PROJECT IDENTIFICATION INFORMATION:

Date of Report: June 28, 2004

EPA Agreement Number: Project #8.

Center Name and Institution of Center Director: Rocky Mountain Regional Hazardous Substance Research Center, Colorado State University

Identifier used by Center for Project: Project #8.

Title of Project: METAL TOXICITY THRESHOLDS FOR IMPORTANT RECLAMATION PLANT SPECIES OF THE ROCKY MOUNTAINS

Investigator(s) and Institution(s): Mark W. Paschke and Edward F. Redente, Department of Forest, Rangeland and Watershed Stewardship, Colorado State University.

Type of Research: Applied


Supplemental Keywords: arsenic, copper, manganese, metal pollution, phytotoxicity, restoration, risk assessment, zinc.

GOAL OF RESEARCH

This research will establish soil metal toxicity thresholds for numerous plant species that are commonly used in reclamation activities in the Rocky Mountains. This information is currently not available and, as a result, ecological risk assessments must rely on toxicity thresholds established for agronomic species. These crop plants have very different physiological characteristics and sensitivity levels than native species used in the reclamation of sites contaminated with metals. As a result, risk assessors may classify sites as phytotoxic to native species and call for intensive remediation activities that may not be necessary. The objective of this work is to provide a better estimate of soil metal toxicity thresholds for four metals and a large number of native plant species (and a few commonly used introduced species). These threshold values would be used by those in the reclamation industry (government regulators and private entities) to more accurately assess risks associated with soil metal contamination, and to better match revegetation plant species to site conditions.

STATEMENT OF WORK

Over the two-year study period we will complete the following tasks in order to establish toxicology thresholds:

1) Mn testing on six grass species, 2) Zn testing on six forb species, 3) As testing on six grass species, 4) As testing on six forb species, 5) Cu testing on six forb species, 6) Mn testing on six forb species, 7) Mn testing on six shrub species.
RELEVANCE OF RESEARCH

This research will determine the metal toxicity thresholds for plants that are commonly used in reclamation efforts in the Rocky Mountains. This work will build on similar previous work by the Investigators, which represents the first comprehensive attempt to establish metal toxicity thresholds for native reclamation plant species of the Western U.S. The research will establish toxicity thresholds for approximately 25 plant species and 4 metals of concern. Such an achievement will have State, National and Global benefits. These threshold values could be used in risk assessments where agronomic species data are inappropriately used in place of threshold values for native species. In addition, these studies will identify plant species and genera that would be suited for reclamation projects involving metal-contaminated soils. This knowledge will lower reclamation costs by reducing planting failures.

PROGRESS

Task 1) Mn testing on six grass species.

Progress to date: Greenhouse experiment was completed in 2003. Lab analyses of the plant materials were completed shortly thereafter. Data from the experiment was analyzed and prepared for publication in the journal “Environmental Pollution”. The manuscript was accepted for publication in June of 2004 after minor revisions. These revisions are currently being made.

Percent completed: 95%

Problems encountered and/or unexpected results: None.

Future activities: Minor revisions to the manuscript will be made and returned to the editor during July, 2004.

Task 2) Zn testing on six forb species.

Progress to date: Greenhouse experiment was completed in 2003. Laboratory analyses of the plant material have been completed and the data are currently being analyzed.

Percent completed: 75%

Problems encountered and/or unexpected results: None.

Future activities: A manuscript will be prepared for submission to the journal “Soil and Sediment Contamination” during 2004.

Task 3) As testing on six grass species.

Progress to date: Greenhouse experiment was completed in 2003. Laboratory analyses of the plant material have been completed and the data have been analyzed. A publication is currently being prepared for submission to the journal “New Phytologist”.

Percent completed: 75%

Problems encountered and/or unexpected results: None.
Future activities: Complete preparation of manuscript and submit for peer-review.

Task 4) As testing on six forb species.
Progress to date: Greenhouse experiment was completed in 2003. Laboratory analyses of the plant material have been completed and the data have been analyzed. A publication is currently being prepared for submission to the journal “Environmental Toxicology and Chemistry”.

Percent completed: 75%

Problems encountered and/or unexpected results: None.

Future activities: Complete preparation of manuscript and submit for peer-review.

Task 5) Cu testing on six forb species.
Progress to date: Greenhouse experiment was completed in 2004. Laboratory analyses of the plant material were completed in May, 2004. Data are currently being analyzed.

Percent completed: 50%

Problems encountered and/or unexpected results: None.

Future activities: After analysis, the data will be synthesized and interpreted. A manuscript will then be prepared for a peer-reviewed journal.

Task 6) Mn testing on six forb species.
Progress to date: Greenhouse experiment was completed in June, 2004. Samples are being prepared for laboratory analyses.

Percent completed: 30%

Problems encountered and/or unexpected results: None.

Future activities: Finish preparation of samples for laboratory analysis followed by lab analysis for tissue Mn. After lab analysis, the data will be analyzed, interpreted, and prepared for publication in a peer-reviewed journal.

Task 7) Mn testing on six shrub species.
Progress to date: Preparations are underway for the greenhouse experiment. Seeds are currently undergoing vernalization to break dormancy.

Percent completed: 5%

Problems encountered and/or unexpected results: None.
Future activities: Conduct the greenhouse experiment, followed by lab analyses, data analyses and publication of results.

SUMMARY OF RELEVANT DATA

Results from Task 1 are provided in Appendix 1, which is a manuscript as submitted to “Environmental Pollution”. This manuscript has been accepted for publication after minor revisions as recommended by reviewers.

SCHEDULE AND SCOPE

Table 1. Schedule of tasks as originally proposed and the current status of each.

<table>
<thead>
<tr>
<th>Period</th>
<th>Task</th>
<th>Status (% completed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY03-04</td>
<td>Mn testing on six grass species</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Zn testing on six forb species</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>As testing on six grass species</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>As testing on six forb species</td>
<td>75</td>
</tr>
<tr>
<td>FY04-05</td>
<td>Cu testing on six forb species</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Mn testing on six forb species</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Mn testing on six shrub species</td>
<td>5</td>
</tr>
</tbody>
</table>
APPENDIX 1
Publication from Task 1 accepted for publication in “Environmental Pollution”.

Manganese Toxicity Thresholds for Restoration Grass Species

Mark W. Paschke*, Alejandro Valdecantos and Edward F. Redente

M.W. Paschke and E.F. Redente, Colorado State University, Department of Forest, Rangeland and Watershed Stewardship, Fort Collins, CO 80523-1472 USA. A. Valdecantos, Fundacion Centro de Estudios Ambientales del Mediterráneo (CEAM), 46980 Paterna, Valencia SPAIN

*Corresponding Author: Mark W. Paschke, Colorado State University, Department of Forest, Rangeland and Watershed Stewardship, Fort Collins, CO 80523-1472.
Phone: 970-491-0760
Fax: 970-491-2339
Email: Mark.Paschke@colostate.edu
ABSTRACT

Manganese toxicity thresholds for restoration plants have not been established. As a result, ecological risk assessments rely on toxicity thresholds for agronomic species, which may differ from those of restoration species. Our objective was to provide Mn toxicity thresholds for grasses commonly used in restoration. We used a greenhouse screening study where seedlings of redtop, slender wheatgrass, tufted hairgrass, big bluegrass, basin wildrye, and common wheat were grown in sand culture and exposed to increasing concentrations of Mn. The LC50, EC50-plant, EC50-shoot, EC50-root, PT50-shoot, and the PT50-root were then determined. Phytotoxicity thresholds and effective concentrations for the restoration species were generally higher than values reported for agronomic species. Our estimates of PT50-shoot for the five restoration grasses range from 41,528 to 120,082 mg Mn kg$^{-1}$. Measures of EC50-plant for these restoration grasses ranged from 877 to >6,000 mg Mn L$^{-1}$. These thresholds might be more useful for risk assessors than those based on crop plants that are widely used.

KEYWORDS
Phytotoxicity, manganese pollution, restoration, risk assessment.

CAPSULE
Mn phytotoxicity thresholds for restoration grasses are provided, which should be more useful for risk assessments of metal-contaminated lands than the currently available and widely used thresholds determined for crop plants.
1. Introduction

Manganese is a common metal in the earth’s crust and its presence in soils mainly results from Mn in the parent material. It is also an essential micronutrient for plants. Mn plays a major role as an enzyme activator and it is an essential constituent of the manganese-containing superoxide dismutase that protects tissues from the toxic oxygen free radicals released in various enzyme reactions (Marschner, 1995).

Human practices have raised Mn content and availability in many soils. Mine tailings and metal smelters increase soil Mn concentration and availability with subsequent effects on vegetation structure and composition (Zheljazkov and Nielsen, 1996; Wong et al., 1983). Long-term and heavy dose applications of sewage-sludge (biosolids) or other organic amendments to agricultural soils and soil anaerobic conditions such as waterlogging or poor drainage may also lead to an increase in the content and availability of Mn and other heavy metals (Ramachandran and D'Souza, 1997).

Little is known about metal toxicity thresholds in perennial rangeland species. Increasing knowledge about the sensitivity to heavy metals of wild, non-agricultural plant species would help land managers make appropriate decisions when planning the restoration of Mn contaminated soils. Much of the research dealing with Mn-phytotoxicity thresholds in plants has been carried out with agricultural species. There is little information about the effects of high Mn levels on those non-agricultural plant species suitable for restoring and revegetating Mn contaminated soils (Prodgers and Inskeep, 1991).

Manganese uptake by plants mainly occurs in the reduced-bivalent form, thus its availability increases in acidic soils or anaerobic conditions. High Mn levels in soil may lead to plant nutrient imbalances, especially in relation to other divalent cations such as Mg\(^{2+}\) and Ca\(^{2+}\) (Marschner, 1995; Cenni et al., 1998) and metals such as Zn (de Varennes et al., 2001). In general, nutrient uptake, especially in relation to elements entering the roots by diffusion, may be hampered by Mn due to Mn inhibition of root hair production and reduction of stomata dimensions (Lidon, 2002). High substrate Mn may thus reduce plant growth due to other nutrient deficiencies instead of Mn toxicity (Langheinrich et al., 1992). In fact, one of the first symptoms associated with Mn toxicity is related to both Ca and Mg deficiencies (Marschner, 1995).

Two plant strategies have been proposed for tolerating high substrate Mn concentrations. One mechanism is related to the internal tissue tolerance by which high Mn concentrations are permitted within the leaves or roots with no apparent toxic effect (Horst, 1988; Ross and Kaye, 1994). The second strategy is to reduce Mn uptake and therefore avoiding high tissue Mn concentration (Quartin et al., 2001). Whole-plant or specific tissue Mn concentration generally increases with increasing substrate concentration of Mn, but different effects are often observed for different species, varieties or genotypes in relation to their sensitivity to Mn (Scott et al., 1998; Lee et al., 1996; Choi et al., 1996). Mahmoud and Grime (1977) observed that the susceptibility of a given species to high Mn levels was related to their ecology and the ability of the species to tolerate acidic soils.

In establishing metal toxicity thresholds for plants it is important to consider several characteristics of toxicity: the quantity and species of metal, the route of exposure, the distribution of the metal both spatially and temporally, the type and severity of injury, and the time needed to produce the injury (Ross and Kaye, 1994). Several methods for describing metal toxicity in plants have been proposed. Most of these have been derived from measures of human or animal health assessments. A discussion of these methods is presented in Ross and Kaye.
(1994). The lethal concentration (LC) is the concentration of a toxin that kills a specified percentage of organisms. Effective concentration (EC) is the concentration of a toxin that produces an observable negative effect in the organism. The phytotoxicity threshold (PT) is the tissue concentration of a plant that corresponds with a defined growth reduction.

Metal toxicity thresholds for plants can be used to estimate a plant’s ability to establish and survive on a contaminated site. Unfortunately, there is a paucity of data on toxicity thresholds for native plant species (Ross and Kaye, 1994) and ironically, there is a lack of information for species that are used to restore heavy metal contaminated sites. Miles and Parker (1979) have identified Cd toxicity thresholds for seven plant species native to northwestern Indiana, and in previous work we have determined Zn (Paschke et al., 2000) and Cu (Paschke and Redente, 2002) toxicity thresholds for a variety of grass species. Others have attempted to establish toxicity thresholds for individual native plant species using a few metals (for example: Pedersen et al., 2000; Symeonidis et al., 1985; Hogan and Rauser, 1979; Ehinger and Parker, 1979). Most work on metal effects on native plant species has focused on relative toxicity of species or ecotypes for selection and use in phytoremediation efforts (for example: Ebbs and Kochian, 1997; Wu and Kruckeberg, 1985; Humphreys and Nicholls, 1984; Pollard, 1980). The vast majority of plant metal toxicity thresholds have been determined for agricultural species (reviewed by Gough et al., 1979).

Due to the paucity of Mn toxicity thresholds established for restoration species, ecological risk assessments and natural resource damage assessments conducted in the United States under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 must rely on toxicity thresholds established for agronomic species. These crop plants may have very different physiological characteristics and sensitivity levels than species used in the restoration of sites contaminated with metals and may therefore be inappropriate for these ecological assessments.

Many metal toxicity thresholds for plants are determined in greenhouse or laboratory experiments by growing plants in nutrient solutions containing known concentrations of metals (reviewed by Macnicol and Beckett, 1985). While these conditions do not mimic field conditions, they may provide a conservative first estimate of toxicity thresholds. Many factors that are lacking in solution culture experiments would be expected to reduce metal toxicity to plants growing in the field. These factors include rhizosphere organisms such as mycorrhizae (Brown and Wilkins, 1985; Bradley et al., 1982; Jones and Hutchinson, 1986; Martino et al., 2000; Van Tichelen et al., 2001) and metal binding with soil organic matter (Alloway, 1990; Stevenson and Ardakani, 1972; Ghosh and Banerjee, 1997) and clays (Alloway, 1990). Thus, toxicity thresholds determined from solution culture experiment would likely be lower than actual field toxicity thresholds.

In previous studies (Paschke and Redente, 2002; Paschke et al., 2000), we have determined zinc and copper toxicity thresholds for several grass species that are commonly used in restoration activities in Western North America. In this paper, we describe a similar study of Mn toxicity thresholds for grass species used in restoration efforts. The objective of this study was to provide a better estimate of Mn toxicity thresholds for five grass species that are commonly used in restoration efforts in the Western United States. Until now, this information has been unavailable and, as a result, ecological risk assessments have relied on Mn toxicity thresholds established for agronomic species.
2. MATERIALS AND METHODS

2.1 Plant growth conditions

A greenhouse screening study was used to determine Mn toxicity thresholds for redtop (Agrostis gigantea Roth.), slender wheatgrass (Elymus trachycaulus [Link] Gould ex Shinners var. Pryor), tufted hairgrass (Deschampsia caespitosa (L.) Beauv.), big bluegrass (Poa ampla J. Presl var. Sherman), basin wildrye (Leymus cinereus [Scribn. & Merr.] A. Löve var. Magnar), and common wheat (Triticum aestivum L. var. Oslo). Common wheat is an agricultural crop and redtop was introduced to North America from Europe; the remaining species are native to the Western U.S. where they are commonly used in restoration and reclamation projects. Wheat seed was obtained from a local agricultural seed supplier and the restoration species were obtained from Granite Seed Company (Lehi, UT, USA), a company that typically supplies the restoration industry. Although previous studies have noted ecotypic metal tolerance variation in native plant species (Symeonidis et al., 1985; Hogan and Rauser, 1979; Ehinger and Parker, 1979), we used seed that would typically be used in the restoration of metal-contaminated sites as an approximation of species toxicity thresholds.

A sand culture technique was used to establish toxicity thresholds because many of these arid and semiarid grass species do not grow well in aerated solution culture. Approximately three seeds of each species were sown directly into 3.8- x 21-cm plastic Cone-tainer™ tubes (Stuewe & Sons, Corvallis, OR, USA). Each tube was filled with approximately 350 cm³ of washed quartz sand (Quikrete® Play Sand) and the sand was covered with approximately 1 cm of perlite to retain moisture at the sand surface. Sand-filled tubes were rinsed daily with approximately 300 ml of water for one week prior to seed sowing. Preliminary tests showed the sand to have a pH of 6.93 (0.01M CaCl₂). Although the pH of the media can be important for Mn availability in bulk soil, it has been demonstrated that the pH of the rhizosphere, which can be much lower than the pH of bulk soil, is the more important measure for determining plant uptake of Mn in greenhouse and field soils (Reisenaur, 1988). Tests of leachate from the sand-filled tubes found no detectable water soluble metals in this media. The pH of water, treatment solutions and plant nutrient solutions were not significantly altered by passage through growth containers filled with sand. A glass wool plug was put in the bottom of each container to keep the soil from escaping through drainage holes. After emergence, seedlings were thinned to one individual per tube.

Manganese treatment began when the seedlings were approximately 4 weeks old. All plants were provided with a complete nutrient solution (Miracle-Gro™ Nutriblend 21-18-18) on alternate days prior to Mn treatments. The fertilizer was applied at standard rate (50 ppm N) via a fertilizer injector. Forty nine seedlings of each of the six species were exposed to one of seven supplemental Mn treatments: 0, 1000, 2000, 3000, 4000, 5000, or 6000 mg Mn L⁻¹. Manganese treatments were administered by application of MnSO₄ solutions on alternate days (MWF) with nutrient solution being added separately (TT). Plants were provided with water as needed on weekends (during the first 30 days of the experiment the small seedlings rarely required weekend watering). Nutrient solution, water and Mn treatments were applied in amounts that saturated the media as evidenced by drainage of solution out of the bottom of the tubes. This treatment regime was continued for 60 days. During the growth period, the greenhouse was maintained at 23 ± 8 °C, with an extended photoperiod of 16 h using 400 W Na vapor lamps that provided approximately 300 µmol m⁻² s⁻¹ of photosynthetically active radiation at a distance of 1.5 m.
2.2 Measures of toxicity

There are numerous measures of metal toxicity thresholds in plants (Ross and Kaye, 1994). In this study, we determined six commonly-used measures of toxicity: The 60-day LC50 (the concentration of metal that kills 50% of the seedlings by 60 days), the 60-day EC50-plant (the concentration of metal that reduces seedling biomass by 50% after 60 days), the 60-day EC50-shoot (the concentration of metal that reduces shoot biomass by 50% after 60 days), the 60-day EC50-root (the concentration of metal that reduces root biomass by 50% after 60 days), the PT50-shoot (the shoot metal concentration corresponding to a 50% seedling biomass reduction), and the PT50-root (the root metal concentration corresponding to a 50% seedling biomass reduction). The LC50 was determined from observations of plant status, alive or dead, at the conclusion of the greenhouse experiment. Sixty days after treatments began, seedlings were harvested and the sand was separated from the roots by gently washing under a stream of water. Roots were separated from shoots and both were dried to constant mass at 55 °C and weighed to determine EC50 values. Treatment effects on root, shoot and plant mass were also evaluated directly using univariate analyses. Differences between control and treatment means were tested using a Tukey's Studentized Range test ($\alpha = 0.05$) on SAS PROC GLM version 8.01 (SAS Institute, Inc., Cary, NC, USA). A subset of root and shoot samples (five plants from each species x Mn treatment combination) were then analyzed for Mn concentrations by HNO$_3$ / HClO$_4$ digestion and analysis by inductively coupled plasma emission spectroscopy at the Soil and Plant Analysis Laboratory at Colorado State University.

Toxicity thresholds were calculated from the data by fitting them to linear and polynomial models using SAS version 8.01 (SAS Institute, Inc., Cary, N.C., USA). The model (either linear or polynomial) that resulted in the best fit to the data, as determined by $R^2$ and p values, was used to calculate each toxicity threshold.

3. RESULTS

Mortality varied greatly by species during the 60-day study period (Table 1). Redtop, basin wildrye and big bluegrass all had high survival with Mn treatment levels of 4000 mg L$^{-1}$ or higher. Tufted hairgrass survived well up to 2000 mg L$^{-1}$, whereas slender wheatgrass and common wheat had very low survival even at the lowest treatment level of 1000 mg L$^{-1}$. These survival rates resulted in high estimates for LC50 (>4500 mg L$^{-1}$) for redtop, basin wildrye and big bluegrass (Table 2), a relatively moderate LC50 for tufted hairgrass (2568 mg L$^{-1}$) and a low LC50 for slender wheatgrass (248 mg L$^{-1}$). Survival of common wheat with the range of Mn concentrations that we tested was too low to calculate an LC50 for this taxa (<1,000 mg L$^{-1}$). Trends in plant size for the various species exposed to Mn were similar to those of survival, with redtop and big bluegrass showing little reduction in plant size relative to other species at the higher treatment levels (Figure 1). Individual big bluegrass plants did show reduced size (in both shoot and root mass) at treatment levels over 1000 mg L$^{-1}$. Whereas, in redtop only root biomass was reduced by Mn additions while shoot biomass increased relative to controls at treatment levels as high as 4000 mg L$^{-1}$ resulting in few significant changes in overall plant size (root plus shoot) across the treatment gradient (Figure 1A). No other species showed a significant increase in growth as a result of the Mn treatments (Figure 1). It should be noted that the fertilizer solution that we provided to all plants twice a week, including controls, contained Mn (7 mg L$^{-1}$) intended to meet the plant’s basic nutritional requirements.

Estimated EC50-shoot values ranged from 707 to greater than 6000 mg Mn L$^{-1}$ (Table 2). Most of the restoration grass species had EC50-shoot values that exceeded the Mn concentration
range tested (> 6,000 mg L\(^{-1}\)). Common wheat (EC50-shoot <1000 mg L\(^{-1}\)) and slender wheatgrass (EC50-shoot = 248 mg L\(^{-1}\)) appeared to be the most sensitive species with respect to Mn effects on shoot growth, with significant reductions in shoot mass occurring at 1000 mg Mn L\(^{-1}\) (Figure 1). Roots of all of these grass species appeared to be more sensitive than shoots to Mn induced growth reductions (Figure 1). Common wheat and slender wheatgrass were again the most sensitive species to Mn, with root growth being significantly reduced at 1000 mg L\(^{-1}\) (Figure 1, Table 2). The effects of Mn on whole plant biomass were similar to those of roots and shoots, with common wheat and slender wheatgrass being sensitive and the majority of the restoration grasses showing less sensitivity to Mn.

Manganese was readily taken up in large amounts by all species (Figure 2). Large amounts of Mn were retained in roots with generally lesser amounts translocated to shoots (Figure 2). Calculated PT50-shoot values ranged from 5,732 mg Mn kg\(^{-1}\) for common wheat to 120,082 mg Mn kg\(^{-1}\) for redtop. Estimated PT50-root values ranged from 6,295 mg Mn kg\(^{-1}\) for common wheat to 151,630 mg Mn kg\(^{-1}\) for Basin wildrye.

4. DISCUSSION

Metal toxicity thresholds in plants can be difficult to determine due to complex interactions between the toxic metal and other nutrient elements, as well as other complex biological and physical factors (Foy et al., 1978). Here, we have identified Mn phytotoxicity thresholds for several important restoration grass species and common wheat using a simplified approach that circumvents many of these experimental pitfalls.

Mean Mn concentrations for shoots in the control treatment, which received 7 mg Mn L\(^{-1}\) in fertilizer solution, were between 204 and 563 mg kg\(^{-1}\). This range is well above the 10 to 30 mg kg\(^{-1}\) general plant Mn deficiency levels reported by Kabata-Pendias (2001). This would seem to indicate that adequate Mn was provided to the plants in the fertilizer solution, but we did observe a significant increase in shoot growth of redtop at Mn applications of up to 4000 mg L\(^{-1}\) (Figure 1A). This hormetic dose-response is typical of many toxicological processes (Calabrese and Baldwin, 2003) but we were surprised to see it at such high Mn treatment levels for this species.

The phytotoxicity thresholds that we have determined for these reclamation species (Table 2) are generally higher than those values that have been reported for agronomic species (Table 3). The one agricultural species that we included in our study, common wheat, was the most sensitive species tested since it nearly always had the lowest values for each threshold type (Table 2). Foy et al. (1973) reported a Mn-EC50-shoot value of >32 mg L\(^{-1}\) and an EC50-root value of 16-32 mg L\(^{-1}\) for wheat. Our estimates of 707 and 632 for these same measures on common wheat are considerably higher. Other estimates of Mn-PT10-shoot for wheat are in the range of 200 to 2561 mg L\(^{-1}\) (Foy et al., 1973; Ohki, 1984; De Marco et al., 1995). These estimates for PT10 (the shoot metal concentration corresponding to a 10% seedling biomass reduction) are not directly comparable to our PT50-shoot estimate of 5732 mg L\(^{-1}\) for common wheat. However, our estimate appears to be reasonable for common wheat given our higher threshold criteria (50% biomass reduction versus 10%).

Few Mn phytotoxicity thresholds have been reported for nonagricultural grass species. Data from Jackson and coworkers (1995) can be used to estimate a Mn-PT50-shoot for buffalograss at >14,100 mg kg\(^{-1}\). Data from Lee and coworkers (1996) indicate a similar Mn-PT50-shoot of >14,900 for Kentucky bluegrass. Our estimates of PT50-shoot for five
nonagricultural grass species range from 41,528 mg Mn kg\(^{-1}\) for tufted hairgrass to 120,082 mg Mn kg\(^{-1}\) for redtop.

Roots appeared to be slightly more negatively affected by Mn than shoots (Figure 1). This differential effect of Mn on roots versus shoots for various species indicates that a more robust measure of effective concentrations may be the EC50-plant. On sites with no existing vegetation, where PT measures are not possible, EC measures could be useful for selecting species and understanding site limitations in restoration planning where they can be related to levels of soil solution Mn. Monitoring soil solution Mn with lysimeters could accomplish this. Our measures of EC50-plant for restoration grasses ranged from 877 to more than 6,000 mg Mn L\(^{-1}\). These Mn phytotoxicity concentrations should be generally applicable to those obtained from lysimeter solutions. Based on EC50-plant values, it appears that slender wheatgrass and common wheat are sensitive to Mn relative to the other restoration grass species. From our data it appears that redtop, tufted hairgrass, Basin wildrye and big bluegrass would all be good species for restoration of Mn contaminated sites. Our observation that the agronomic species used in this experiment (common wheat) was the least Mn tolerant species is important because it indicates that risk assessments conducted using thresholds for agronomic species may call for remediation efforts that might not be justifiable where restoration grass species are to be used.

5. CONCLUSIONS

The Mn toxicity thresholds reported here for restoration grass species are generally high relative to common wheat and to values for other agronomic species reported in the literature (Table 3). Under field conditions, Mn stress would act synergistically with other environmental factors (for example: competition, disease, herbivory) and would result in greater mortality than was observed in this simple greenhouse study. We recognize that toxicity thresholds reported here are only approximations of what might be observed in the field due to the assumptions implicit in the experimental design. Nevertheless, these thresholds should be more useful for risk assessors than the currently available and widely used thresholds determined for crop plants using similar methodology.

ACKNOWLEDGEMENTS

This research was made possible through funding from the Colorado Agriculture Experiment Station, Project Number COL006600 and U.S. EPA – Science to Achieve Results (STAR) Program Grant #R-82951501-0. We would like to thank Daniel LeCain and the USDA-ARS Rangeland Resources Research Unit for the generous use of their greenhouse facilities to conduct this research. Fundacion CEAM is funded by Generalitat Valenciana and Bancaixa.

REFERENCES


Table 1. Percent survival (after 60 days) of grass species exposed to various Mn treatment levels. These survival values were used to estimate LC50’s (Table 2). Values are raw scores for survival of all of the seedlings in the experiment. Values for n are shown after each percent survival value. The number of seedlings (n) used in each species by treatment combination varied due to lack of germination in some of the tubes. The original number of tubes planted for each species by treatment combination was 49.

<table>
<thead>
<tr>
<th>Treatment Mn (mg L⁻¹)</th>
<th>Species</th>
<th>Redtop</th>
<th>Slender Wheatgrass</th>
<th>Tufted Hairgrass</th>
<th>Basin Wildrye</th>
<th>Big Bluegrass</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 (36)</td>
<td>100 (49)</td>
<td>100 (48)</td>
<td>98 (49)</td>
<td>98 (48)</td>
<td>94 (49)</td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td>89 (38)</td>
<td>18 (49)</td>
<td>100 (47)</td>
<td>96 (49)</td>
<td>100 (48)</td>
<td>2 (49)</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>89 (37)</td>
<td>0 (49)</td>
<td>76 (46)</td>
<td>94 (49)</td>
<td>89 (44)</td>
<td>0 (49)</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>86 (44)</td>
<td>0 (49)</td>
<td>9 (46)</td>
<td>73 (48)</td>
<td>60 (35)</td>
<td>0 (49)</td>
</tr>
<tr>
<td>4000</td>
<td></td>
<td>61 (44)</td>
<td>0 (49)</td>
<td>0 (49)</td>
<td>71 (48)</td>
<td>68 (22)</td>
<td>0 (49)</td>
</tr>
<tr>
<td>5000</td>
<td></td>
<td>53 (45)</td>
<td>0 (49)</td>
<td>0 (46)</td>
<td>53 (49)</td>
<td>44 (16)</td>
<td>0 (49)</td>
</tr>
<tr>
<td>6000</td>
<td></td>
<td>13 (43)</td>
<td>0 (48)</td>
<td>4 (45)</td>
<td>38 (47)</td>
<td>33 (48)</td>
<td>0 (49)</td>
</tr>
</tbody>
</table>
Table 2. Estimated manganese toxicity thresholds for lethal concentration (LC50), effective concentrations (EC50-shoot, EC50-root and EC50-plant), and phytotoxicity thresholds (PT50-shoot and PT50-root) for restoration grasses and common wheat. Values for LC50 and EC50s are mg Mn L\(^{-1}\), values for PT50s are mg Mn kg\(^{-1}\). Model statistics are shown for each estimate.

<table>
<thead>
<tr>
<th></th>
<th>Redtop</th>
<th>Slender Wheatgrass</th>
<th>Tufted Hairgrass</th>
<th>Basin Wildrye</th>
<th>Big Bluegrass</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LC50</strong></td>
<td>4604</td>
<td>248</td>
<td>2568</td>
<td>5403</td>
<td>4736</td>
<td>&lt;1000(^a)</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.85)</td>
<td>(R^2 = 0.49)</td>
<td>(R^2 = 0.81)</td>
<td>(R^2 = 0.93)</td>
<td>(R^2 = 0.92)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(p = 0.0033)</td>
<td>(p = 0.0818)</td>
<td>(p = 0.0056)</td>
<td>(p = 0.0004)</td>
<td>(p = 0.0007)</td>
<td></td>
</tr>
<tr>
<td><strong>EC50 shoot</strong></td>
<td>&gt;6000(^a)</td>
<td>886</td>
<td>&gt;6000(^a)</td>
<td>&gt;6000(^a)</td>
<td>&gt;6000(^a)</td>
<td>707</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.32)</td>
<td>(p &lt; 0.0001)</td>
<td></td>
<td>(R^2 = 0.55)</td>
<td>(p &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td><strong>EC50 root</strong></td>
<td>&gt;6000(^a)</td>
<td>868</td>
<td>&gt;6000(^a)</td>
<td>&gt;6000(^a)</td>
<td>&gt;6000(^a)</td>
<td>632</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.36)</td>
<td>(p &lt; 0.0001)</td>
<td></td>
<td>(R^2 = 0.59)</td>
<td>(p &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td><strong>EC50 plant</strong></td>
<td>&gt;6000(^a)</td>
<td>877</td>
<td>&gt;6000(^a)</td>
<td>&gt;6000(^a)</td>
<td>&gt;6000(^a)</td>
<td>667</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.35)</td>
<td>(p &lt; 0.0001)</td>
<td></td>
<td>(R^2 = 0.58)</td>
<td>(p &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td><strong>PT50 shoot</strong></td>
<td>120082</td>
<td>63446</td>
<td>41528</td>
<td>70944</td>
<td>67241</td>
<td>5732</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.44)</td>
<td>(R^2 = 0.75)</td>
<td>(R^2 = 0.61)</td>
<td>(R^2 = 0.67)</td>
<td>(R^2 = 0.61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(p &lt; 0.0001)</td>
<td>(p &lt; 0.0001)</td>
<td>(p = 0.0015)</td>
<td>(p &lt; 0.0001)</td>
<td>(p &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td><strong>PT50 root</strong></td>
<td>&gt;48000(^a)</td>
<td>&gt;29000(^a)</td>
<td>36865</td>
<td>151630</td>
<td>&gt;26900(^a)</td>
<td>6295</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.66)</td>
<td>(R^2 = 0.60)</td>
<td>(R^2 = 0.66)</td>
<td>(R^2 = 0.60)</td>
<td>(R^2 = 0.96)</td>
<td>(p = 0.0006)</td>
</tr>
</tbody>
</table>

\(^a\) Estimated threshold is outside the data range and can only be estimated based upon the highest or lowest treatment level for LC50 and EC50s or mean tissue concentration for PT50s.
Table 3. Mn phytotoxicity thresholds (PT) and effective concentrations (EC) for grasses that have been published, or have been calculated here from published data.

<table>
<thead>
<tr>
<th>Plant taxa</th>
<th>Threshold</th>
<th>Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>corn (<em>Zea mays</em> L.)</td>
<td>EC10-shoot</td>
<td>400</td>
<td>(Fageria, 2001)</td>
</tr>
<tr>
<td></td>
<td>PT50-shoot</td>
<td>~ 4000</td>
<td>(Fageria, 2001)</td>
</tr>
<tr>
<td>rice (<em>Oryza sativa</em> L.)</td>
<td>EC10-shoot</td>
<td>560</td>
<td>(Fageria, 2001)</td>
</tr>
<tr>
<td></td>
<td>PT50-shoot</td>
<td>&gt;5000</td>
<td>(Fageria, 2001)</td>
</tr>
<tr>
<td>triticale (<em>×Triticosecale rimpauli</em> Wittm. [<em>Triticum aestivum</em> × <em>Secale cereale]</em>)</td>
<td>EC50-shoot</td>
<td>&gt;25</td>
<td>(Quartin et al., 2001)</td>
</tr>
<tr>
<td></td>
<td>EC50-root</td>
<td>&lt;25</td>
<td>(Quartin et al., 2001)</td>
</tr>
<tr>
<td>wheat (<em>Triticum aestivum</em> L.)</td>
<td>PT10-shoot</td>
<td>396-2561</td>
<td>(Foy et al., 1973)</td>
</tr>
<tr>
<td></td>
<td>EC50-shoot</td>
<td>&gt;32</td>
<td>(Foy et al., 1973)</td>
</tr>
<tr>
<td></td>
<td>EC50-root</td>
<td>16-32</td>
<td>(Foy et al., 1973)</td>
</tr>
<tr>
<td></td>
<td>PT10-shoot</td>
<td>200-1100</td>
<td>(Ohki, 1984)</td>
</tr>
<tr>
<td></td>
<td>PT10-shoot</td>
<td>373</td>
<td>(Ohki, 1985)</td>
</tr>
<tr>
<td></td>
<td>PT10-shoot</td>
<td>570</td>
<td>(De Marco et al., 1995)</td>
</tr>
<tr>
<td>Plant taxa</td>
<td>Threshold</td>
<td>Value$^a$</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>------------</td>
<td>-----------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><strong>Nonagricultural species:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>buffalo grass (Buchloe dactyloides (Nutt.) Engelm.)</td>
<td>EC50-shoot</td>
<td>&gt;659</td>
<td>(Jackson et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>PT50-shoot</td>
<td>&gt;14100</td>
<td>(Jackson et al., 1995)</td>
</tr>
<tr>
<td>colonial bentgrass (Agrostis capillaris L.)</td>
<td>EC50-shoot</td>
<td>~100</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
<tr>
<td></td>
<td>EC50-root</td>
<td>~100</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
<tr>
<td>Kentucky bluegrass (Poa pratensis L.) (cv. Touchdown)</td>
<td>EC50-shoot</td>
<td>&gt;659</td>
<td>(Lee et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>PT50-shoot</td>
<td>&gt;14900</td>
<td>(Lee et al., 1996)</td>
</tr>
<tr>
<td>sheep fescue (Festuca ovina L.)</td>
<td>EC50-shoot</td>
<td>&gt;200</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
<tr>
<td></td>
<td>EC50-root</td>
<td>&gt;200</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
<tr>
<td>tall oatgrass (Arrhenatherum elatius (L.) Beauv. ex J.&amp; K. Presl)</td>
<td>EC50-shoot</td>
<td>5-25</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
<tr>
<td></td>
<td>EC50-root</td>
<td>2-25</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
<tr>
<td>wavy hairgrass (Deschampsia flexuosa (L.) Trin.)</td>
<td>EC10-shoot</td>
<td>312</td>
<td>(Kroeze et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>EC10-root</td>
<td>&gt;312</td>
<td>(Kroeze et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>EC50-shoot</td>
<td>&gt;312</td>
<td>(Kroeze et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>EC50-root</td>
<td>&gt;312</td>
<td>(Kroeze et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>EC50-shoot</td>
<td>&gt;200</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
<tr>
<td></td>
<td>EC50-root</td>
<td>&gt;200</td>
<td>(Mahmoud and Grime, 1977)</td>
</tr>
</tbody>
</table>

$^a$ Values for phytotoxicity thresholds (PT’s) are mg kg$^{-1}$ plant tissue; values for effective concentrations (EC’s) are mg L$^{-1}$. 
FIGURE LEGENDS

Figure 1. Effect of Mn on plant biomass presented as a percentage of the control means. Panel A shows redtop, slender wheatgrass and tufted hairgrass. Panel B shows basin wildrye, big bluegrass and common wheat. Thin bars represent the standard error of the mean \( n = \) between 49 and 1 depending on mortality of test plants during the study period. Treatment means that are significantly different from the corresponding control mean at \( \alpha = 0.05 \) by using a Tukey's Studentized Range test are indicated by an asterisk (*).

Figure 2. Relationships between plant tissue Mn concentrations and growth reduction in shoots and roots of various grass species growing in sand culture and exposed to supplemental Mn treatments ranging from 0 (control) to 6000 mg Mn L\(^{-1}\). Error bars represent the standard error of the mean. Note that axes for shoots and roots are not scaled uniformly.
Figure 1A

[Bar chart showing the percentage of control mean for Shoot Mass, Root Mass, and Plant Mass for Redtop, Slender Wheatgrass, and Tufted Hairgrass across different treatments (mg Mn L⁻¹ x 1000)].
Figure 1B

Percentage of Control Mean

Shoot Mass

Root Mass

Plant Mass

Basin Wildrye

Big Bluegrass

Wheat

Treatment (mg Mn L\(^{-1}\) x 1000)
Figure 2.

Percentage of Control Mean

Shoot Mass

Redtop

Slender Wheatgrass

Tufted Hairgrass

Basin Wildrye

Big Bluegrass

Wheat

Root Mass

Redtop

Slender Wheatgrass

Tufted Hairgrass

Basin Wildrye

Big Bluegrass

Wheat

Tissue Mn (mg kg\(^{-1}\) x 1000)