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Phytotoxicity of zinc and manganese to seedlings grown in soil contaminated by zinc smelting

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ABSTRACT

Historic emissions from two zinc smelters have injured the forest on Blue Mountain near Palmerton, Pennsylvania, USA. Seedlings of soybeans and five tree species were grown in a greenhouse in a series of mixtures of smelter-contaminated and reference soils and then phytotoxic thresholds were calculated. As little as 10% Palmerton soil mixed with reference soil killed or greatly stunted seedlings of most species. Zinc was the principal cause of the phytotoxicity to the tree seedlings, although Mn and Cd may also have been phytotoxic in the most contaminated soil mixtures. Calcium deficiency seemed to play a role in the observed phytotoxicity. Exposed soybeans showed symptoms of Mn toxicity. A test of the effect of liming on remediation of the Zn and Mn phytotoxicity caused a striking decrease in Sr-nitrate extractable metals in soils and demonstrated that liming was critical to remediation and restoration.

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1. Introduction

Historic emissions from two Zn smelters have destroyed the ridgetop forest on Blue Mountain near Palmerton, PA, including a section of the Appalachian National Scenic Trail. This area has been the subject of a recently settled Natural Resource Damage Assessment (Trustees of the Palmerton Zinc Pile Superfund Site, 2011). A barren area of about 600–800 ha is surrounded by a larger area of reduced canopy cover and reduced plant vigor. Zinc is phytotoxic to both seedlings and mature trees on Blue Mountain (Beyer et al., 2011; Jordan, 1975) and Mn and Cd, also emitted by the smelters, may contribute to the phytotoxicity. We investigate the phytotoxicity of Blue Mountain soil to five indigenous species of tree seedlings and to soybeans, a species that has been the subject of several metal phytotoxicity studies.

Jordan (Buchauer, 1973; Jordan, 1975) experimentally demonstrated that tree seedlings grew better in soil collected far from the Palmerton smelters than in soil close to the smelters, that Zn caused chlorosis and marginal necrosis in seedling foliage, and that Zn inhibited root growth and was the probable cause of the observed phytotoxicity. Our study builds on these findings and develops quantitative criteria necessary for evaluating phytotoxicity and for in use in ecological risk assessment. The phytotoxic thresholds that we calculate, expressed as Zn concentrations in roots, leaves and soils (total and extractable Zn), provide a means to evaluate possible Zn phytotoxicity at other contaminated sites – to gauge the severity of an exposure, to determine cause and effect of an injury, and to develop cleanup concentrations, if necessary. Assessments are most reliable when based on concentrations in both soils and tissues, as well as on observations on the foliage. Our study also considers the possibility that Mn is phytotoxic, the role of Ca in the observed phytotoxicity, and the beneficial effect of liming to remediate the site.

Zinc phytotoxicity has been demonstrated in soils contaminated by smelters and mining waste, incinerators, excessive applications of fertilizers and pesticides, burned rubber residues, galvanized materials, livestock manures and biosolid sewage sludge (Chaney, 1993). Zinc tends to be accumulated to a greater extent in roots than in leaves, interfering with root growth and elongation, thereby limiting a plant's uptake of water and nutrients (Castiglione et al., 2007; Disante et al., 2010; Påhlsson, 1989). Because Zn^{2+}'s activity in soil increases with acidity, liming counters its phytotoxicty (Lott, 1958). High Zn concentrations may induce Fe-deficient chlorosis (Chaney, 1993) and interfere with Ca metabolism. In field-grown peanuts (*Arachis hypogaea* L.), toxicity has been found to be associated with foliar Ca to Zn ratios less than 50 (Parker et al., 1990). Phytotoxic concentrations of both Zn and Cd in tree leaves are associated with chlorosis, wilting, and stunting of leaves and with reduced initiation and growth of roots, especially root laterals

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(Mitchell and Fretz, 1977). Symptoms of Zn toxicity overlap with those of nutrient deficiencies and other phytotoxic metals; thus, diagnoses are usually based on a combination of observations with comparisons to toxicity thresholds measured as extractable soil metal concentrations or as total Zn in plant tissue.

Cadmium is usually associated with Zn and, on a mass basis, is more phytotoxic than Zn. Because it is generally much less abundant ($<1\%$ Zn level in geogenic sources) in the environment than Zn, however, it is usually less toxic to plants but may be a concern to humans consuming crops and to wildlife. The two metals interact and are best evaluated together. Because high soil Zn concentrations inhibit Cd uptake by plants, Cd generally does not reach concentrations toxic to plants or consumers, but accumulation may become a concern when the Zn to Cd ratio is abnormally low or when agronomic conditions greatly favor accumulation of Cd in plants, such as in rice (Chaney and Ryan, 1994). We also consider the possibility of Mn phytotoxicity in the tree species studied, partly as a result of observing phytotoxic symptoms of Mn in the soybeans in the course of the study.

2. Methods

2.1. Collection of site and reference soils

The soils from Palmerton and the reference site belonged to the Hazleton series (Loamy-skeletal, siliceous, active, mesic Typic Dystrudept), which are found on ridgetops along Blue Mountain and were formed in residuum of acid gray, brown, or red sandstone. Hazleton soils have a very strongly acid ochric horizon and natural low fertility (https://soilseries.sc.egov.usda.gov/OSD_Docs/H/HAZLETON.html March 7, 2013). Deposition of zinc oxide from the smelters has increased soil pH near Palmerton (Beyer et al., 2011; Buchauer, 1973). Hazleton soil was collected from the ridgetop of Blue Mountain (40° 47′ 57.494″ N, 75° 34′ 27.786″ W), close to the Appalachian Trail, in an oak forest showing toxic effects from the Palmerton smelters. Soil for the study was dug to about 10 cm deep and included the highly decomposed lower layer of litter, which is abnormally thick in the area (Strojan, 1978). Reference Hazleton soil (40° 47′ 57.494″ N, 75° 34′ 27.786″ W) was collected similarly from an uncontaminated forest on Blue Mountain, by Highway 309. Each batch of soil was sieved (4 mm), mixed thoroughly on a tarp, and stored at an intermediate moisture content at the Beltsville Agricultural Center, where trials were conducted.

2.1.1. Experiment 1 – phytotoxicity comparison among species

Soil treatments were mixed in a cement mixer and named according to the percentage of Palmerton soil, by weight (0% [reference], 10%, 20%, 40%, 60%, 80% and 100%). Reference soil was added to bring the total in each treatment to 100%. Soil treatments were prepared for two trials, three species in each. After significant phytotoxicity was observed in the 10% Palmerton soil in the first trial, a 5% Palmerton soil treatment was added to the second trial.

Gray birch (*Betula populifolia*), chestnut oak (*Quercus prinus*), northern red oak (*Quercus rubra*), and eastern white pine (*Pinus strobus*) seeds were purchased from Sheffield's Seed Co., (Locke, N.Y.), red maple (*Acer rubrum*) seeds were collected locally, and 'Williams 82' soybeans (*Glycine max*) were obtained from the Beltsville Agricultural Research Center. The five tree species grow on Blue Mountain, near Palmerton. Prepared soils were added to 15-cm pots, six replicates per treatment. In the first trial, 5 seeds of red maple, 5 seeds of white pine, and 4 northern red oak acorns were planted per pot and, in the second, 3 gray birch seeds, 4 chestnut oak acorns and 6 soybeans. Pots were placed in a greenhouse, with supplementary light on cloudy days. To reduce variability among pots, we added seeds to those pots with poor germination, bringing the number of seedlings up to the number of seeds initially planted. In this way, we avoided confusing phytotoxic effects with effects resulting from ungerminated seeds. Since seedlings of different species grew at different rates, experiments were terminated when plants in the reference soil were well established (4 weeks for soybeans, 11 weeks for northern red oak, 12 weeks for chestnut oak, 17 weeks for gray birch and 18 weeks for red maple and white pine).

At harvest, the top of each seedling was cut at the root collar. Soybeans tops were left intact but leaves were cut off from the woody stems of the other species. Roots were carefully removed from soil and all tissues were washed in sodium lauryl sulfate, rinsed in deionized water, air-dried, and weighed. The contents of each pot, rather than the individual plants, were considered the experimental units. Tissues were oven-dried overnight at 70° C before being chemically analyzed. Roots were scanned and the total root length per pot was estimated with the use of the WinRHIZO© (Regent Instruments Inc., Canada) computer program.

2.1.2. Experiment 2 – liming to reduce phytotoxicity

Reagent grade powdered amorphous $CaCO_3$ plus $MgCO_3$ ($MgCO_3$ replaced 10% of the $CaCO_3$ application alkalinity) was mixed into reference and contaminated soils (mixture of 40% Palmerton and 60% reference) at progressively higher weights to achieve two series of 6 soil pH's from unamended to slightly over 7. At the highest treatment, the rate was 50 g lime per kg of soil, dry weight. Red oak acorns were planted in soils that had previously been wetted, allowed to partially dry for 10 days, and remixed. The experiment was terminated after 11 weeks.

2.2. Chemical methods

We collected subsamples of soil from each of the treatment groups in experiment 1 before we commenced the trials, air dried and digested them with hot, concentrated HNO_3, and then quantified Zn, Cd, Mn, Cu, Fe and Pb by atomic absorption spectrometry. Additional subsamples of these soils were sent to the Pennsylvania State Analytical Services Laboratory in University Park, PA, to be analyzed for soil pH (1:1 soil to water, by volume), cation exchange capacity, nitrate, ammonium N and Mehlich 3 extractable elements (Zn, S, Al, P, K, Mg, Ca). The Mehlich 3 extractant is a combination of acetic and nitric acids, ammonium fluoride, and the chelator EDTA that is commonly used to estimate plant available trace elements. Organic matter contents of the reference and Palmerton soils were measured as loss on ignition at 450 °C. Subsamples of soil collected at the end of the trials from all pots in both experiments were extracted into a 1:4 soil to 10 mM $Sr(NO_3)_2$ solution (Helmke et al., 1997) that was shaken and filtered. Zinc, Cd, Mn, Al, Ca, and Mg were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES), using Y as an internal standard. Final soil pH's in experiment 2 were measured in a 1:2 soil to water solution. Plant samples were weighed and then ashed in a 480 °C oven for 16 h. After cooling, the ash was digested with 2 mL concentrated HNO_3, swirled and taken to dryness. The residue was dissolved in 10 mL of 3 N HCl, filtered through Whatman #40 filter paper and brought to volume in a 25 mL volumetric flask using 0.1 N HCl (final concentration, 1 N HCl). Concentrations of Zn, Cd, Mn, Cu, and Ca were quantified by ICP-OES. We included in our results only those seedlings whose leaves or tops (soybeans) weighed at least 20 mg. Quality control of all metal analyses was monitored at the Beltsville Agricultural Research Center by the use of blanks, duplicates and NIST reference soils and plant materials, all of which remained within acceptable limits.

2.3. Statistics

Statistical tests were run by the programs SigmaPlot© and SigmaStat© (Systat Software Inc., San Jose, CA). Plant tissues weights were treated as averages per pot by treatment. Because plant weights were not normally distributed, they were first examined with a Kruskal–Wallis one way analysis of variance on ranks and, if significant at $p < 0.05$, evaluated further with Dunn's Method to identify differences from reference values. Spearman rank correlation coefficients were calculated to estimate the degree of correlation between variables. The PT_{50} (phytotoxicity threshold), which is comparable to the "EC_{50}," (effective concentration), is defined here as the concentration of a metal that causes a fifty percent reduction in a measure, such as leaf weight. We fitted phytotoxicity data to 3-variable logistic curves, which are sigmoid curves that directly estimate the PT_{50}. Confidence intervals for the PT_{50}'s were estimated by sight from 95% confidence points along the curve but could not be estimated for red maple because of severe toxicity occurring in this species even at 10% Palmerton soil. Concentrations below detection limits were treated as one half the detection limit in calculations.

3. Results and discussion

3.1. Experiment 1 – phytotoxicity among species compared

3.1.1. Soils

The reference Hazleton soil was relatively infertile, having suboptimal concentrations of K, Mg, and P and Ca (Table 1). Growth of northern red oak on Hazleton soils has previously been shown to be limited by low concentrations of Ca and P (Sharpe et al., 1993). A comparison to national mean agricultural soil metal concentrations (Holmgren et al., 1993) suggests that the reference soil had typical concentrations of Zn, Cd and Cu but that the Pb concentration was to some extent elevated (compare to published means of 56.5 mg Zn/kg, 0.265 mg Cd/kg, 29.6 mg/kg Cu/kg and 12.3 mg Pb/kg). The 21 mg Mn/kg detected in the reference soil was well below the national average in surficial materials of 550 mg Mn/kg (Shacklette and Boerngen, 1984).

Concentrations of Zn, Cd, Mn, Cu, and Pb, metals associated with the ores smelted, increased as expected across the eight treatments. For example, as the fraction of contaminated soil in the mixtures increased from 0% to 100%, Zn concentrations extractable with hot nitric acid increased from 37 mg/kg to 3200 mg/kg (Table 1).