Influence of Heavy Metal Leaf Contaminants on the in vitro Growth of Urban-Tree Phylloplane-Fungi

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Abstract. The surfaces of urban woody vegetation are contaminated with varying amounts of numerous metallic compounds, including Cd, Cu, Mn, Al, Cr, Ni, Fe, Pb, Na, and Zn. To examine the possibility that these metals may affect phylloplane fungi, the above cations were tested in vitro for their ability to influence the growth of numerous saprophytic and parasitic fungi isolated from the leaves of London plane trees. Considerable variation in growth inhibition by the metals was observed. Generally *Aureobasidium pullulans*, *Epicoccum* sp., and *Phialophora verrucosa* were relatively tolerant; *Gnomonia platani*, *Cladsporium* sp., and *Pleurophomella* sp. were intermediate; and *Pestalotiopsis* and *Chaetomium* sp. were relatively sensitive to the incorporation of certain metals into solid and liquid media. If similar growth inhibitions occur in nature, competitive abilities or population structures of plant surface microbes may be altered by surface metal contamination. Metals causing the greatest and broadest spectrum growth suppression included Ni, Zn, Pb, Al, Fe, and Mn.

Introduction

The relationship between air pollution and microorganisms is an important and incompletely appreciated topic [1, 7, 12, 16, 17]. Organisms that exist in the phylloplane for all or part of their life-cycle are particularly vulnerable to the influence of air contaminants [21]. Since the leaves and twigs of urban and roadside plants are superficially contaminated with numerous trace metals [18–20, 22] and because several of these metals are essential or toxic to microbes under certain conditions [15], it is appropriate to examine the effects of particulate metal contaminants on plant surface fungi.

This study examined the influence of ten metals, all found to be common contaminants of urban tree leaf and twig tissue [20] on the growth of selected saprophytic and parasitic fungi isolated from foliar surfaces of streetside London plane trees in downtown New Haven. Of particular interest was an assessment of differential heavy metal response of non-pathogenic and pathogenic fungi.

Materials and Methods

Leaf washing and impression techniques [3] were employed to isolate phylloplane fungi form the leaves of mature London plane (*Platanus acerifolia* [Ait.] Willd.) growing in downtown New

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Haven. The following fungi were consistently isolated from various crown positions and at different times during the growing season. Those existing primarily saprophytically included; *Aureobasidium pullulans* (deBary) Arn., *Caetomium* sp., *Cladosporium* sp., *Epicoccum* sp., and *Phialophora verrucosa* Medlar. Those existing primarily parasitically included *Gnomonia platani* Kleb., *Pestalotiposis* sp., and *Pleurophomella* sp. These organisms were maintained in plate culture and evaluated in vitro to determine their tolerance to several metals known to be superficial contaminants of urban tree-leaf surfaces.

The metals included in this investigation were Cd, Cu, Mn, Al, Cr, Ni, Fe, Pb, Na, and Zn. In every test a nitrate salt was used with the cation concentration equivalent to the average or maximum leaf burden (dry weight basis) of deciduous tree species sampled in the fall in New Haven, Connecticut [20]. The average concentrations in parts per million (μg/gm) were Cd 1.5, Cu 8.7, Mn 469, Al 503, Cr 2.8, Ni 10, Fe 404, Pb 110, Na 373, and Zn 130. The maximum concentrations in parts per million (μg/gm) were Cd 2.2, Cu 18, Mn 1311, Al 783, Cr 7.4, Ni 19, Fe 791, Pb 275, Na 977, and Zn 265.

The in vitro tests performed included linear extension in plate culture and dry weight in liquid culture. No dry weight determinations were made with *P. verrucosa*, *Pleurophomella* sp., or *Cladosporium* sp. Media were amended with appropriate concentrations of the metal nitrates. Incubation was at 20°C. The pH of all growth media with all metal amendments was within the range 5.0 to 5.8.

Linear extension was determined by placing inverted agar plugs of the fungi in the center of Petri plates containing 15 ml of potato dextrose agar (Difco). Several defined media were tested, but none was acceptable for all fungi. Mean colony diameters were calculated from four replicate plates after 7 days growth. Treatments consisted of plates amended with both average and maximum concentrations of all metals. One treatment involved all metals combined at both concentrations. Control plates did not receive nitrate salt amendment.

Dry weight values were obtained following inoculation of 250-ml Erlenmeyer flasks containing 50 ml of potato dextrose broth. Treatments consisted of medium amended with average concentrations only. Control media were amended with potassium nitrate in appropriate amounts to provide the same nitrate concentration as the various test-metal flasks. Four replicate flasks were shaken at 175 rpm for 7 days. The mycelium was harvested, dried at 80°C, and weighed.

Results

Linear extension in plate culture showed that *A. pullulans* growth was significantly reduced by average and maximum concentrations of Fe, Al, Mn, and Ni and by maximum concentrations of Na and Cd (Fig. 1A). Linear growth of *Chaetomium* sp. was reduced by all metals except Ni and Na at the average concentration and by all cations at the maximum concentration (Fig. 1B). Linear extension of *Cladosporium* sp. was reduced by average and maximum concentrations of Fe, Al, Zn, and Ni and by maximum concentrations of Na, Mn, and Pb (Fig. 1C). Linear growth of *Epicoccum* sp. was suppressed by average and maximum concentrations of Fe, Al, and Mn and maximum additions of Cu, Ni, and Zn (Fig. 1D). *P. verrucosa* linear development was very tolerant of trace metal amendment and was significantly reduced only by average and maximum concentrations of Fe and Al (Fig. 1E). Incorporation of all metals together in potato dextrose agar plates in either average or maximum concentration com-

pletely inhibited the growth of all saprophytic fungi. The addition of certain metal nitrates significantly stimulated the linear extension of all saprophytic fungi except *Chaetomium* sp. and *P. verrucosa*. Maximum additions of Pb $(NO_3)_2$ significantly stimulated the linear growth of *A. pullulans* and *Epicoccum* sp. The growth of *Epicoccum* sp. in particular was increased by the incorporation of several metal nitrates.

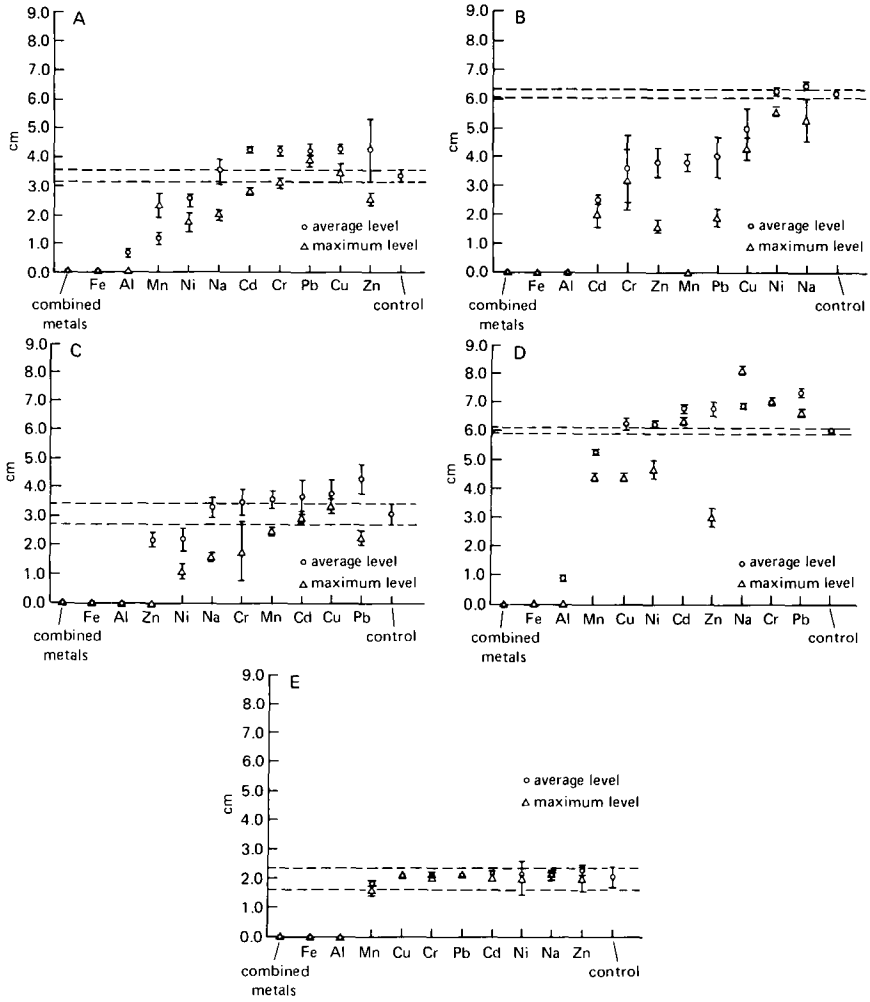

Fig. 1. Linear extension of saprophytic fungi isolated from the leaf surfaces of urban *Platanus acerifolia* and grown on potato dextrose agar amended with various trace metals in average and maximum concentrations equivalent to urban-tree leaf-contamination. Data represent four replicate plates grown at 20°C for 7 days. Means ± 95% confidence intervals. **A.** *Aureobasidium pullulans*. **B.** *Chaetomium* sp. **C.** *Cladosporium* sp. **D.** *Epicoccum* sp. **E.** *Phialophora verrucosa*.

Linear extension of *G. platani* was reduced by average and maximum concentrations of Fe, Al, Ni, and Zn and by maximum concentrations of Mn, Na, and Pb (Fig. 2A). Mycelial extension of *Pestalotiopsis* sp. was lessened by average and maximum levels of Fe, Al, and Pb and maximum levels of Cd and Ni (Fig. 2B). Linear extension of *Pleurophomella* sp. was reduced by average levels of Fe and Al and by eight metals (Fe, Al, Cd, Zn, Pb, Ni, Cu, and Mn) at the maximum concentration (Fig. 2C).

Dry weight data obtained from the shake culture experiment show significant growth suppression of *A. pullulans* only by Fe (Fig. 3A); *Chaetomium* sp. by all metals except Na, Cu, and Cr (Fig. 3B); and *Epicoccum* sp. by Fe, Al, and Zn (Fig. 3C).

Dry weight data for parasitic species show growth suppression of *G. platani* by Zn, Al, and Ni (Fig. 4A) and of *Pestalotiopsis* sp. by Zn, Al, Fe, Cd, and

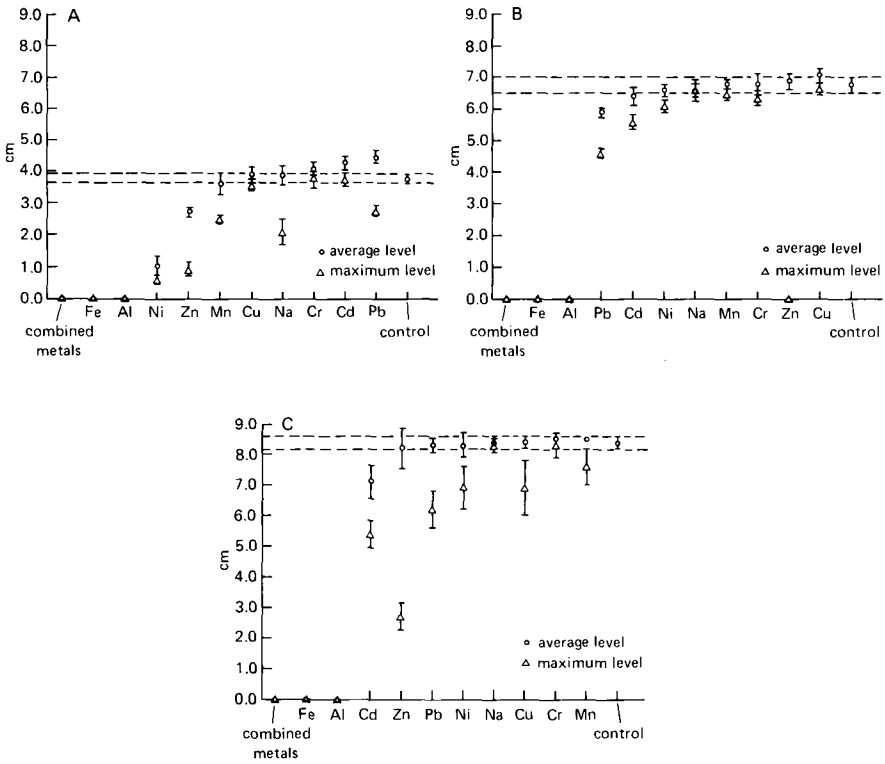

Fig. 2. Linear extension of parasitic fungi isolated from the leaf surfaces of urban *Platanus acerifolia* and grown on potato dextrose agar amended with various trace metals in average and maximum concentrations equivalent to urban-tree leaf-contamination. Data represent four replicate plates grown at 20°C for 7 days. Means ± 95% confidence intervals. **A.** *Gnomonia platani*. **B.** *Pestalotiopsis* sp. **C.** *Pleurophomella* sp.

Pb (Fig. 4B). Significant stimulation by metal nitrates was not observed in the dry weight experiment.

Discussion

The evidence presented supports the suggestion that there is no correlation between saprophytic or parasitic activity and sensitivity to trace metals in vitro. Both linear extension and dry weight data indicate that the saprophytic *Chaetomium* sp. is very sensitive to numerous metals. *Aureobasidium pullulans*, *Epicoccum* sp. and especially *P. verruosa*, on the other hand, appear much more tolerant. Of the parasites, *G. platani* appears more tolerant than *Pestalotiopsis* sp. and *Pleurophomella* sp.

The stimulation of growth observed in the linear extension experiment was probably not a result of cationic but rather of the anionic fraction of the metal nitrate amendment. The nitrate salts provided increased nitrogen substrate rela-

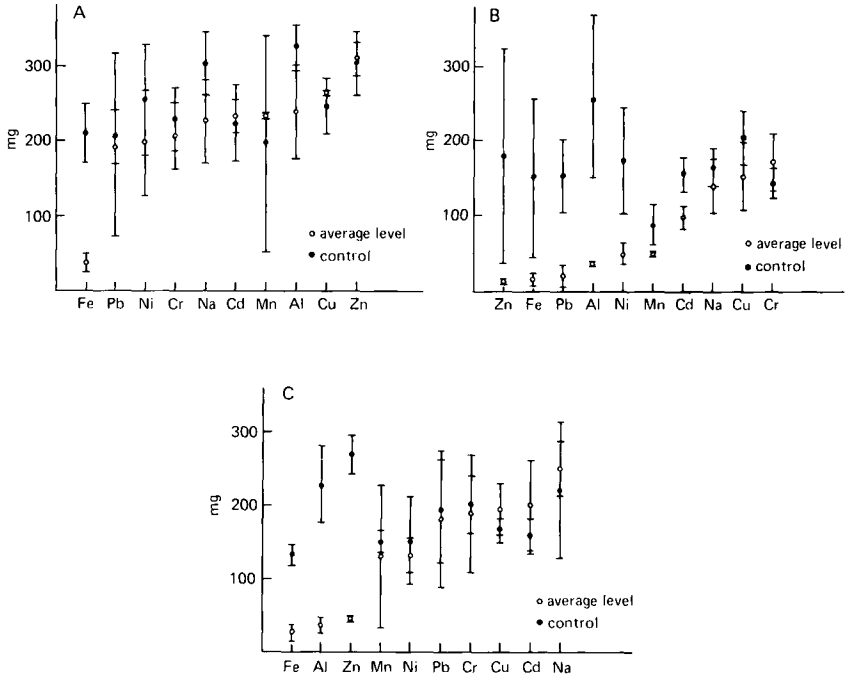

Fig. 3. Dry weight of saprophytic fungi isolated from the leaf surfaces of urban *Platanus acerifolia* and grown in potato dextrose broth amended with various trace metals in average concentrations equivalent to urban-tree leaf contamination. Data represent four replicate flasks shaken at 20°C for 7 days. Means ± 95% confidence intervals. **A.** *Aureobasidium pullulans*. **B.** *Chaetomium* sp. **C.** *Epicoccum* sp.

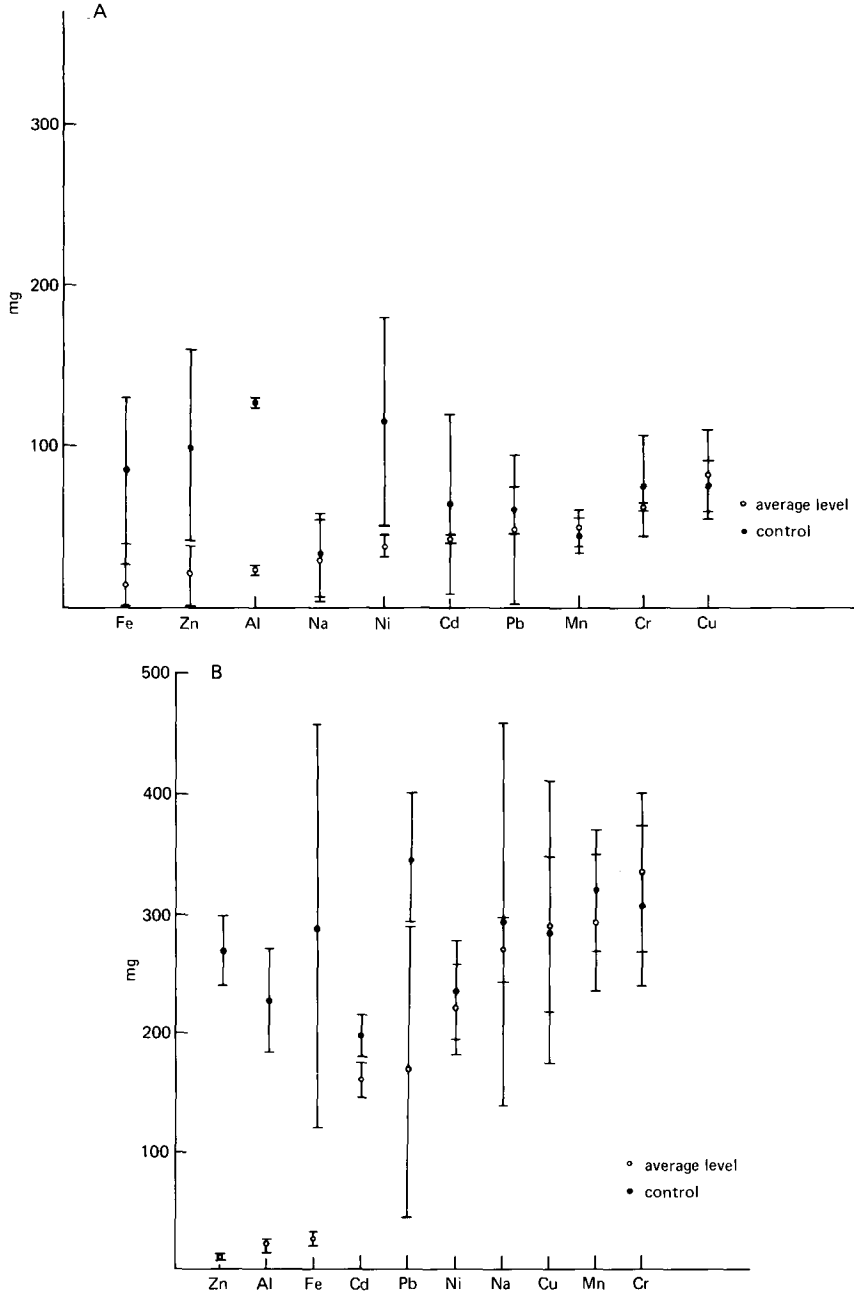

Fig. 4. Dry weight of parasitic fungi isolated from the leaf surfaces of urban *Platanus acerifolia* and grown on potato dextrose broth amended with various trace metals in average concentrations equivalent to urban-tree leaf contamination. Data represent four replicate flasks shaken at 20°C for 7 days. Means ± 95% confidence intervals. **A.** *Gnomonia platani*. **B.** *Pestalotiopsis* sp.

tive to the non-nitrate controls. When comparable nitrate concentrations were supplied in the liquid culture experiment, none of the metal nitrate amendments was stimulatory. Another explanation of stimulation might involve the detoxification hypothesis advanced by Englander and Corden [4] and employed in their explanation of the ability of high concentration of Cu and Fe to stimulate the growth of *Endothia parasitica* (Murr.) And.

Metals exhibiting the broadest spectrum growth suppression were Fe, Al, Ni, Zn, Mn, and Pb. The general fungitoxicity of metal cations is presumably related to the strength of their cell surface covalent binding power [24] or electronegativity [15]. Certain of the metals shown to have broad-spectrum toxicity in this experiment (Ni, Zn, and Pb) are members of standard lists of heavy metals exhibiting toxicity to a broad range of fungal species [8, 23, 24]. The other metals shown to be significant by these data (Al, Fe, and Mn) are presumably toxic because of their extremely high concentration. The numerous and varied particulate sources in urban atmospheres [2, 5] release quantities of particules high in Al, Fe and Mn [2, 6, 11, 14] and accounts for the high values we have recorded on leaves [20] and hence have employed in the in vitro tests.

Unfortunately it is difficult to extrapolate from in vitro observations to the natural environment. The chemistry of the trace-element compounds on leaf surfaces in the field is unknown. Nitrate salts were employed to provide a common anion and completely soluble compounds. In nature the trace metals probably occur as less soluble oxides, halides, sulfates, or phosphates. The dosages employed were arbitrary and may be relatively high, as late season concentrations based on dry leaf weights were employed. Other concentrations would have been no less arbitrary, however, as the use of any natural product medium will cause alteration of available metal concentrations due to binding by media components [3]. Accurate determination of thresholds of fungal trace-metal influence will require the use of synthetic-defined media and dose-response curves prepared over a range of concentrations bracketing those known to occur in nature. Since the metals were reacted with the fungi individually, it is possible that important antagonistic, additive, or synergistic interactions were overlooked. Because the phylloplane has a complex microflora [9], with much interaction between pathogenic and nonpathogenic microbes [5, 10], it is possible that trace-element impact on organisms that influence the fungi examined in this study may be more significant in nature than direct metal impact on these test organisms.

In spite of these limitations, we conclude that our data support the importance of determining more specifically the relationships between trace metals deposited on plant surfaces and microorganisms inhabiting these surfaces. The hypothesis that trace metals in urban, industrial, and roadside environments may alter species composition of foliar microbial ecosystems is worthy of investigation.

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