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Environmental Contamination and Toxicology

Cadmium-Nickel Toxicity Interactions Towards a Bacterium, Filamentous Fungi, and a Cultured Mammalian Cell Line

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Emissions of metals from pollution sources usually consist of mixtures rather than individual metals. Such mixtures are deposited into aquatic and onto terrestrial environments. For example, atmospheric emissions from the smelting complex at Sudbury, Ontario, Canada, contain multiple metals, including cadmium (Cd) and nickel (Ni), which are deposited into surrounding lakes (Nriagu et al. 1982).

The response of the biota to exposure to individual metals may differ from its response to multiple metals, as mixtures of metals may interact antagonistically or synergistically in their resultant toxicity. For example, Wong et al. (1978, 1982) studied the toxicity of a mixture of ten metals (i.e., Cd, Ni, copper, iron, lead, mercury, zinc, arsenate, dichromate, and selenite) towards growth and photosynthesis of freshwater algae and cyanobacteria. Although each metal in this mixture was at a concentration equivalent to its Great Lakes Quality Objective, this mixture was strongly inhibitory. The present study evaluated the effects of a combination of Cd and Ni on the freshwater bacterium, Aeromonas hydrophila, the terrestrial fungi, Trichodema viride and Aspergillus niger, and the mammalian cell line, BALB/c mouse 3T3 fibroblasts. This particular spectrum of target cells was selected because studies in the literature show a wide variety of possible interactions between Cd and Ni in their combined toxicities towards bacteria (Ainsworth et al. 1980), cyanobacteria (Stratton and Corke 1979; Prasad and Prasad 1982; Whitton and Shehata 1982), slime molds (Chin et al. 1978), isolated rat hepatocytes (Jacobs et al. 1956; Stacey and Klaassen 1981), and rats (Tandon et al. 1984).

MATERIALS AND METHODS

A. hydrophila was inoculated into test tubes containing broth, consisting of 1% glucose and 0.5% neopeptone, adjusted to pH 7, and grown overnight in a rotating drum housed in a 28°C incubator. The bacterial suspension was then diluted with additional broth to yield 80% transmittance at 420 nm, and 0.25 ml aliquots of the suspension were then inoculated into tubes containing 3 ml of

broth, unamended or amended with $CdCl_2 \cdot 2.5H_2O$ and/or $NiCl_2 \cdot 6H_2O$ and adjusted to pH 7. The tubes were placed in a rotating drum and after 20 hr of growth at 28°C, turbidity was measured at 420 nm (Babich and Stotzky 1983).

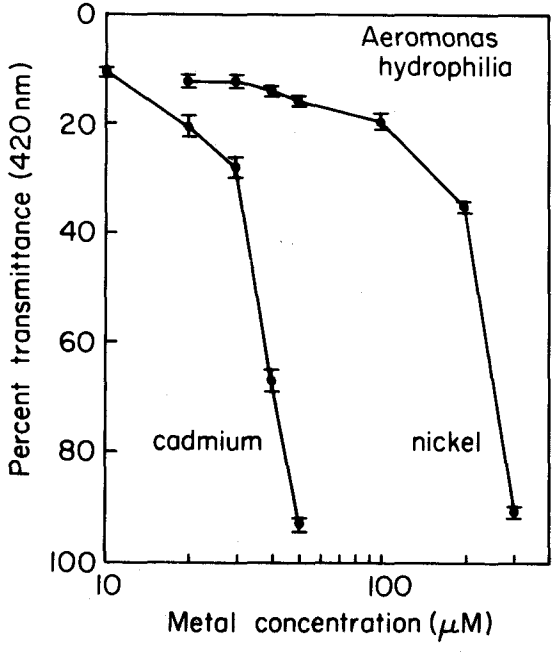
T. viride and A. niger were grown in modified Sabouraud dextrose agar (pH 7) and after incubation for several days at 28°C, 4 mm circular plugs cut with a sterile cork borer were transferred, with the mycelial growth up, to the center of petri dishes containing medium unamended or amended with metals. After incubation for up to 5 days, the diameters of mycelial growth were measured and radial growth rates, in mm/day, were calculated (Babich and Stotzky 1983).

BALB/c mouse 3T3 fibroblasts were grown in a humidified, 5% CO_2 atmosphere at 37°C in Dulbecco's modified Eagle's medium, supplemented with 10% fetal calf serum, 100 units/ml penicillin G, 100 µg/ml streptomycin, and 1.25 µg/ml Fungizone. The cells were trypsinized in a solution containing 0.05 g trypsin and 0.02g versene in 100 ml of phosphate-buffered saline. Cytotoxicity after a 24-hr exposure of 3T3 cells to the toxicants was determined by the reduction of the lysosomal uptake and retention of the vital dye, neutral red (Borenfreund and Puerner 1985a,b), and by a protein-based cell growth assay (Shopsis and Eng 1985). Cytotoxicity was also determined after a 4-hr exposure to metals, by measuring the inhibition of uridine uptake into intracellular pools (Shopsis and Sathe 1984; Shopsis 1985).

RESULTS AND DISCUSSION

Growth of A. hydrophila was reduced by 50% in the presence of 35 µM Cd or 0.22 mM Ni (Figure 1). A mixture of Cd and Ni produced neither a synergistic nor antagonistic interaction in their combined toxicity towards growth of the bacterium. Addition of 10 µM Cd had no effect on the toxicity of 0.01 to 0.3 mM Ni towards growth of A. hydrophila (Figure 2).

The mycelial growth rate of T. viride was reduced by 50% in the presence of 70 µM Cd or 3.7 mM Ni and that of A. niger by 40 µM Cd or 6.1 mM Ni (Figure 3). Combinations of Cd and Ni interacted synergistically in their combined toxicity to T. viride, but interacted antagonistically in their combined toxicity to A. niger. When T. viride was grown on medium amended with 3.0 to 4.2 mM Ni, the mycelial growth rates were further reduced in the presence of 50 µM Cd, a concentration of Cd that by itself was noninhibitory to growth (Figure 4). A synergistic interaction was also noted, although to a lesser extent, when T. viride was grown on media amended with 60 to 100 µM Cd in the presence of 3.3 mM Ni (data not shown). Conversely, the relative toxicity of 4.5 to 6.5 mM Ni to growth of A. niger was reduced in the presence of 30 µM Cd (Figure 4). An antagonistic interaction, although to a lesser extent, between Cd and Ni was also noted when A. niger was grown on media containing 30 to 60 µM Cd in the presence of 6.0 mM Ni (data not shown).



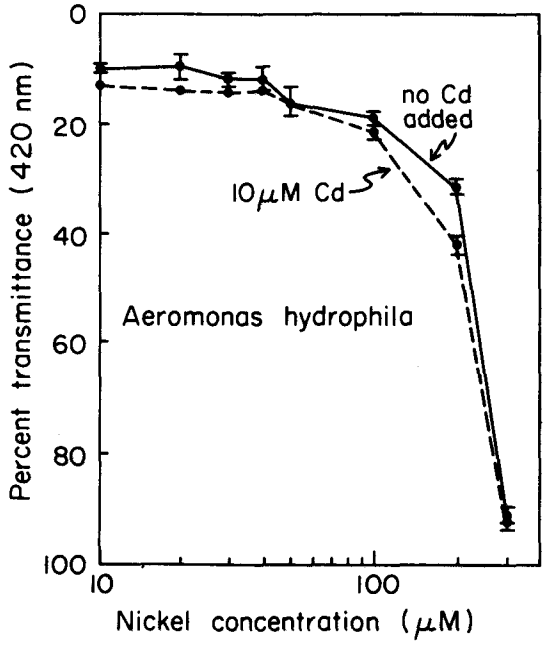
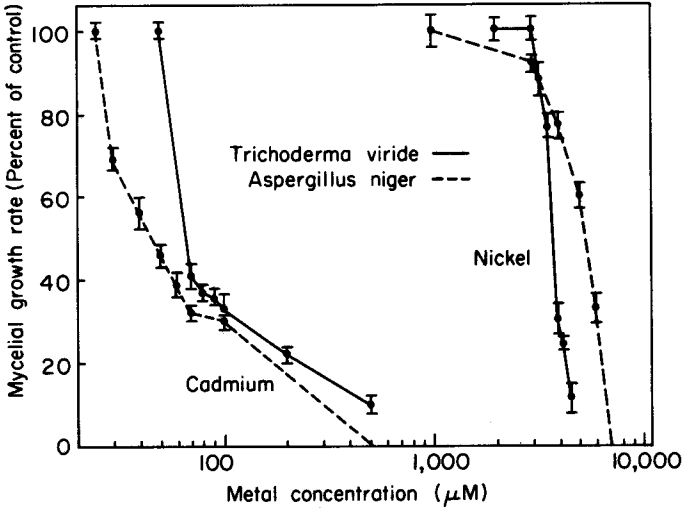


Figure 1 (Left). The effect of cadmium and nickel on growth, after 20 hr, of Aeromonas hydrophila. The data are expressed as the means ± SEM.
Figure 2 (Right). The effect of nickel, at 10 to 300 μM, in the absence and presence of 10 μM cadmium, on growth, after 20 hr, of Aeromonas hydrophila. The data are expressed as the means ± SEM.



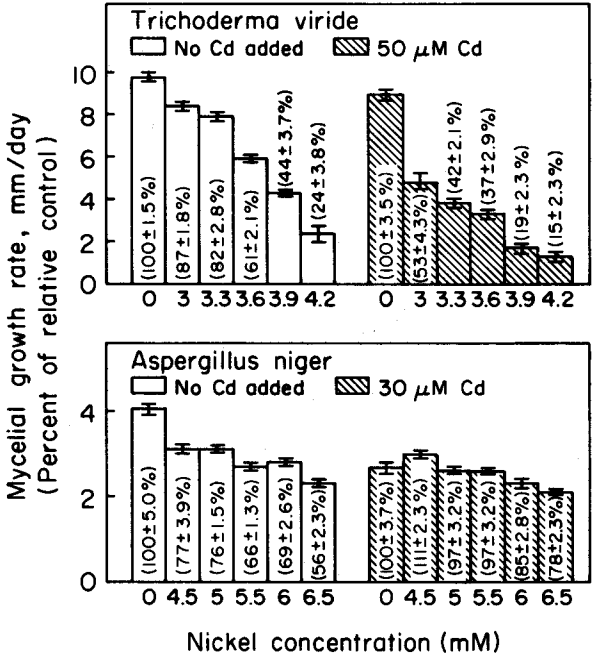
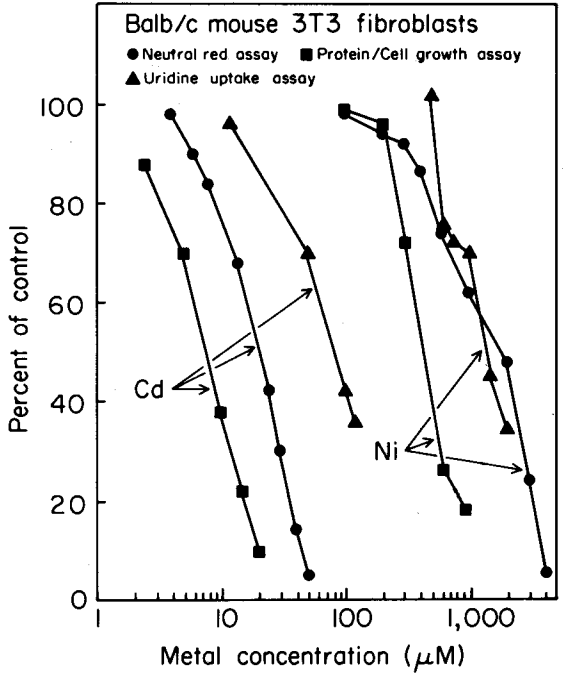


Figure 3 (Left). The effect of cadmium and nickel on the mycelial growth rates of Aspergillus niger and Trichoderma viride. In the absence of metal amendments, the growth rates, in mm/day, were 4.1 and 9.8 for A. niger and T. viride, respectively. The data are expressed as the means ± SEM.

Figure 4 (Right). The effect of combinations of cadmium and nickel on the mycelial growth rates of Trichoderma viride and Aspergillus niger. The numbers in parentheses are the relative percent of control values; the data are expressed as the means ± SEM.



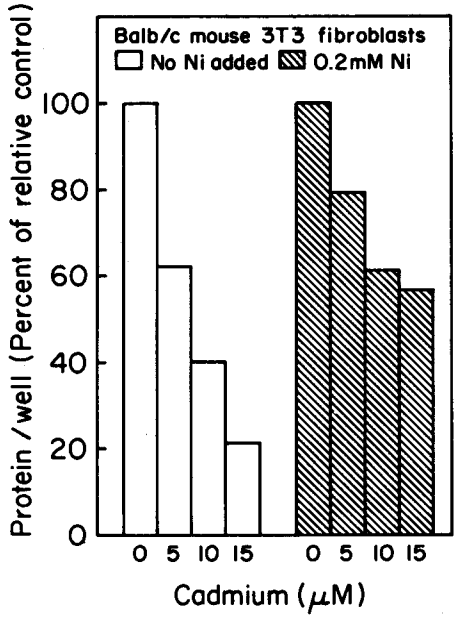
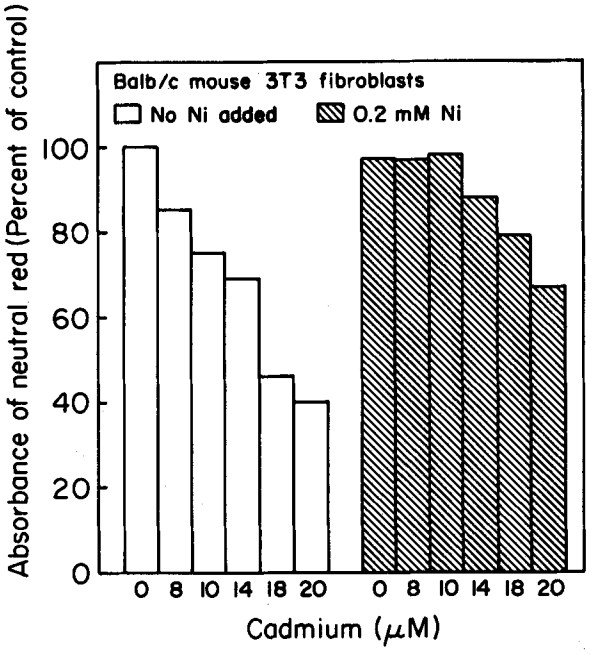


Figure 5 (Left). The effect of cadmium and nickel on lysosomal uptake of neutral red, cell growth, and uridine uptake of BALB/c mouse 3T3 fibroblasts. In the uridine uptake assay the exposure was for 4 hr, while in the two other assays the exposure was for 24 hr. The data are expressed as the means, with the SEM being within ± 5% of the means.

Figure 6 (Right). The effect of 5 to 15 μM cadmium, in the absence or presence of 0.2 mM nickel, on cell growth, as determined by protein analysis, of BALB/c mouse 3T3 fibroblasts. The data are expressed as the means, with the SEM being within ± 5% of the means.



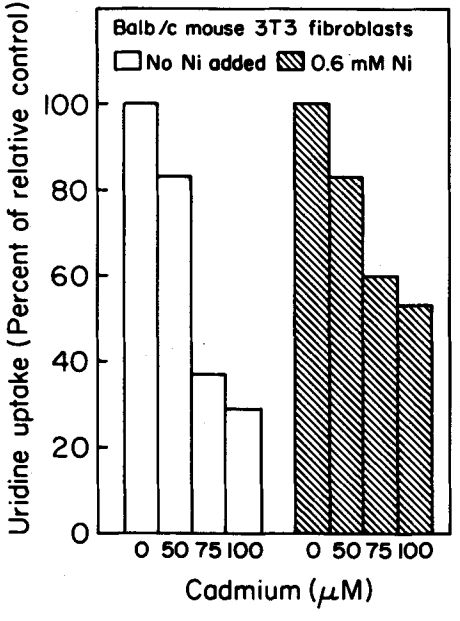


Figure 7 (Left). The effect of 8 to 20 μM cadmium, in the absence and presence of 0.2 mM nickel, on lysosomal uptake of neutral red by BALB/c mouse 3T3 fibroblasts. The data are expressed as the means, with the SEM being within ± 5% of the means.

Figure 8 (Right). The effect of 50 to 75 μM cadmium, in the absence and presence of 0.6 mM nickel, on the uptake of labeled uridine by BALB/c mouse 3T3 fibroblasts. The data are expressed as the means, with the SEM being within ± 5% of the means.

The concentration causing a 50% reduction in cell growth, in lysosomal uptake of neutral red, and in the uptake of labeled uridine by the 3T3 fibroblasts was 9, 20, and 70 μM Cd, respectively, and 0.42, 1.8, and 1.5 mM Ni, respectively (Figure 5). In each cytotoxicity assay with the 3T3 cells, a combination of Cd and Ni interacted antagonistically. Thus, the toxicity of 5 to 15 μM Cd to cell growth was reduced in the presence of 0.2 mM Ni (Figure 6). Similarly, while lysosomal uptake of neutral red by viable 3T3 cells was reduced by 14 to 20 μM Cd, the addition of 0.2 mM Ni protected against Cd toxicity (Figure 7). However, 0.1 mM Ni was insufficient to protect, as determined by neutral red uptake, against the toxicity of 14 to 20 μM Cd (data not shown). Cadmium, at 70 to 110 μM, reduced the uptake of labeled uridine by 3T3 cells; this inhibitory effect of Cd was reduced in the presence of 0.6 mM Ni (Figure 8). However, 0.4 mM Ni was insufficient to protect against the toxicity of 50 to 125 μM Cd, as assayed by the inhibition of uridine uptake (data not shown).

In each assay, Cd was more toxic to the target cells than Ni, although differential sensitivity to the relative toxicity of each metal was evident in each assay. For example, the concentration of Cd causing 50% toxicity to growth of A. hydrophila was about 7X less than that of Ni, whereas the concentration of Cd causing 50% inhibition of growth of A. niger was about 150X less than that of Ni. In the neutral red assay, the concentration of Cd causing a 50% toxicity to the 3T3 cells was about 100X less than that of Ni. The greater toxicity of Cd than of Ni was consistent with their softness parameters (σ_p), i.e., 0.081 for Cd and 0.126 for Ni (Williams et al. 1982). The mechanisms accounting for the Cd-Ni interactions, whether synergistic or antagonistic, are not known. However, these interactions are apparently not related to competition between cations of similar size, as the nonhydrated ionic radii of Ni^{2+} and Cd^{2+} are 0.69 and 1.14 A, respectively (Weast and Selby 1969). Furthermore, the σ_p values of Cd and Ni are sufficiently dissimilar, suggesting that each metal interacts with different organic moieties to induce a toxic effect.

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