Effect of Plant Species and Benomyl on Lead Concentration and Removal from Lead-enriched Soil

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Abstract. Some agricultural soils in North America are lead (Pb)-enriched as a result of the application of lead arsenate (PbHAsO₄) insecticide. A controlled-environment experiment was conducted with Pb-enriched Canning soil series in Nova Scotia, Canada, to evaluate the remediation potential of 10 plant species in combination with the fungicide benomyl applied as a soil drench to suppress mycorrhizae. Overall, the highest biomass was provided by yellow poppy followed by Indian mustard and thorn apple. The application of benomyl increased Pb concentration in thorn apple tissue but not in the other crops. The phytoremediation potential (Pb removal with the harvested biomass) was higher with clary sage, alyssum, garden sage, and Indian mustard with benomyl treatments and lower in the Swiss chard, thorn apple without benomyl, and in the geranium with benomyl treatments. The results suggest that some plants can be used for phytoremediation of mildly Pb-contaminated soils.

Some agricultural soils in Nova Scotia and other parts of North America are Pb-enriched as a result of the application of PbHAsO₄ insecticide in apple orchards several decades ago (Chisholm, 1972; MacLean and Langille, 1973). Although the Pb concentrations in these soils are relatively low and are not toxic to most crops, Pb accumulation in horticultural crops such as carrots and potato that are the most widely grown horticultural crops in Nova Scotia and Atlantic Canada is a major concern.

Lead is an extremely toxic element, especially for young children. Significant adverse health effects as a result of elevated blood Pb levels in children have been found (Bugroon et al., 1995). Lead is known to affect heme biosynthesis, the nervous system, and blood pressure. Plants growing in unpolluted soils generally accumulate between 0.01 and 3 mg Pb/kg dry weight (Shacklette, 1980). Average total Pb concentrations in agricultural soils worldwide vary between 5 and 30 ppm (mg·kg⁻¹) (Alloway, 1990). Total Pb concen-

trations above 100 ppm (mg·kg⁻¹) in soils are regarded as toxic for plants (Kabata-Pendias and Pendias, 1992). Research has indicated that as a result of its high affinity for soils, the retention time for Pb in soils could be up to 5000 years (Friedland, 1990; Kabata-Pendias and Pendias, 1992).

Phytoextraction appears to be the most suitable and cost-effective alternative to conventional approaches (such as excavation or dredging) for remediation of mildly heavy metal-polluted agricultural soils (Brown, 1995; Chaney, 1995; Cunningham and Ow, 1996; McGrath et al., 2002; Vamerali et al., 2010). Research conducted to identify metal-tolerant plants for use in cleansing polluted soils has identified some wild and cultivated species that absorb metals in significantly elevated concentrations (Brown et al., 1995; Chaney, 1995; Kumar et al., 1995; Vamerali et al., 2010). However, wild plants can be unsuitable for use as phytoextractors of agricultural soils contaminated with heavy metals because their wild nature makes cultivation difficult. In addition, production of a wild species generates no revenue, making the growth of such plants unattractive to farmers with contaminated soils. Related promising crop plants for phytoextraction seem to be Indian mustard [*Brassica juncea* (L.) Czern.], clary sage (*Salvia sclarea* L.), garden sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* L.), sunflower (*Helianthus annuus* L.), scented geranium (*Pelargonium* sp.), common motherwort (*Leonurus cardiaca* L.), horehound (*Marrubium vulgare* L.), hemp (*Cannabis sativa* L.), and willow (*Salix* sp.) (Dan et al., 2002; Vamerali et al., 2010; Xiong

and Feng, 2001; Zheljaskov and Nielsen, 1996; Zheljaskov et al., 2008a, 2008b).

Previous research indicated that mycorrhizae play a role in heavy metal accumulation in plant shoots (Dueck et al., 1986; Khan et al., 2000; Wong et al., 2007). Reduction of mycorrhizal associations by treating the plants with systemic fungicide was shown to significantly improve Pb translocation from plant roots to shoots and subsequent Pb accumulation in shoots (Burke et al., 2000; Wong et al., 2007). Reduced mycorrhizal associations might be used as a tool for increasing Pb accumulation by plants. The usefulness of various plants for phytoextraction of Pb in mildly contaminated Nova Scotia soils is unknown.

The objective of this study was to evaluate the phytoextraction potential of 10 plant species with or without the application of the fungicide benomyl on Pb accumulation in harvestable plant parts and Pb removal. The fungicide benomyl was applied as a soil drench in an attempt to suppress vesicular-arbuscular mycorrhizae development on crop roots.

Materials and Methods

Experiment. The experiment was conducted in the Cox greenhouse of Nova Scotia Agricultural College under natural daylight with day temperatures of 22 to 25 °C and a night temperature of 18 to 19 °C.

The Pb-enriched soil was obtained from a grower's field in Canning, Nova Scotia, with a PbHAsO₄ application history. The total Pb concentration of the soil was ≈109 mg·kg⁻¹. The soil was a sandy loam with 47% sand, 48.2% silt, and 4.8% clay with pH of 6.2 and cation exchange capacity of 17.9.

Plant growing conditions. In each pot (20-cm diameter and 15-cm high plastic containers, Classic 600; Nursery Supplies, Inc., Fairless Hills, PA), 3 kg of air-dried soil was used. Certified seeds of the 10 plant species, namely alyssum (*Alyssum maritimum* ssp. *Benthami*, synonym *Lubularia maritima*), black mustard (*Brassica nigra* L.), clary sage (*Salvia sclarea* L.), garden sage (*Salvia officinalis* L.), Indian mustard [*Brassica juncea* (L.) Czern.], Swiss chard (*Beta vulgaris* L.), thorn apple (*Datura innoxia* Mill.), white mustard (*Sinapis alba* L.), yellow poppy (*Glaucium flavum* Grantz), and zonal geranium (*Pelargonium xhortorum*), were used. The 10 plants were started from seed by direct seeding in the pots. After emergence, plants were thinned down to an equal number per pot within a species.

The experimental design was a two-factor factorial design with four replications. The factors were plant species (nine or 10 levels) and benomyl application (two levels). Irrigation was provided separately for the 10 plant species by watering each pot with enough water to maintain plant growth and development but to avoid any significant leaching out of the pots. Plastic saucers, 31 cm in diameter, were placed under each pot to collect occasional leaching and to ensure that no Pb or nutrients were lost from the system.

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Phosphorus (P) as triple superphosphate and potassium (K) fertilizers as potassium chloride (3 g of P and 4 g of K/pot) and half of the nitrogen (N) (2.5 g N/pot) as ammonium nitrate were provided at seeding through incorporation in the soil. The second half of N was provided 30 d after emergence as a topdress.

Application of benomyl for suppression of mycorrhizae. Benomyl (trade name Benlate®; DuPont, Canada) applied as a soil drench at concentrations as low as 25 mg·kg⁻¹ was shown to provide mycorrhizae-free soil in pots for up to 90 d (Habte, 1997). However, our preliminary experimentation with the application of benomyl indicated a mycorrhizal-suppressing effect for up to 30 d. Hence, we applied benomyl twice as a soil drench at concentrations of 50 mg·kg⁻¹. An important consideration when applying fungicides (i.e., benomyl) to soil for suppressing mycorrhizae is that adverse effects on other soil microflora may occur (Paul et al., 1989; Torstensson and Wessen, 1984). Hence, although some of the plant species tested in this study such as Alyssum, mustards, and Swiss chard do not have mycorrhizal associations, we applied benomyl to all species to keep the experiment balanced and to observe any other potential effect of the fungicide on soil microflora that may affect Pb accumulation in shoots. DuPont discontinued the production of Benlate® as a result of numerous lawsuits (DuPont, 2011), but the chemical was not recalled and the license has not expired, making it available.

Sampling and measurements of lead in plant tissue and soil samples. All plants were harvested at the same time, 78 d after the establishment, by cutting the aboveground shoots ≈2 cm above the soil surface and recording fresh weight. Plants were dried in a drying oven at 70 °C for 72 h, and dry weight was recorded. Representative subsamples from each treatment combination and replicate were ground for analysis of Pb. Soil samples were taken at harvest with a soil probe (six probes/pot), air-dried, and submitted to Pb analysis. The concentration of Pb in tissue and soil samples was determined after nitric acid digestion using the method of Zarcinas et al. (1987) as described previously (Zheljazzkov and Warman, 2004; Zheljazzkov and McNeil, 2008). As a result of the relatively low concentrations of Pb in tissue and soil, and to improve accuracy, we used relatively large samples (4 g of tissue or soil). Samples were digested for at least 8 h in 250-mL digestion tubes, and nitric acid (5 mL at least three times) was added during the digestion process. The available Pb concentrations in soil were determined using extraction with 1 M Mg(NO₃)₂ to extract the exchangeable fraction of Pb as described by Luo and Christie (1998).

Statistical analyses. The effects of species (nine or 10 plant species) and benomyl (with and without benomyl) on dry weight yield of plants, available Pb in the soil, and tissue Pb concentration and Pb accumulation in shoots were determined using a two-factor factorial design. Only the main effect of species was

significant on dry yield and available Pb in the soil, whereas the interaction between species and benomyl was significant on tissue Pb concentration and Pb accumulation in shoots. Consequently, multiple means comparison was conducted to compare the 10 species in terms of mean dry yield and available Pb in the soil using the least significant difference method at the 5% level and produce letter groupings in the GLM procedure of SAS (SAS Institute Inc., 2008). However, the 18 treatment combinations of species and benomyl were compared in terms of mean tissue Pb concentration and Pb accumulation in shoots using the least squares means statement of the GLM procedure in SAS (SAS Institute Inc., 2008), and letter groupings were generated at the 1% level of significance to protect Type I experimentwise (family) error rate from overinflation. For all four response variables, the analysis of variance model assumptions on the error terms, namely normal distribution and constant variance, were verified by examining the residuals as described in Montgomery (2009).

Results and Discussion

Under the conditions of the experiment, yellow poppy provided the highest dry biomass yield followed by Indian mustard and

thorn apple (Fig. 1). The crops that provided less dried biomass per pot included alyssum, black mustard, clary sage, garden sage, Swiss chard, and geranium. White mustard provided similar yield as the crops with the lower biomass production with the exception of clary sage. Also, white mustard yields were not significantly different from thorn apple yields (Fig. 1).

The concentration of available Pb in the soil was measured at harvest to evaluate the potential effect of crops on the bioavailability of Pb in soil. The highest concentration of available Pb was in the pots with geranium, Swiss chard, and thorn apple and the lowest was in the soil with clary sage (Fig. 2).

Benomyl was applied to suppress mycorrhizae and to promote Pb accumulation in plant shoots. The application of benomyl significantly increased Pb concentration in thorn apple tissue but not in the other crops (Fig. 3). Thorn apple is a mycorrhizal plant (Zheljazzkov, 2005). This result indicated that mycorrhizae suppression to improve Pb accumulation in shoots may work in relatively few species. Because fungicides can affect soil microflora (Paul et al., 1989; Torstensson and Wessen, 1984), benomyl might subsequently have an effect on Pb accumulation in shoots. We applied benomyl to all plants; however, the results demonstrated that benomyl application did not affect Pb accumulation

Fig. 1. Mean dry yield (g/pot) for the 10 plant species. Means sharing the same letter are not significantly different.

Fig. 2. Mean available lead in the soil (mg·kg⁻¹) for the 10 plant species. Means sharing the same letter are not significantly different.

Fig. 3. Mean lead concentration in plant shoots ($\text{mg}\cdot\text{kg}^{-1}$) for the nine species without or with benomyl applied. Means sharing the same letter are not significantly different.

Fig. 4. Mean lead accumulation in shoots ($\mu\text{g}/\text{pot}$) for the nine species without or with benomyl applied. Means sharing the same letter are not significantly different.

in shoots in non-mycorrhizal species (black mustard, Indian mustard, Swiss chard, or white mustard).

Of all species, clary sage accumulated the highest concentration of Pb in the above-ground biomass. However, the accumulation of Pb in alyssum, garden sage in the benomyl treatment, and white mustard without benomyl was not statistically different from Pb accumulation in clary sage (Fig. 4). The lowest accumulation of Pb in shoots was in Swiss chard, thorn apple with no benomyl, and in geranium (Fig. 4). The phytoremediation potential (Pb removal with the harvested biomass) was greatest with clary sage, alyssum, garden sage with benomyl, and Indian mustard and lower in the Swiss chard, thorn apple without benomyl, and in the geranium with benomyl treatments (Fig. 4).

Concluding Remarks

Mycorrhizae can play different roles in Pb uptake depending on the concentration of Pb in soil. For example, Wong et al. (2007) reported that mycorrhizal colonization increased Pb by vetiver grass (*Vetiveria zizanioides*) in soil with relatively low Pb

concentration but decreased it in soil with relatively high Pb concentration. Our results on Pb accumulation in shoots of thorn apple support this and previous reports on potential use of mycorrhizae for improving Pb accumulation in shoots.

Benomyl application as a soil drench did not have an effect on Pb accumulation in shoots in non-mycorrhizal species (Black mustard, Indian mustard, Swiss chard, or white mustard) or in most mycorrhizal species.

Indian mustard and other related mustards are used widely for studies on phytoremediation because they are considered metal accumulators (Vamerali et al., 2010). However, our study demonstrated that clary sage may accumulate higher concentrations of Pb than Indian mustards, black mustard, or white mustards. This result further supports a previous report on metal accumulation in clary sage (Zheljzkov and Nielsen, 1996; Zheljzkov and Jeljazkova, 1996). Nevertheless, although Pb accumulation is important; more important is the total Pb removal from the site with the harvestable biomass. This study demonstrated that Pb accumulation and removal with harvestable plant parts from Pb-enriched Nova Scotia

soil was higher with alyssum, black mustard, clary sage, garden sage, Indian mustard, thorn apple with benomyl application, and white mustard and lower with Swiss chard, thorn apple with no benomyl applied and geranium.

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