE 2. *Effect of temperature on the toxicity of zinc to A. niger*

Zn concn[a] (mM)	Growth rate	
	25°C	37°C
None	9.6 ± 0.56[b]	10.6 ± 0.51
	(100 ± 5.9)[c]	(100 ± 4.8)
0.01	9.3 ± 0.57	11.2 ± 0.46
	(100 ± 5.3)	(105 ± 2.0)
0.1	9.2 ± 0.55	10.4 ± 0.49
	(96 ± 1.4)	(93 ± 2.3)
1	8.9 ± 0.63	10.4 ± 0.52
	(92 ± 1.8)	(98 ± 1.7)
10	4.1 ± 0.23[d]	5.6 ± 0.64[d]
	(44 ± 1.2)	(51 ± 4.1)

[a] Zn, as $ZnSO_4 \cdot 7H_2O$, added to Sabouraud dextrose agar (Difco).
[b] Mean radial growth, in millimeters per day, ± standard error of the mean.
[c] Mean percent of control ± standard error of the mean.
[d] Significant difference at $P = 0.001$.

TABLE 1. *Effect of zinc on growth of fungi*

Zn concn[a] (mM)	Growth rate				
	R. solani	*F. solani*	*C. echinulata*	*A. niger*	*T. viride*
None	7.8 ± 0.34[b]	4.6 ± 0.13	10.0 ± 0.08	9.6 ± 0.56	16.0 ± 0.39
	(100 ± 4.3)[c]	(100 ± 2.8)	(100 ± 0.7)	(100 ± 5.9)	(100 ± 2.4)
0.01	7.8 ± 0.32	4.7 ± 0.14	9.7 ± 0.23	9.3 ± 0.57	15.9 ± 0.16
	(100 ± 4.1)	(103 ± 3.0)	(97 ± 2.3)	(100 ± 5.3)	(99 ± 1.0)
0.1	7.7 ± 0.28	4.6 ± 0.20	9.8 ± 0.11	9.2 ± 0.55	16.0 ± 0.36
	(99 ± 3.6)	(100 ± 4.5)	(98 ± 1.1)	(96 ± 1.4)	(100 ± 2.2)
1	7.1 ± 0.19	4.4 ± 0.20	10.3 ± 0.30	8.9 ± 0.63	17.6 ± 4.90
	(91 ± 2.5)	(96 ± 4.5)	(103 ± 3.0)	(92 ± 1.8)	(110 ± 3.0)
10	0[d]	0.6 ± 0.12[d]	2.1 ± 0.17[d]	4.1 ± 0.23[d]	7.2 ± 0.39[d]
	(0)	(13 ± 2.6)	(21 ± 1.6)	(44 ± 1.2)	(45 ± 2.5)

[a] Zn, as $ZnSO_4 \cdot 7H_2O$, added to Sabouraud dextrose agar (Difco).
[b] Mean radial growth, in millimeters per day, ± standard error of the mean.
[c] Mean percent of control ± standard error of the mean.
[d] Significant difference at $P = 0.001$.

in the absence of Zn^{2+} (Table 3).

In the absence of Zn^{2+}, growth of *F. solani* was initially reduced at 0.1 M NaCl and progressively decreased as the concentration of NaCl was increased to 1 M. These concentrations of NaCl did not significantly alter the response of the fungus to 1 mM Zn^{2+} added to the various concentrations of NaCl (Table 3).

The growth of *A. niger*, in the absence of Zn^{2+}, was initially reduced by 0.5 M NaCl, with greater reductions occurring at 1 M NaCl. In the absence of NaCl, 1 mM Zn^{2+} did not adversely influence growth, but, in the presence of 0.5 or 1 M NaCl, *A. niger* was progressively sensitive to Zn. At 10 mM Zn^{2+}, growth rates were decreased and, as the concentration of NaCl increased, the toxicity of Zn increased until growth was totally inhibited at 1 M NaCl (Table 3).

Although 0.1 M NaCl slightly enhanced the growth of *C. echinulata* in the absence of Zn, higher concentrations of NaCl reduced growth. The extent of inhibition by 10 mM Zn^{2+} was not affected by concentrations of NaCl to 1 M. For example, at 0 or 1 M NaCl, a 10 mM concentration of Zn^{2+} reduced growth to approximately 20% of the controls (i.e., no Zn + no NaCl and no Zn + 1 M NaCl, respectively) (Table 3).

In the absence of Zn^{2+}, growth of *T. viride* was initially reduced at 0.5 M NaCl, with a greater reduction occurring at 1 M NaCl. In the absence of NaCl, 10 mM Zn^{2+} significantly reduced growth, but the tolerance to Zn increased as the concentration of NaCl increased. For example, in the absence of NaCl, 10 mM Zn^{2+} reduced growth to 36% of the control (i.e., no Zn + no NaCl), whereas in the presence of 1 M NaCl, an equivalent concentration of Zn reduced growth only to 72% of the control (i.e., no Zn + 1 M NaCl) (Table 3).

The influence of temperature on the sensitivity of *A. niger* to varying concentrations of Zn in the absence and presence of 0.5 M NaCl was also studied. At 25°C, mycelial growth was initially reduced at a concentration of 0.1 mM Zn^{2+} in the presence of 0.5 M NaCl, whereas in the absence of NaCl toxicity was initially evident only at 10 mM Zn^{2+}. At 37°C, reductions in growth were initially evident at 10 mM Zn^{2+}, regardless of the absence or presence of NaCl, although toxicity was significantly greater in the presence of NaCl (Table 4).

Bacteria. The influence of increasing concentrations of NaCl on the survival of bacteria in phthalate buffer after a 3-h exposure to 10 mM Zn^{2+} or Mg^{2+} was studied. Because Mg^{2+} does not form complex coordination complexes with Cl^-, differences in bacterial survival with increasing concentrations of NaCl in the presence of Mg^{2+} probably reflected the influence of increased osmotic pressure.

In the presence of Mg^{2+}, the addition of 0.1 M NaCl reduced survival of *E. coli* to about 15% of the control (i.e., Mg^{2+} + no NaCl), and at higher concentrations of NaCl, survival was reduced to 2% or less of the control. In the absence of NaCl, 10 mM Zn^{2+} decreased the survival of *E. coli* to about 20% of the control (i.e., Mg^{2+} + no NaCl); however, in the presence of 0.1 M NaCl, the

TABLE 3. *Effect of different concentrations of sodium chloride on the toxicity of zinc to growth of fungi*

Treatment[a]	Growth rate			Treatment	Growth rate		
	R. solani	*F. solani*	*A. niger*		*A. niger*	*C. echinulata*	*T. viride*
No NaCl, no Zn	7.6 ± 0.31[b]	5.0 ± 0.25	9.3 ± 0.19	No NaCl, no Zn	9.3 ± 0.19	9.4 ± 0.29	14.6 ± 0.70
No NaCl, 1 mM Zn	6.9 ± 0.31 (90 ± 4.1)[c]	4.3 ± 0.36 (85 ± 4.6)	9.9 ± 0.08 (106 ± 0.8)	No NaCl, 10 mM Zn	5.1 ± 0.24[d] (54 ± 2.5)	1.8 ± 0.19[d] (20 ± 2.0)	5.3 ± 0.52[d] (36 ± 3.6)
0.1 M NaCl, no Zn	6.1 ± 0.36	3.8 ± 0.36	9.7 ± 0.09	0.1 M NaCl, no Zn	9.7 ± 0.09	10.2 ± 0.21	14.4 ± 0.77
0.1 M NaCl, 1 mM Zn	6.1 ± 0.25 (100 ± 4.1)	3.7 ± 0.46 (97 ± 3.2)	9.6 ± 0.12 (98 ± 1.2)	0.1 M NaCl, 10 mM Zn	3.3 ± 0.73[d] (34 ± 7.5)	1.5 ± 0.25[d] (15 ± 2.4)	7.2 ± 0.90[d] (50 ± 6.3)
0.5 M NaCl, no Zn	0.8 ± 0.15	2.5 ± 0.10	8.4 ± 0.09	0.5 M NaCl, no Zn	8.4 ± 0.09	7.7 ± 0.40	9.8 ± 0.49
0.5 M NaCl, 1 mM Zn	1.9 ± 0.06[d] (231 ± 6.9)	2.4 ± 0.26 (96 ± 7.6)	6.9 ± 0.11[d] (82 ± 1.4)	0.5 M NaCl, 10 mM Zn	0.3 ± 0.16[d] (5 ± 2.7)	1.0 ± 0.12[d] (13 ± 1.6)	6.6 ± 0.57[d] (68 ± 5.9)
1 M NaCl, no Zn	0	1.9 ± 0.23	6.2 ± 0.15	1 M NaCl, no Zn	6.2 ± 0.15	3.1 ± 0.15	5.0 ± 0.30
1 M NaCl, 1 mM Zn	0 (0)	1.5 ± 0.20 (79 ± 4.3)	3.8 ± 0.12[d] (61 ± 1.9)	1 M NaCl, 10 mM Zn	0[d] (0)	0.6 ± 0.08[d] (18 ± 3.0)	3.6 ± 0.35[e] (72 ± 7.0)

[a] Zn, as $ZnSO_4 \cdot 7H_2O$, and NaCl added to Sabouraud dextrose agar (Difco).
[b] Mean radial growth, in millimeters per day, ± standard error of the mean.
[c] Mean percent of control ± standard error of the mean (control = no Zn at equivalent NaCl concentration).
[d] Significant difference at $P = 0.001$.
[e] Significant difference at $P = 0.01$.

toxicity of Zn was lessened, as survival was 60% of the control (i.e., Mg^{2+} + 0.1 M NaCl). Above 0.1 M NaCl, the toxicity of Zn increased, with survival being 20, 2, and 0% in the presence of 0.5, 1, and 5 M NaCl, respectively (Table 5).

In the presence of Mg^{2+}, *P. aeruginosa* showed no significant decreases in survival with additions of NaCl up to 1 M, but survival was greatly reduced at 5 M NaCl. A concentration of 10 mM Zn^{2+} was not toxic in the presence of 0, 0.1, 0.5, or 1 M NaCl, but, in the presence of 5 M NaCl, Zn toxicity was enhanced, and survival was only 13% of the control (i.e., Mg^{2+} + 5 M NaCl) (Table 5).

Survival of *B. cereus* at 10 mM Zn^{2+} was progressively increased by additions of NaCl to 0.5 M, but, thereafter, was progressively decreased as the NaCl concentration was increased to 5 M. Compared with the control (i.e., Mg^{2+} + no NaCl), 10 mM Zn^{2+} enhanced survival of *B. cereus*; however, 0.1 M NaCl decreased this enhancement, and, at higher NaCl concentrations, survival in the presence of Zn was approximately equivalent to that with Mg^{2+} (Table 5).

N. corallina was not sensitive to 10 mM Zn^{2+} either in the absence or presence of NaCl (Table 5).

Coliphages. The influence of increasing con-

TABLE 4. *Influence of temperature on the toxicity of zinc in the absence and presence of sodium chloride on growth of A. niger*

Zn concn[a] (mM)	25°C		37°C	
	No NaCl	0.5 M NaCl	No NaCl	0.5 M NaCl
None	7.9 ± 0.35[b]	6.3 ± 0.07	9.0 ± 0.35	4.3 ± 0.08
	(100 ± 4.4)[c]	(100 ± 1.1)	(100 ± 3.9)	(100 ± 1.9)
0.01	7.5 ± 0.23	5.7 ± 0.47	9.6 ± 0.25	4.0 ± 0.12
	(96 ± 2.4)	(91 ± 7.5)	(106 ± 2.2)	(93 ± 4.4)
0.1	7.5 ± 0.20	5.7 ± 0.23[d]	8.9 ± 0.15	4.1 ± 0.27
	(96 ± 2.8)	(90 ± 3.6)	(99 ± 2.5)	(97 ± 4.5)
1	7.1 ± 0.10	4.8 ± 0.16[e]	8.7 ± 0.15	3.9 ± 0.26
	(91 ± 3.6)	(76 ± 2.5)	(97 ± 2.8)	(94 ± 5.2)
10	3.5 ± 0.70[e]	0[e]	4.6 ± 0.11[e]	0.2 ± 0.03[e]
	(44 ± 1.9)	(0)	(51 ± 3.1)	(4 ± 0.1)

[a] Zn, as $ZnSO_4 \cdot 7H_2O$, added to Sabouraud dextrose agar (Scott).
[b] Mean radial growth, in millimeters per day, ± standard error of the mean.
[c] Mean percent of control ± standard error of the mean (control = no Zn at an equivalent NaCl concentration and temperature).
[d] Significant difference at $P = 0.05$.
[e] Significant difference at $P = 0.001$.

TABLE 5. *Effect of different concentrations of sodium chloride on the toxicity of zinc to bacteria*

Treatment[a]	Colony-forming units (× 10^{-1}) per 0.1 ml[b]			
	E. coli	*P. aeruginosa*	*B. cereus*	*N. corallina*
10 mM Mg, no NaCl	35.53 ± 1.919	2.86 ± 0.405	13.13 ± 2.164	37.00 ± 3.055
10 mM Zn, no NaCl	7.22 ± 0.964[c]	2.77 ± 0.297	58.13 ± 0.125[c]	39.00 ± 3.512
	(20 ± 2.7)[d]	(98 ± 4.2)	(490 ± 9.8)	(106 ± 9.0)
10 mM Mg, 0.1 M NaCl	4.95 ± 0.444	2.78 ± 0.265	26.00 ± 3.201	31.00 ± 3.500
10 mM Zn, 0.1 M NaCl	2.99 ± 0.641[e]	3.11 ± 0.333	48.38 ± 6.728[e]	34.33 ± 1.965
	(60 ± 9.5)	(112 ± 4.8)	(188 ± 20.7)	(112 ± 6.7)
10 mM Mg, 0.5 M NaCl	0.61 ± 0.223	2.55 ± 0.240	45.25 ± 6.537	40.83 ± 2.186
10 mM Zn, 0.5 M NaCl	0.08 ± 0.025[e]	2.34 ± 0.326	57.00 ± 2.102	40.67 ± 2.728
	(20 ± 5.6)	(91 ± 6.2)	(134 ± 19.5)	(100 ± 6.4)
10 mM Mg, 1 M NaCl	0.66 ± 0.142	2.14 ± 0.210	16.28 ± 2.571	27.00 ± 3.691
10 mM Zn, 1 M NaCl	0.01 ± 0.007[e]	2.21 ± 0.256	17.48 ± 2.208	25.13 ± 3.561
	(2 ± 1.7)	(103 ± 5.8)	(112 ± 16.0)	(93 ± 5.6)
10 mM Mg, 5 M NaCl	0.02 ± 0.006	0.56 ± 0.108	0.46 ± 0.054	34.83 ± 2.455
10 mM Zn, 5 M NaCl	0	0.08 ± 0.004[f]	0.38 ± 0.056	36.00 ± 0.764
	(0)	(13 ± 4.7)	(83 ± 9.6)	(104 ± 7.1)

[a] Mg added as $MgSO_4 \cdot 7H_2O$; Zn added as $ZnSO_4 \cdot 7H_2O$.
[b] Mean bacterial titer ± standard error of the mean after 3 h of incubation.
[c] Significant difference at $P = 0.001$.
[d] Mean percent of control ± standard error of the mean (control = Mg at an equivalent NaCl concentration).
[e] Significant difference at $P = 0.05$.
[f] Significant difference at $P = 0.01$.

centrations of NaCl on the toxicity of Zn to coliphages was also studied, with Mg^{2+} again serving as the control. In the presence of 10 mM Mg^{2+}, increasing the concentration of NaCl to 5 M did not significantly affect survival of T1, T7, and P1, but survival of φ80 was slightly enhanced at 5 M NaCl. As compared with the controls (i.e., Mg^{2+} + equivalent NaCl concentrations), 10 mM Zn^{2+} in the presence of 0, 0.1, and 0.5 M NaCl did not significantly reduce survival of the coliphages, but at higher NaCl concentrations Zn was toxic (Table 6).

Mechanism. Experiments were performed to determine whether the enhanced toxicity of Zn in the presence of NaCl was the result of a synergistic effect between Zn^{2+} and the elevated osmotic pressures or of the formation of the various Zn-Cl species ($ZnCl^+$, $ZnCl_2$, $ZnCl_3^-$, $ZnCl_4^{2-}$) that may exert greater toxicities than divalent Zn^{2+}. *A. niger* and *E. coli* were selected as the test organisms, as previous studies had demonstrated that the toxicity of Zn towards these microbes was enhanced by high NaCl concentrations; as a control, experiments were also performed with *C. echinulata,* the tolerance of which to Zn was independent of the NaCl concentration (Tables 3 and 5). The organisms were exposed to the inhibitory concentration of Zn in the presence of NaCl, $NaNO_3$, glucose, or xylose.

In the absence of Zn, NaCl and $NaNO_3$ were more inhibitory towards growth of *A. niger* and *C. echinulata* (Table 7) and decreased survival of *E. coli* to a greater extent (Table 8) than did glucose and xylose at equivalent theoretical osmotic pressures. These differences in microbial response may have resulted either from differential sensitivities to high levels of these solutes or from utilization of the sugars as a source of

TABLE 7. *Influence of different salts and sugars at equivalent calculated osmotic pressures on the toxicity of zinc to growth of fungi*

Treatment[a]	Growth rate	
	A. niger	*C. echinulata*
0.5 M NaCl, no Zn	6.6 ± 0.17[b]	7.2 ± 0.17
	(100 ± 2.5)[c]	(100 ± 2.4)
0.5 M NaCl, 10 mM Zn	0[d]	0.6 ± 0.02[d]
	(0)	(8 ± 0.3)
0.5 M $NaNO_3$, no Zn	4.0 ± 0.17	4.3 ± 0.26
	(100 ± 5.6)	(100 ± 5.9)
0.5 M $NaNO_3$, 10 mM Zn	0[d]	0.4 ± 0.09[d]
	(0)	(9 ± 1.9)
1 M glucose, no Zn	8.2 ± 0.31	6.5 ± 0.15
	(100 ± 3.7)	(100 ± 2.3)
1 M glucose, 10 mM Zn	1.5 ± 0.11[d]	0.5 ± 0.07[d]
	(18 ± 1.3)	(4 ± 1.0)
1 M xylose, no Zn	9.1 ± 0.23	4.8 ± 0.13
	(100 ± 2.5)	(100 ± 2.7)
1 M xylose, 10 mM Zn	2.6 ± 0.3[d]	0.7 ± 0.08[d]
	(28 ± 2.9)	(14 ± 1.7)

[a] Zn, as $ZnSO_4 \cdot 7H_2O$, added to Sabouraud dextrose agar (Scott).
[b] Mean radial growth, in millimeters per day, ± standard error of the mean.
[c] Mean percent of control ± standard error of the mean (control = no Zn + equivalent molar concentration of osmotic agent).
[d] Significant difference at $P = 0.001$.

TABLE 6. *Effect of different concentrations of sodium chloride on the toxicity of zinc to coliphages*

Treatment[a]	Plaque-forming units (×10⁶) per 0.1 ml[b]			
	T1	T7	φ80	P1
10 mM Mg, no NaCl	4.80 ± 0.338	0.96 ± 0.201	0.76 ± 0.150	3.61 ± 0.267
10 mM Zn, no NaCl	5.17 ± 0.695	0.79 ± 0.203	0.77 ± 0.128	3.19 ± 0.195
	(107 ± 9.9)[c]	(82 ± 4.4)	(104 ± 6.3)	(89 ± 3.4)
10 mM Mg, 0.1 M NaCl	5.05 ± 0.791	1.03 ± 0.135	0.79 ± 0.127	4.35 ± 0.470
10 mM Zn, 0.1 M NaCl	4.51 ± 0.753	0.98 ± 0.130	0.77 ± 0.106	3.71 ± 0.350
	(89 ± 3.3)	(95 ± 2.7)	(99 ± 3.6)	(88 ± 11.6)
10 mM Mg, 0.5 M NaCl	4.83 ± 0.451	1.38 ± 0.324	0.83 ± 0.093	4.10 ± 0.194
10 mM Zn, 0.5 M NaCl	4.57 ± 0.321	1.09 ± 0.271	0.08 ± 0.083	3.66 ± 0.286
	(96 ± 4.0)	(80 ± 8.1)	(106 ± 2.1)	(89 ± 5.6)
10 mM Mg, 1 M NaCl	5.89 ± 0.784	1.11 ± 0.309	0.99 ± 0.079	4.35 ± 0.236
10 mM Zn, 1 M NaCl	3.34 ± 0.897	0.50 ± 0.087	0.64 ± 0.070[d]	2.80 ± 0.500[e]
	(58 ± 15.1)	(50 ± 8.1)	(68 ± 8.0)	(64 ± 7.9)
10 mM Mg, 5 M NaCl	6.18 ± 1.450	1.43 ± 0.221	1.01 ± 0.213	5.73 ± 0.512
10 mM Zn, 5 M NaCl	4.03 ± 0.510	0.35 ± 0.101[f]	0.50 ± 0.100[d]	1.65 ± 0.215[d]
	(65 ± 8.5)	(23 ± 4.8)	(50 ± 3.6)	(30 ± 5.5)

[a] Mg added as $MgSO_4 \cdot 7H_2O$; Zn added as $ZnSO_4 \cdot 7H_2O$.
[b] Mean coliphage titer ± standard error of the mean after 3 h of incubation.
[c] Mean percent of control ± standard error of the mean (control = Mg at an equivalent NaCl concentration).
[d] Significant difference at $P = 0.001$.
[e] Significant difference at $P = 0.05$.
[f] Significant difference at $P = 0.005$.

TABLE 8. *Influence of different salts and sugars at equivalent calculated osmotic pressures on the toxicity of zinc to E. coli*

Treatment[a]	Colony-forming units ($\times 10^1$) per 0.1 ml[b]
1 M NaCl, 10 mM Mg	8.3 ± 2.57
1 M NaCl, 10 mM Zn	0.7 ± 0.32[c]
	(11 ± 4.7)[d]
1 M $NaNO_3$, 10 mM Mg	2.5 ± 0.41
1 M $NaNO_3$, 10 mM Zn	0.5 ± 0.05[e]
	(18 ± 4.0)
2 M glucose, 10 mM Mg	28.0 ± 2.40
2 M glucose, 10 mM Zn	19.0 ± 3.50[e]
	(65 ± 8.2)
2 M xylose, 10 mM Mg	36.0 ± 5.50
2 M xylose, 10 mM Zn	17.0 ± 3.10[f]
	(49 ± 4.4)

[a] Mg added as $MgSO_4 \cdot 7H_2O$; Zn added as $ZnSO_4 \cdot 7H_2O$.

[b] Mean bacterial titer ± standard error of the mean after 3 h of incubation.

[c] Significant difference at $P = 0.02$.

[d] Mean percent of control ± standard error of the mean (control = Mg + equivalent molar concentration of osmotic agent).

[e] Significant difference at $P = 0.001$.

[f] Significant difference at $P = 0.01$.

substrate. However, these differences did not interfere with the interpretation of the influence of these osmotic agents on Zn toxicity, as each experimental value was compared with the appropriate control (e.g., with *E. coli*, 10 mM Zn^{2+} + 1 M $NaNO_3$ was compared with 10 mM Mg^{2+} + 1 M $NaNO_3$).

A concentration of either 0.5 M NaCl or $NaNO_3$ enhanced the toxicity of Zn to *A. niger* to a greater extent than did 1 M glucose or xylose, which gave equivalent calculated osmotic pressures (Table 7). Similarly, the toxicity of Zn to *E. coli* was enhanced to a significantly greater extent in the presence of 1 M NaCl or $NaNO_3$ than in the presence of 2 M glucose or xylose (Table 8). The toxicity of Zn to *C. echinulata* was approximately equivalent in the presence of 0.5 M NaCl or $NaNO_3$ and of 1 M glucose or xylose (Table 7).

DISCUSSION

A concentration of 10 mM Zn^{2+} decreased the survival of *E. coli*, enhanced that of *B. cereus*, but did not affect that of *P. aeruginosa* and *N. corallina* after 3 h of incubation in buffer. Differential sensitivities to Zn^{2+} were also noted with fungi, the sequence of sensitivity being *R. solani* > *F. solani* > *C. echinulata* > *A. niger*, *T. viride*. In comparison with other divalent heavy metals, such as Cd^{2+}, Pb^{2+}, or Hg^{2+} (9), a relatively high concentration of Zn^{2+} was required to inhibit growth rates of fungi and to reduce survival of bacteria. This greater tolerance to Zn^{2+} may have reflected the differential sorption of Zn^{2+} to ionogenic sites on the surface of cells, as studies with frog kidney cells and normal lymphoid and transformed Burkitt lymphoma cell lines demonstrated that Zn^{2+} sorbed to surface ionogenic sites different from those that bound Ca^{2+}, Cd^{2+}, and Pb^{2+} (29, 30). The greater tolerance to Zn^{2+} may have also resulted from the requirement of Zn^{2+} as a micronutrient. For example, both *A. niger* and *P. aeruginosa* were comparatively tolerant of high Zn^{2+} concentrations, and Zn^{2+} is required for growth of *A. niger* (58) and for maintaining the integrity of the cell wall of *P. aeruginosa* (20). The mechanism responsible for the enhancement of survival of *B. cereus* in the presence of Zn^{2+} is unknown, but Zn^{2+} is an essential micronutrient for growth (21) and sporulation (26) of *Bacillus* species. Low concentrations of Zn^{2+} are apparently required by all bacteria, including actinomycetes, and fungi (3, 14, 24). However, the mechanisms responsible for the toxicity of elevated concentrations of Zn^{2+} are essentially undefined (45), although it has been suggested that excess Zn^{2+} may interfere with the metabolism of Mg^{2+} (1, 35).

The survival of T1, T7, P1, and ϕ80 coliphages was not adversely influenced after 3 h of incubation in the presence of 10 mM Zn^{2+}, in agreement with other studies that have shown that 10 mM Zn^{2+} [as $Zn(NO_3)_2$] did not affect the viability of T2 coliphage (33).

Depending on the anionic composition of the environment, divalent Zn^{2+}, similar to Cd^{2+}, Pb^{2+}, and Hg^{2+}, forms a variety of complex ionic and molecular coordination complexes. In these studies, experiments were performed at pH 6 or below to avoid the formation of hydroxylated Zn species. In the absence of competing anions but in the presence of 0.1 M NaCl, about 75% of the Zn occurs as Zn^{2+}, 20% occurs as $ZnCl^+$, and 5% occurs as $ZnCl_2$. In the presence of 0.5 M NaCl, approximately 40% of the Zn occurs as Zn^{2+}, 35% occurs as $ZnCl^+$, 20% occurs as $ZnCl_2$, and 5% occurs as $ZnCl_3^-$. The concentration of divalent Zn^{2+} becomes insignificant as the concentration of Cl^- is further increased. Thus, with 1 M NaCl, about 5% of the Zn exists as Zn^{2+}, 20% exists as $ZnCl^+$, 30% exists as $ZnCl_2$, 30% exists as $ZnCl_3^-$, and 15% exists as $ZnCl_4^{2-}$. At 5 M NaCl, the anionic Zn-Cl species predominate, because there is no Zn^{2+} and about 5% of the Zn occurs as $ZnCl^+$, 10% occurs as $ZnCl_2$, 30% occurs as $ZnCl_3^-$, and 55% occurs as $ZnCl_4^{2-}$ (25). These different forms of Zn may exert differing toxicities to microbes and viruses.

The microbes and viruses were grouped into three categories depending on their response to

Zn in the presence of increasing concentrations of NaCl. The first category included those microbes whose response to Zn was unaffected by the concentrations of NaCl tested: the inhibition by Zn of the mycelial growth of *F. solani* and *C. echinulata* and of the survival of *N. corallina* was independent of NaCl. The second group included those microbes and viruses whose sensitivity to Zn increased as the concentration of NaCl increased: the mycelial growth of *A. niger*; the survival of *P. aeruginosa* and of the coliphages, T1, T7, P1, and ϕ80; and the enhancement of survival of *B. cereus* by Zn were reduced by increasing the concentration of NaCl. In these systems, the anionic Zn-Cl species were apparently more toxic than was Zn^{2+}. The third category included those microbes whose sensitivity to Zn decreased as the concentration of NaCl increased: the toxicity of Zn for the mycelial growth of *T. viride* and *R. solani* was lessened as the concentration of NaCl increased, indicating a greater sensitivity to Zn^{2+} than to the anionic Zn-Cl species.

There appear to be few studies in the literature that evaluate the influence of Cl^- or of the inorganic anionic environment in general on the toxicity of Zn^{2+} or other heavy metal pollutants to microbes and viruses. Studies with T2 coliphage showed that, although Zn^{2+} did not affect viability of the virus, anionic Zn-cyanide complexes [i.e., $Zn(CN)_3^-$ and $Zn(CN)_4^{2-}$] at equivalent concentrations of Zn were toxic, apparently by rupturing thiol ester bonds in the bacteriophage tail (34). However, other studies have shown that Cd^{2+} was more inhibitory to growth of a "mixed microflora" from activated sludge than was $Cd(CN)_4^{2-}$ (16) and that greater toxicities towards growth of bacteria and fungi were exerted by $CdOH^+$ than by comparable concentrations of Cd^{2+} (6, 7, 8).

The toxicity of Zn^{2+} to *A. niger* was approximately equivalent at 25 and 37°C, but, in the presence of 0.5 M NaCl, *A. niger* was more sensitive to Zn at 25 than at 37°C. The greater tolerance to Zn in the presence of NaCl at 37°C may have reflected the enhanced physiological state of the fungus, because in the absence of NaCl or Zn^{2+} both mycelial growth and sporulation were greater when the fungus was grown at 37 than at 25°C.

The increased toxicity of Zn in the presence of NaCl was most probably the result of a synergistic interaction between Zn and Cl^- (i.e., the formation of Zn-Cl species) and not of a synergism between Zn^{2+} and elevated osmotic pressures. The toxicity of Zn to *A. niger* and *E. coli* was increased to a greater extent with concentrations of NaCl or $NaNO_3$ that would result in anionic Zn-Cl or Zn-NO_3 species than in the presence of concentrations of glucose or xylose calculated to yield equivalent osmotic pressures and where Zn would exist only as Zn^{2+}. The sensitivity of *C. echinulata* to Zn was independent of high concentrations of NaCl or of the other osmotic agents, indicating that the anionic Zn-Cl complexes and Zn^{2+} exerted similar toxicities.

Although this model system focused on Zn, the results of these investigations have implications for the toxicity to microbes and other biological systems of other heavy metal pollutants, particularly those heavy metals that form complexes with Cl^- at concentrations lower than those required for Zn^{2+}. For example, in seawater with an average Cl^- concentration of 0.56 M, Cd exists mainly as $CdCl_2$ and $CdCl_3^-$, and Hg exists as $HgCl_3^-$ and $HgCl_4^{2-}$ (25). Thus, the form and resulting toxicity of a heavy metal pollutant depend, in part, on the anionic composition of the environment into which the pollutant is deposited. In assessing the toxicity of pollutants to the biota, attention must therefore be focused on the specific physicochemical abiotic factors of the recipient environment, which may mediate or potentiate the toxicity of the contaminant (9).

LITERATURE CITED

1. **Abelson, P. H., and E. Aldous.** 1950. Ion antagonisms in microorganisms: interference of normal magnesium metabolism by nickel, cobalt, cadmium, zinc, and manganese. J. Bacteriol. **60**:401-413.
2. **Adiga, P. R., K. S. Sastry, and P. S. Sarma.** 1962. The influence of iron and magnesium on the uptake of heavy metals in metal toxicities in *Aspergillus niger*. Biochim. Biophys. Acta **64**:546-548.
3. **Alexander, M.** 1977. Introduction to soil microbiology. John Wiley & Sons, Inc., New York.
4. **Auld, D. S., H. Kawaguchi, D. M. Livingston, and B. L. Vallee.** 1974. Reverse transcriptase from avian myeloblastosis virus: a zinc metalloenzyme. Biochem. Biophys. Res. Commun. **57**:967-972.
5. **Babich, H., and G. Stotzky.** 1974. Air pollution and microbial ecology. Crit. Rev. Environ. Contr. **4**:353-421.
6. **Babich, H., and G. Stotzky.** 1977. Sensitivity of various bacteria, including actinomycetes, and fungi to cadmium and the influence of pH on sensitivity. Appl. Environ. Microbiol. **33**:681-695.
7. **Babich, H., and G. Stotzky.** 1977. Reductions in the toxicity of cadmium to microorganisms by clay minerals. Appl. Environ. Microbiol. **33**:696-705.
8. **Babich, H., and G. Stotzky.** 1977. Effect of cadmium on fungi and on interactions between fungi and bacteria in soil: influence of clay minerals and pH. Appl. Environ. Microbiol. **33**:1059-1066.
9. **Babich, H., and G. Stotzky.** 1978. Effects of cadmium on the biota: influence of environmental factors. Adv. Appl. Microbiol. **23**:55-117.
10. **Babich, H., and G. Stotzky.** 1978. Influence of pH on inhibition of bacteria, fungi, and coliphages by bisulfite and sulfite. Environ. Res. **15**:405-417.
11. **Bartlett, L., F. W. Rabe, and W. H. Funk.** 1974. Effects of copper, zinc, and cadmium on *Selanastrum capricornutum*. Water Res. **8**:179-185.
12. **Bruland, K. W., K. Bertine, M. Koide, and E. D. Goldberg.** 1974. History of heavy metal pollution in

southern California coastal zone. Environ. Sci. Technol. **8**:425–432.
13. **Buchauer, M. J.** 1973. Contamination of soil and vegetation near a zinc smelter by zinc, cadmium, copper, and lead. Environ. Sci. Technol. **7**:131–135.
14. **Byrne, A. R., V. Ravnik, and L. Kosta.** 1976. Trace element concentrations in higher fungi. Sci. Total Environ. **6**:65–78.
15. **Carter, J. W., and I. L. Cameron.** 1973. Toxicity bioassay of heavy metals in water using *Tetrahymena pyriformis*. Water Res. **7**:951–961.
16. **Cenci, G., and G. Morozzi.** 1977. Evaluation of the toxic effects of Cd^{2+} and $Cd(CN)_4^{2-}$ ions on the growth of mixed microbial population of activated sludges. Sci. Total Environ. **7**:131–143.
17. **Chaney, W. R., J. M. Kelly, and R. C. Strickland.** 1978. Influence of cadmium and zinc on carbon dioxide evolution from litter and soil from a black oak forest. J. Environ. Qual. **7**:115–119.
18. **Chvapil, M.** 1973. New aspects in the biological role of zinc: a stabilizer of macromolecules and biological membranes. Life Sci. **13**:1041–1049.
19. **Coleman, J. E.** 1974. The role of Zn (II) in transcription by T7 RNA polymerase. Biochem. Biophys. Res. Commun. **60**:641–648.
20. **Eagon, R. G., G. P. Simmons, and K. J. Carson.** 1965. Evidence for the presence of ash and divalent metals in the cell wall of *Pseudomonas aeruginosa*. Can. J. Microbiol. **11**:1041–1043.
21. **Feeney, R. E., H. D. Lightbody, and J. A. Garibaldi.** 1947. Zinc as an essential element for growth and subtilin formation by *Bacillus subtilis*. Arch. Biochem. **15**: 13–17.
22. **Giese, A. C.** 1968. Cell physiology. W. B. Saunders, Philadelphia.
23. **Gingell, S. M., R. Campbell, and M. H. Martin.** 1976. The effect of zinc, lead, and cadmium pollution on the leaf surface microflora. Environ. Pollut. **11**:25–37.
24. **Guirard, B. M., and E. E. Snell.** 1962. Nutritional requirements of microorganisms, p. 33–94. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 4. Academic Press Inc., New York.
25. **Hahne, H. C. H., and W. Kroontje.** 1973. Significance of pH and chloride concentration on behavior of heavy metal pollutants: mercury (II), cadmium (II), zinc (II), and lead (II). J. Environ. Qual. **2**:444–450.
26. **Halvorson, H.** 1962. Physiology of sporulation, p. 223–264. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 4. Academic Press Inc., New York.
27. **John, M. K., C. J. VanLaerhoven, and C. H. Cross.** 1975. Cadmium, lead, and zinc accumulation in soils near a smelter complex. Environ. Lett. **10**:25–35.
28. **Jordan, M. J., and M. P. Lechevalier.** 1975. Effects of zinc-smelter emissions on forest soil microflora. Can. J. Microbiol. **21**:1855–1865.
29. **Kiremidjian, L., and G. Stotzky.** 1975. Influence of mono- and multivalent cations on the electrokinetic properties of *Rana pipiens* kidney cells. J. Cell. Physiol. **85**:125–134.
30. **Kiremidjian-Schumacher, L., and G. Stotzky.** 1976. Influence of mono- and multivalent cations on the electrokinetic properties of normal human lymphoid and Burkitt lymphoma cells. Experientia **32**:312–314.
31. **Klein, D. H., and P. Russell.** 1973. Heavy metals: fallout around a power plant. Environ. Sci. Technol. **7**:357–358.
32. **Korant, B. D., J. C. Kauer, and B. E. Butterworth.** 1974. Zinc ions inhibit replication of rhinoviruses. Nature (London) **248**:588–590.
33. **Kozloff, L. M., and M. Lute.** 1957. Viral invasion. II. The role of zinc in bacteriophage invasion. J. Biol. Chem. **288**:529–536.
34. **Kozloff, L. M., M. Lute, and K. Henderson.** 1957. Viral invasion. I. Rupture of thiol ester bonds in the bacteriophage tail. J. Biol. Chem. **228**:511–528.
35. **Laborey, F., and J. Lavollay.** 1973. Sur la nature des antagonismes responsables de l'interaction des ions Mg^{++}, Cd^{++}, et Zn^{++} dans croissance d'*Aspergillus niger*. C. R. Acad. Sci. **276**:529–532.
36. **Lagerwerff, J. V., and A. W. Specht.** 1970. Contamination of roadside soil and vegetation with cadmium, nickel, lead, and zinc. Environ. Sci. Technol., **4**:583–586.
37. **Marsh, P. B., M. E. Simpson, and M. W. Trucksess.** 1975. Effects of trace metals on the production of aflatoxins by *Aspergillus parasiticus*. Appl. Microbiol. **30**: 52–57.
38. **Nash, T. H., III.** 1972. Simplification of the Blue Mountain lichen communities near a zinc factory. Bryologist **75**:315–324.
39. **Nickerson, W. J.** 1946. Inhibition of fungus respiration: a metabolic bioassay method. Science **103**:484–486.
40. **Ou, J. T., and T. F. Anderson.** 1972. Effect of Zn^{2+} on bacterial conjugation: inhibition of mating pair formation. J. Bacteriol. **111**:177–185.
41. **Parsons, J. D.** 1977. Effects of acid mine wastes on aquatic ecosystems. Water Air Soil Pollut. **7**:333–354.
42. **Peterson, E. A., and H. Katznelson.** 1956. The effect of trace elements on growth of *Helminthosporium sativum* and several related species. Can. J. Microbiol. **2**: 441–446.
43. **Pickett, A. W., and A. C. R. Dean.** 1976. Cadmium and zinc sensitivity and tolerance in *Klebsiella* (*Aerobacter*) *aerogenes*. Microbios **15**:79–91.
44. **Prask, J. A., and D. J. Plocke.** 1971. A role for zinc in the structural integrity of the cytoplasmic ribosomes of *Euglena gracilis*. Plant Physiol. **48**:150–155.
45. **Ross, I. S.** 1975. Some effects of heavy metals on fungal cells. Trans. Br. Mycol. Soc. **64**:175–193.
46. **Ruhling, A., and G. Tyler.** 1973. Heavy metal pollution and decomposition of spruce needle litter. Oikos **24**: 402–416.
47. **Ruthven, J. A., and C. Cairns, Jr.** 1973. Response of fresh-water protozoan artificial communities to metals. Protozoology **20**:127–135.
48. **Smith, W. H., B. J. Staskawicz, and R. S. Harkov.** 1978. Trace-metal pollutants and urban-tree leaf pathogens. Trans. Br. Mycol. Soc. **70**:29–33.
49. **Somers, E.** 1961. The fungitoxicity of metal ions. Ann. Appl. Biol. **49**:246–253.
50. **Throneberry, G. O.** 1973. Some physiological responses of *Verticillium albo-atrum* to zinc. Can. J. Bot. **51**: 57–59.
51. **Tyler, G.** 1976. Heavy metal pollution, phosphatase activity, and mineralization of organic phosphorus in forest soils. Soil Biol. Biochem. **8**:327–332.
52. **Tzagoloff, H., and D. Pratt.** 1964. The initial steps in infection with coliphage M13. Virology **24**:372–380.
53. **Vega, R. R., and D. LeTourneau.** 1974. The effect of zinc on growth and sclerotial formation in *Whetzelinia sclerotiorum*. Mycologia **66**:256–264.
54. **Westing, A. H.** 1969. Plants and salt in the roadside environment. Phytopathology **59**:1174–1181.
55. **White, A., P. Handler, and E. L. Smith.** 1973. Principles of biochemistry. McGraw-Hill Book Co., New York.
56. **Wilson, D. O.** 1977. Nitrification in three soils amended with zinc sulfate. Soil Biol. Biochem. **9**:277–280.
57. **Winslow, C.-E. A., and E. T. Haywood.** 1931. The specific potency of certain cations with reference to their effect on bacterial viability. J. Bacteriol. **22**:49–69.
58. **Wold, W. S. M., and I. Suzuki.** 1976. The citric acid fermentation by *Aspergillus niger*: regulation by zinc of growth and acidogenesis. Can. J. Microbiol. **22**: 1083–1092.