ZINC TOXICITY THRESHOLDS FOR RECLAMATION FORB SPECIES

MARK W. PASCHKE*, LAURA G. PERRY and EDWARD F. REDENTE
Colorado State University, Department of Forest, Rangeland and Watershed Stewardship, Fort Collins, CO 80523-1472, USA
*(*author for correspondence, e-mail: Mark.Paschke@colostate.edu, Tel.: 970 491 0760, Fax: 970 491 2339)*

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Abstract. Zinc toxicity thresholds for reclamation plants are largely unknown. As a result, ecological risk assessments often rely on toxicity thresholds for agronomic species, which may differ from those of restoration species. Our objective was to provide Zn toxicity thresholds for forb species that are commonly used in reclamation activities. We used a greenhouse screening study where seedlings of yarrow (*Achillea millefolium* L.), Bigelow's tansyaster (*Machaeranthera bigelovii* (Gray) Greene var. *bigelovii*), blue flax (*Linum perenne* L. var. Appar), alfalfa (*Medicago sativa* L. var. Ladak), Palmer's penstemon (*Penstemon palmeri* Gray), and Rocky Mountain penstemon (*Penstemon strictus* Benth. var. Bandera) were grown in sand culture and exposed to increasing concentrations of Zn. Lethal concentrations (LC50 – substrate Zn concentration resulting in 50% mortality), effective concentrations (EC50 – substrate Zn concentration resulting in 50% biomass reduction), and phytotoxicity thresholds (PT50 – tissue Zn concentration resulting in 50% biomass reduction) were then determined. Phytotoxicity thresholds and effective concentrations for these reclamation species were relatively consistent between species. Our estimates of PT50-shoot for these species range from 1258 to 3214 mg Zn kg^{-1}. Measures of EC50-plant for these restoration forbs ranged from 82 to 214 mg Zn L^{-1}. These thresholds might be more useful for risk assessors working on reclamation sites than those based on non-reclamation species that are widely used.

Keywords: phytotoxicity, zinc pollution, restoration, risk assessment

1. Introduction

Zinc is a natural constituent of soils in terrestrial ecosystems. It usually occurs in low concentrations and does not pose a toxicity problem for plants and animals. Zinc is a required element for plant growth as it serves an important role in plant structure and function (Kabata-Pendias and Pendias, 2001). However, increased concentrations of Zn in soils can lead to toxic effects in plants (Chaney, 1993). Potentially toxic quantities of Zn in soils largely result from anthropogenic sources (Chaney, 1993). These include mining and smelting activities, textile and microelectronics industries, pyrometallurgical industries, fossil fuel combustion, agriculture sources (fertilizers, manures and pesticides), corrosion of metals and waste disposal such as land application of sewage sludge (Alloway, 1995; Chaney, 1993; Freedman and Hutchinson, 1981; Kabata-Pendias and Pendias, 2001; Ross, 1994).

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Little is known about metal toxicity thresholds in revegetation plant 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 Zn contaminated soils. Much of the research dealing with Zn-phytotoxicity thresholds in plants has been carried out with agricultural species. There is little information about the effects of high Zn levels on those non-agricultural plant species suitable for restoring and revegetating Zn contaminated soils (Prodgers and Inskeep, 1991).

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, 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, 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: Ehinger and Parker, 1979; Hogan and Rauser, 1979; Pedersen *et al.*, 2000; Symeonidis *et al.*, 1985). Most work on metal effects on native plants has focused on relative toxicity to species or ecotypes for selection and use in phytoremediation efforts (for example: Ebbs and Kochian, 1997; Humphreys and Nicholls, 1984; Pollard, 1980; Wu and Kruckeberg, 1985). The vast majority of plant metal toxicity thresholds have been determined for agricultural species (see reviews by Gough *et al.* (1979) and Macnicol and Beckett (1985)).

Due to the paucity of Zn 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 lab experiments by growing plants in nutrient solutions containing known concentrations of metals (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 alleviate metal toxicity stress to plants growing in the field. These factors include rhizosphere organisms such as mycorrhizae (Bradley *et al.*, 1982; Brown and Wilkins, 1985; Jones and Hutchinson, 1986; Martino *et al.*, 2000; Van Tichelen *et al.*, 2001) and metal binding with soil organic matter (Alloway, 1990; Ghosh and Banerjee, 1997; Stevenson and Ardakani, 1972) and clays (Alloway, 1990). Other factors present in the field such as herbivory, competition and pathogens could act synergistically with metals to reduce a plant's ability to tolerate high metal concentrations. Thus, toxicity thresholds determined from solution culture experiments would likely be different than actual field toxicity thresholds. However, given the extreme heterogeneity associated with soil organisms, organic matter, herbivory, competition and pathogens, both within and between sites, it can easily be argued that metal toxicity data derived from solution culture experiments under controlled conditions have broader utility than field tests.

In previous studies (Paschke and Redente, 2002; Paschke *et al.*, 2000), we have determined Zn and Cu 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 Zn toxicity thresholds for perennial forb species that are commonly used in restoration efforts. The objective of this study was to provide a better estimate of Zn toxicity thresholds for six forb 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 Zn toxicity thresholds established for agronomic species.

2. Materials and Methods

2.1. Plant growth conditions

A greenhouse screening study was used to determine Zn toxicity thresholds for yarrow *(Achillea millefolium* L.), Bigelow's tansyaster *(Machaeranthera bigelovii* (Gray) Greene var. *bigelovii),* blue flax *(Linum perenne* L. var. Appar), alfalfa *(Medicago sativa* L. var. Ladak), Palmer's penstemon *(Penstemon palmeri* Gray), and Rocky Mountain penstemon (*Penstemon strictus* Benth. var. Bandera). Alfalfa is an agricultural crop and is sometimes used as a reclamation species. Yarrow is a circumpolar species and blue flax is a naturalized reclamation species. The remaining species are native to the Western U.S. where they are commonly used in restoration and reclamation projects. Seed was 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 (Ehinger and Parker, 1979; Hogan and Rauser, 1979; Symeonidis *et al*., 1985), we used seed that would typically be used in the restoration of metal-contaminated sites to approximate species toxicity thresholds.

A sand culture technique was used to establish toxicity thresholds because many of these arid and semiarid species do not grow well in aerated solution culture. Approximately five seeds of each species were sown directly into 3.8- × 21-cm plastic Cone-tainer ™ tubes (Stuewe & Sons, Corvallis, OR, USA). Each tube was filled with approximately 350 cm^3 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 (0.01 M $CaCl_3$). Although the pH of the media can be important for Zn 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 metals in greenhouse and field soils (Reisenauer, 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.

Zn 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 Zn 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 Zn treatments: 0, 100, 200, 300, 400, 500, or 600 mg Zn L^{-1}. It should be noted that the fertilizer solution that we provided to all plants twice a week, including controls, contained Zn (0.58 mg L^{-1}) intended to meet the plant's basic nutritional requirements. Zinc treatments were administered by application of $ZnSO_4$ solutions on alternate days (MWF) with nutrient solution being added separately (TT) to maintain Zn in a plant-available form and avoid precipitation of Zn at high treatment levels. 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 Zn 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 $23 \pm 8\,^{\circ}C$, with an extended photoperiod of 16 h using 400 W Na vapor lamps that provided approximately 300 $\mu mol\, m^{-2}\, s^{-1}$ 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 using a hydro pneumatic root elutriator. Roots were separated from shoots and both were dried to constant mass at 65 °C and weighed to determine EC50 and PT50 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 Tukeys Studentized Range test ($\alpha = 0.05$) on SAS PROC GLM version 8.01 (SAS Institute, Inc., Gary, NC, USA). A subset of root and shoot samples (six plants from each species × Zn treatment combination) were then analyzed for Zn 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., Gary, N.C., USA). Mass data were examined as a percent of the mean mass for each species in the controls. 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. Confidence intervals (95%) for the thresholds were calculated using methods for inverse predictions (Nester *et al.*, 1996). Resulting confidence intervals are large because the error includes both the error associated with the model and the error associated with the inverse of the model.

3. Results

Mortality varied by species during the 60-day study period (Table I). Blue flax had high survival at 200 and 300 mg L^{-1} Zn and modest survival at 400 mg L^{-1}. The other species showed relatively poor survival at 400 mg L^{-1} and above. Yarrow showed a rapid decline in survival between 100 and 200 mg L^{-1}. Calculated LC50s (Table I) were rather uniform for these six forb species relative to our previous experience with other species (Paschke et al. 2000), ranging from 190 to 424 mg L^{-1}.

TABLE I

Percent survival (after 60 days) and resulting sixty-day zinc lethal concentrations (LC50) for reclamation forb species

	Species					
Zn (mg L^{-1})	Yarrow	Bigelow's tansyaster	Blue flax	Alfalfa	Palmer's penstemon	Rocky Mtn. penstemon
0	100[a] (49)[b]	100 (49)	100 (49)	100 (49)	100 (49)	100 (49)
100	100 (49)	100 (49)	100 (49)	98 (49)	90 (49)	59 (49)
200	20 (49)	67 (49)	100 (49)	75 (49)	22 (49)	37 (49)
300	0 (49)	33 (49)	78 (49)	22 (49)	19 (48)	35 (49)
400	0 (49)	12 (49)	53 (49)	6 (49)	0 (49)	22 (49)
500	0 (49)	2 (49)	10 (49)	4 (49)	0 (48)	2 (49)
600	2 (49)	2 (49)	0(49)	8 (49)	0 (49)	0 (49)
Model	$86.92 - 0.1838x$	$103.63 - 0.1947x$	$119.45 - 0.1882x$	$102.02 - 0.1904x$	$86.83 - 0.1794x$	$82.39 - 0.1531x$
R^2	0.71	0.92	0.89	0.86	0.80	0.90
P	0.0177	0.0005	0.0015	0.0027	0.0069	0.0011
LC50	201	275	369	273	205	212
95% CI	424	190	234	265	333	217

Survival values were used to estimate LCSO's.

[a]Values are raw scores for survival of all of the seedlings in the experiment.

[b]The number of seedlings (n) used in each species by treatment combination. This number 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 II

Sixty-day zinc effective concentrations (EC50-shoot, EC50-root and EC50-plant) for reclamation forb species

Species	Plant part	Model	R^2	p	EC50	95% CI
Palmer's penstemon	Shoot	$105.37 - 0.1492x$	0.19	<0.0001	371	383
Yarrow	Shoot	$102.96 - 0.2404x$	0.24	<0.0001	220	231
Blue flax	Shoot	$102.94 - 0.4074x + 4.33^{-04}x^2$	0.77	<0.0001	156	88
Alfalfa	Shoot	$97.89 - 0.3743x + 4.11^{-04}x^2$	0.61	<0.0001	154	118
Rocky Mountain penstemon	Shoot	$102.03 - 0.4883x + 6.81^{-04}x^2$	0.52	<0.0001	130	136
Bigelow's tansyaster	Shoot	$100.44 - 0.5207x + 8.90^{-04}x^2$	0.54	<0.0001	123	102
Blue flax	Root	$101.17 - 0.4122x + 5.38^{-04}x^2$	0.40	<0.0001	156	171
Bigelow's tansyaster	Root	$104.17 - 0.5906x + 9.76^{-04}x^2$	0.42	<0.0001	113	134
Alfalfa	Root	$94.99 - 0.5064x + 6.49^{-04}x^2$	0.61	<0.0001	102	105
Palmer's penstemon	Root	$99.73 - 0.7779x + 1.70^{-03}x^2$	0.67	<0.0001	77	62
Rocky Mountain penstemon	Root	$96.11 - 0.8121x + 1.56^{-03}x^2$	0.77	<0.0001	65	57
Yarrow	Root	$100.64 - 1.1113x + 3.60^{-03}x^2$	0.65	<0.0001	56	49
Palmer's penstemon	Plant	$98.83 - 0.2279$x	0.46	<0.0001	214	197
Blue flax	Plant	$101.90 - 0.4102x + 4.94^{-04}x^2$	0.61	<0.0001	156	118
Bigelow's tansyaster	Plant	$101.72 - 0.5447x + 9.20^{-04}x^2$	0.59	<0.0001	119	94
Alfalfa	Plant	$96.12 - 0.4552x + 5.57^{-04}x^2$	0.66	<0.0001	118	96
Yarrow	Plant	$97.25 - 0.4077x$	0.58	<0.0001	116	110
Rocky Mountain penstemon	Plant	$98.37 - 0.6885x + 1.23^{-03}x^2$	0.76	<0.0001	82	65

Values are mg Zn L^{-1}.

Trends in plant size for the various species exposed to Zn (Figure 1) were similar to those of survival. All of the species tested here showed significant reduction of plant growth by 100 or 200 mg L^{-1} Zn. Similar trends in species response to Zn were observed for shoot and root growth, with both above- and below-ground plant parts showing reduced growth in the presence of increasing Zn.

Estimated EC50-shoot values varied little between species and ranged from 123 to 371 mg Zn L^{-1} (Table II). Roots of all of these species appeared to be more sensitive than shoots to Zn induced growth reductions as evidenced by lower EC50-root values (ANOVA, $F_{1.10} = 5.59$, Table II). The effects of Zn on whole plant biomass were similar to those of roots and shoots, with the species showing a narrow range of EC50-plant values. Based upon EC50-plant thresholds, Palmer's penstemon appears to be the most tolerant (EC50-plant = 214) and Rocky Mountain penstemon (EC50-plant = 82) the least tolerant of the species tested.

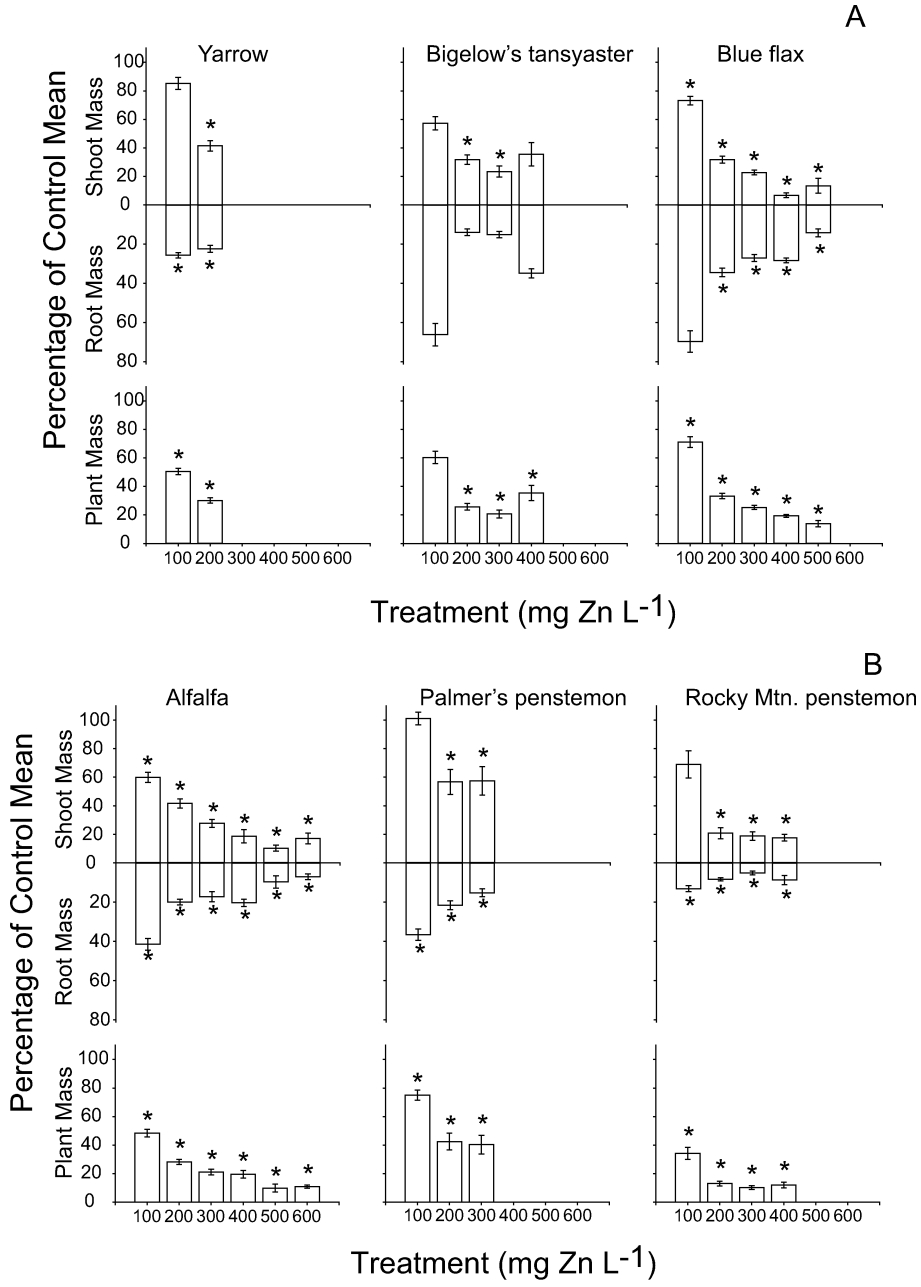

Figure 1. Effect of Zn on plant biomass presented as a percentage of the control means. Panel A shows yarrow, Bigelow's tansyaster and blue flax. Panel B shows alfalfa, Palmer's penstemon and Rocky Mountain penstemon. Thin bars represent the standard error of the mean ($n =$ between 49 and 2 depending 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 (*).

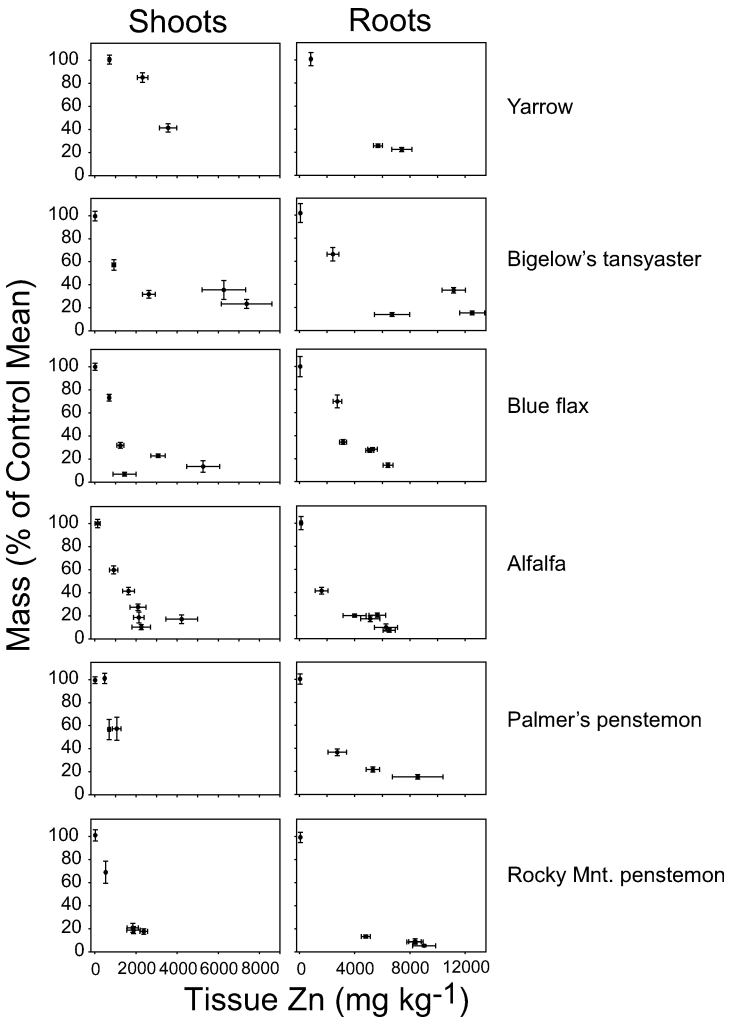

Figure 2. Relationships between plant tissue Zn concentrations and growth reduction in shoots and roots of various reclamation forb species growing in sand culture and exposed to supplemental Zn treatments ranging from 0.58 (control) to 600 mg Zn L^{-1}. Error bars represent the standard error of the mean. Note that axes for shoots and roots are not scaled uniformly. The relationship between these two variables was used to calculate PT50 values (Table III).

Zinc was readily taken up by all species (Figure 2). Roughly twice as much Zn was recovered from roots relative to shoots (Figure 2). Of the species tested, Bigelow's tansyaster contained the highest levels of tissue Zn in both shoots and roots (Figure 2). Calculated PT50-shoot values ranged from 1,258 mg Zn kg^{-1} for Palmer's penstemon to 3,214 mg Zn kg^{-1} for yarrow (Table III). Estimated PT50-root values ranged from 1,543 mg Zn kg^{-1} for alfalfa to 6,018 mg Zn kg^{-1} for Bigelow's tansyaster.

TABLE III
Sixty-day zinc phytotoxicity thresholds (PT50-shoot and PT50-root) for reclamation forb species

Species	Plant part	Model	R^2	p	PT50	95% CI
Yarrow	Shoot	$128.5 - 2.44^{-02}x$	0.84	<0.0001	3214	1352
Bigelow's tansyaster	Shoot	$99.15 - 2.44^{-0.2}x + 1.65^{-06}x^2$	0.76	<0.0001	2400	1674
Rocky Mountain penstemon	Shoot	$116.14 - 3.55^{-02}x$	0.48	<0.0001	1912	2284
Blue flax	Shoot	$88.46 - 2.97^{-02}x + 2.9^{-06}x^2$	0.74	<0.0001	1523	1151
Alfalfa	Shoot	$101.96 - 4.79^{-02}x + 5.76^{-06}x^2$	0.67	<0.0001	1281	928
Palmer's penstemon	Shoot	$112.08 - 4.93^{-02}x$	0.38	0.0014	1258	1354
Bigelow's tansyaster	Root	$152.23 - 2.47^{-02}x^2 + 1.28^{-06}x^2$	0.48	<0.0001	6018	4060
Yarrow	Root	$119.55 - 2.54^{-02}x + 1.63^{-06}x^2$	0.89	<0.0001	3536	1128
Blue flax	Root	$148.21 - 4.13^{-02}x + 3.55^{-06}x^2$	0.52	<0.0001	3322	2082
Palmer's penstemon	Root	$89.02 - 0.0140x + 6.07^{-07}x^2$	0.76	<0.0001	3243	2459
Rocky Mountain penstemon	Root	$100.63 - 2.17^{-02}x + 1.31^{-06}x^2$	0.96	<0.0001	2802	679
Alfalfa	Root	$88.92 - 2.90^{-02}x + 2.45^{-06}x^2$	0.80	<0.0001	1543	1106

Values are mg Zn kg^{-1}.

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, 1978). Here, we have identified Zn phytotoxicity thresholds for several important reclamation forb species using a simplified approach that circumvents many of these experimental pitfalls.

The effective concentrations (EC50s) that we have determined for these reclamation species (EC50-shoot = 123 to 371 mg Zn L^{-1}, Table II) are generally comparable with the Zn EC50s that have been published for dicots (EC50-shoot = 43–996 mg Zn L^{-1}, Table IV), although nearly all previously published levels are from crop species. The one crop species that we included in our study, alfalfa, was found to have a lower EC50-shoot value than a previous published report for alfalfa (Boawn, 1971). However, we used different methodology than the previous report. Past studies using more similar methods as our study (Jordan, 1975; Langille and Batteese, 1974) have reported EC50-shoot values in other dicots (Table IV) that are slightly lower (39–80 mg L^{-1}) than the values we have determined for reclamation forb species (123–371 mg L^{-1}) (Table II).

No Zn phytotoxicity thresholds (PT50s) have been reported for nonagricultural forb species. Therefore, comparisons of our values to published values are of limited utility. For the crop plants that have been examined, reports for PT50-shoot range

TABLE IV

Zinc phytotoxicity thresholds (PT) and effective concentrations (EC) for agricultural or reclamation forb species that have 2 been published, or have been calculated here from published data

Plant taxa	Threshold	Value[a]	Media	Zn form	Reference
Alfalfa (*Medicago sativa* L.)	EC50-shoot	>500	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Common beat (*Beta vulgaris* L.)	EC50-shoot	~764	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Garden lettuce (*Lactuca sativa* L.)	EC50-shoot	~950	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Pea (*Pisum sativum* L.)	EC50-shoot	>500	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Potato (*Solatium tuberosum* L.)	EC50-shoot	43	Soln.	$ZnSO_4$	(Langille and Batteese, 1974)
Potato (*Solanum tuberosum* L.)	EC50-root	39	Soln.	$ZnSO_4$	(Langille and Batteese, 1974)
Red oak (*Quercus rubra* L.)	EC50-shoot	~80	Soln.	$ZnSO_4$	(Jordan, 1975)
Spinach (*Spinacia oleracea* L.)	EC50-shoot	~844	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Tomato (*Solanum lycopersicum* L. var. *lycopersicum)*	EC50-shoot	~996	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Cassava (*Manihot esculenta* Crantz)	PT10-shoot	330	Soln.		(Howeler *et al.*, 1982)
Clover (*Trifolium subterraneum* L.)	PT10-shoot	250–300	Soln.	$ZnSO_4$	(Millikan, 1963)
Alfalfa (*Medicago sativa* L.)	PT50-shoot	>345	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Common beat (*Beta vulgaris* L.)	PT50-shoot	>1000	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Garden lettuce (*Lactuca sativa* L.)	PT50-shoot	>660	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Garden lettuce (*Lactuca sativa* L.)	PT50-shoot	475	Soil	$ZnSO_4$	(Chang *et al.*, 1992, MacLean and Dekker, 1978)
Pea (*Pisum sativum* L.)	PT50-shoot	>522	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Potato (*Solanum tuberosum* L.)	PT50-shoot	2303	Soln.	$ZnSO_4$	(Langille and Batteese, 1974)
Spinach (*Spinacia oleracea* L.)	PT50-shoot	>945	Soil	$Zn(NO_3)_2$	(Boawn, 1971)
Tomato (*Solanum lycopersicum* L. var. *lycopersicum)*	PT50-shoot	>514	Soil	$Zn(NO_3)_2$	(Boawn, 1971)

[a]Values for effective concentrations (EC's) are mg L^{-1}; values for phytotoxicity thresholds (PT's) are mg kg^{-1} plant tissue.

from >345 for alfalfa to 2,303 for potato (Table IV). The comparison of our PT50 for alfalfa to the published value (Boawn, 1971) is of limited value since different techniques were used. Our estimates of PT50-shoot for six forb species range from 1,258 mg Zn kg^{-1} for Palmer's penstemon to 3,214 mg Zn kg^{-1} for yarrow. At the higher treatment levels, we observed shoot tissue Zn concentrations (Figure 2) that fall within the range of concern for animals that might be grazing on such plants (Gough *et al.,* 1979).

In a previous study, using similar techniques, we determined Zn toxicity thresholds for five reclamation grass species (Paschke *et al.*, 2000). In that study, we found EC50-plant values of between 84 and 222 mg L^{-1} (compared to 82–214 mg L^{-1} in this study) and PT50-shoot values of between 2,449 and 5,026 mg kg^{-1} (compared to 1258–3214 mg kg^{-1} in this study). The notable difference between the previous grass study and this forb study is in the measured LC50 values. In this study, LC50 values are between 201 and 369 mg Zn L^{-1}. In the grass study, LC50 values were all >500 mg Zn L^{-1}, as mortality of the grass species never exceeded 4% even at the highest (500 mg Zn L^{-1}) treatment level (Paschke *et al.*, 2000). The higher mortality observed for these forb species suggests that the grass species tested may generally be more tolerant of Zn-contaminated sites than the reclamation forbs examined here. However, thresholds for individual species should be considered along with other factors when determining a seed mixture for a reclamation site.

For most species, roots appeared to be slightly more affected by Zn than shoots (Figure 1). This differential effect of Zn on roots versus shoots for most 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 reclamation planning where they can be related to levels of soil solution Zn. Monitoring soil solution Zn with lysimeters could accomplish this. Our measures of EC50-plant ranged from 82 to 214 mg Zn L^{-1}. These Zn effective concentrations should be generally applicable to those obtained from lysimeter solutions. Under field conditions, Zn 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.

5. Conclusions

Based on EC50-plant values, it appears that there is little separation of these forb species in terms of their tolerance to high substrate Zn. From our data it appears that of the species tested, Palmer's Penstemon or blue flax would be the best forb species for restoration of Zn contaminated sites. Palmer's penstemon exhibited a relatively high EC50-plant value (214 mg L^{-1}) and blue flax had a high survival

rate at substrate Zn levels up to 400 mg L^{-1}. The thresholds provided here should be more useful for risk assessors than the currently available and widely used thresholds determined for non-reclamation plants using similar methodology.

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