

## HEAVY METAL TOLERANCE OF INLAND SALTGRASS (*DISTICHLIS SPICATA*)

R. A. Prodgers<sup>1</sup> and W. P. Inskeep<sup>2</sup>

**ABSTRACT.**—Inland saltgrass (*Distichlis spicata*) occurs on at least two metal-contaminated sites in southwestern Montana. As a result of mining, milling, and smelting activities, soils have elevated concentrations of copper, zinc, and manganese. One soil is acidic (upper horizons), slightly saline, and moderately sodic. The other soil is alkaline, nonsaline, and nonsodic. The fact that inland saltgrass grows on these soils and does not accumulate dangerous levels of metals makes it a candidate species for revegetating hardrock mining and other metal-polluted sites.

*Key words:* inland saltgrass, *Distichlis spicata*, preadaptation, metal-contaminated soils.

*Distichlis spicata* (L.) Green var. *stricta*, (Torr.) Beetle, commonly known as inland saltgrass, is a perennial, shallow-rooted, warm-season grass that grows from hard, scaly rhizomes. Palatability is low but is enhanced by heavy cropping, which also promotes a dense sod (Weaver 1954).

*D. spicata* is most commonly found on moist saline soils in the Great Plains. Daubenmire (1974) referred to inland saltgrass as an example of plant zonation around a saline basin; saltgrass was found in the wettest and saltiest sites occupied by vascular plants. Elsewhere, other species may exhibit greater salt tolerance (Dodd et al. 1964, Unger 1974). The soil salt content associated with saltgrass communities can range at least from 0.03 to 5.6% (Unger 1974). Inland saltgrass can tolerate an average soil electrical conductivity (EC) of 66 mmhos/cm in the upper 10 cm of soil (Unger 1969). However, in mixed-prairie communities where saltgrass is abundant in east central Montana, saltgrass occur on soils with ECs of  $\leq 2$  (Prodgers 1978). Saltgrass abundance appears to increase in response to grazing intensity in mixed communities adjacent to more saline sites.

The relative abundance of cations and anions in soils of saltgrass communities varies with soil horizon and site. The predominant cation is usually sodium, but magnesium, potassium, or calcium may be abundant (Dodd et al. 1964, Dodd and Coupland 1966a, Ludwig and McGinnies 1978). Principal anions are sulfate, bicarbonate, and chloride.

Prodgers (1978) reported sodium adsorption ratios (SARs)  $>60$  for saltgrass community soils. Ludwig and McGinnies (1978) reported an SAR of 4 in the A horizon and 79 in the C horizon. Soil pHs for inland saltgrass communities commonly range from 7 to 10 (Poole 1980).

The ability of saltgrass to grow on salty sites may depend on its ability to exclude salts. *Distichlis spicata* can survive salt-stressed environments by a process in which vacuolar compartmentation of salt is followed by osmotic compartmentation in the cytoplasm via an organic solute such as proline (Daines and Gould 1985). Inland saltgrass maintains high osmotic pressure in the cell sap. Dodd and Coupland (1966b) measured osmotic pressures in *D. spicata* of 22–48 atmospheres in a saline meadow community. This suggests that the membranes of saltgrass can extract water from the matrix of saline soils. Efficient membranes might also exclude salts and metals from critical sites in the plant. *Distichlis* has been reported to secrete salts, allowing it to survive what would otherwise be excessive ionic accumulation (Frey-Wyssling 1935, as cited in Unger 1974).

We speculate that salt-excluding mechanisms may preadapt saltgrass to grow on metal-rich sites and suggest that, if so, saltgrass might be planted on metal-contaminated sites to provide cover. Due to the rhizomatous growth form, saltgrass spreads vegetatively and controls soil erosion. Low

<sup>1</sup>2715 Ottawa, Butte, Montana 59701.

<sup>2</sup>Department of Plant and Soil Science, Montana State University, Bozeman, Montana 59717.

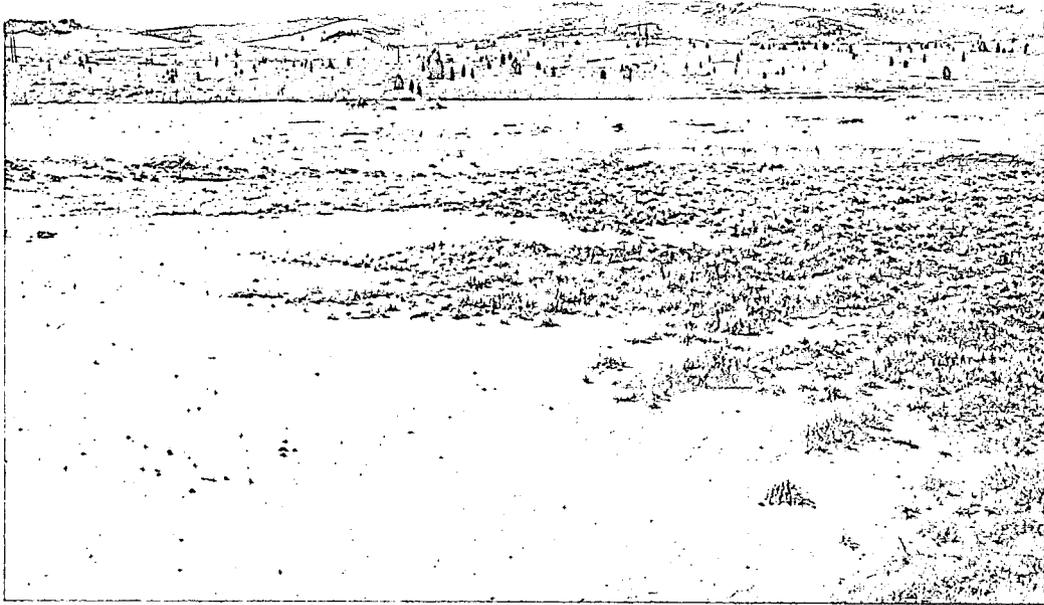


Fig. 1. Inland saltgrass community on streambank tailings near Ramsay, Montana. Floods deposited tailings and mining detritus along stream. Contagious distribution of vegetatively spreading saltgrass community is evident.

palatability is desirable for revegetation of metal-contaminated sites.

To determine the metal tolerance and metal-excluding capabilities of saltgrass, we measured the metal concentrations of saltgrass tissues and associated soils at two contaminated sites. Comparison is made between acceptable metal levels in livestock forage and normal metal concentrations in soils.

#### SITES AND METHODS

Two *Distichlis spicata* communities were investigated on metal-rich soils in southwestern Montana. One community is situated on stream-deposited hardrock mine tailings along Silver Bow Creek near Ramsay, Montana, Section 22, T3N, R9W, at an elevation of 1615 m (Fig. 1). The tailings originated at upstream milling and smelting operations along the creek from 1860 to 1910. A series of floods in the 1890s and a major flood in 1908 washed tailings into the creek. Subsequent floods since 1908 (particularly in 1948, 1975, 1980, and 1981) have re-deposited tailings

along the creek (CH2M Hill 1989). These tailings deposits now occur along the creek in distinctively colored strata, most of which are rich in heavy metals and low in pH due to pyrite oxidation. Some taller plants, such as willows, have survived tailings deposits, but shorter vegetation was buried and current grass and forb plant communities represent colonization by adapted species.

The second community occurs on an upland Mollisol derived from frost-worked travertine a few hundred meters from the copper smelter stack near Anaconda, Montana, in Section 12, T4N, R11W, at an elevation of 1740 m (Fig. 2). This site has received heavy metal deposits and sulfur dioxide fumigation from the smelter stack dating roughly from 1900 to 1980. Vegetation within several miles of the stack has been dramatically affected by stack emissions. Erosion from a nearby road has resulted in soil deposition in the *Distichlis spicata* community. Inland saltgrass does not typically occur on upland Mollisols.

Three soil pits were located to encompass surficial variability within each community.



Fig. 2. Inland saltgrass-Great Basin wildrye plant community near copper smelter at Anaconda, Montana. Although Great Basin wildrye is more conspicuous in the photo due to its height, saltgrass has greater canopy coverage. Heavy metals in soils were deposited as airborne fallout from the copper smelter (background) and through deposition of erosional sediments.

Soil profiles were described and .5 kg of soil was collected from each horizon at each pit. Particle size was determined following the procedure of Day (1965). Electrical conductivity, SAR, and pH were determined from

saturated paste extracts. Soils were digested in 4M HNO<sub>3</sub> at 70 C overnight (Chang et al. 1987) for total analysis of copper, manganese, and zinc. Extractable metal concentrations were determined using DTPA (Lindsay and

TABLE 1. Profile description for streambank mine tailings soil near Ramsay, Montana.

Horizon	Depth (cm)	Texture	Structure	Consistency	Roots	pH <sup>a</sup>	EC <sup>a</sup> (mmhos/cm)	SAR <sup>a</sup>
A	0-13	very fine sandy loam	massive	slightly hard very friable nonsticky, nonplastic	none (underground culms)	4.0-4.9	4-8	1-4
C1	13-20	fine and very fine sandy loam	massive	soft very friable nonsticky, nonplastic	culms, rhizomes, very few medium, fine, and very fine roots	5.4-7.6	4-8	2-5
C2	20-33	very fine sandy loam	massive	hard very friable nonsticky, nonplastic	common medium, few fine and very fine roots	5.4-7.6	4-8	2-5
Ab	33-46	very fine sandy loam	massive	slightly hard friable slightly sticky, slightly plastic	few rhizomes, few fine and very fine roots	6.5-7.6	2-10	2-9
C1b	46-74	silt loam	massive	soft friable slightly sticky, slightly plastic	few fine and very fine roots	7.8-7.9	1-7	2-13
C2b	74-100	silt loam	massive	slightly hard friable slightly sticky, slightly plastic	few fine and very fine roots	7.8-7.9	1-7	2-13
2Cb	100-127	very fine sandy loam	massive	soft friable nonsticky, nonplastic	very few fine and very fine roots	7.5-8.0	1-3	3-18

<sup>a</sup>Ranges based on three field subsamples of each horizon.

Norvell 1969, Soltanpour et al. 1976) in the absence of triethanolamine. Copper, zinc, and manganese concentrations in digestates and DTPA extracts were analyzed using inductively coupled plasma emission spectroscopy (ICP).

Soil profile descriptions are summarized in Tables 1 and 2. At the streambank tailings site, near-surface horizons are acidic, but pH increases in the deeper, poorly aerated horizons. These tailings generate acid in an oxidized environment. This soil is slightly saline, but sodium is not excessive, although borderline in some horizons. At the smelter site, where the soil parent material is frost-worked travertine, pH is slightly alkaline, the soil is nonsaline, and due to the predominance of calcium, SARs are <1.

Plant communities were sampled by esti-

mating canopy coverage (Daubenmire 1959) in 20 × 50-cm quadrats placed at 1-m intervals (smelter) or 3-m intervals (tailings) along linear transects. Due to the shapes of the plant communities, the tailings *D. spicata* community was sampled along a single 75-m transect ( $n = 25$ ), whereas the smelter community was sampled along three transects with total length of 40 m ( $n = 40$ ). The larger sample size was appropriate for the more heterogeneous community at the smelter site. Aboveground plant tissue samples were clipped above 3 cm at random intervals along transects, separated into foliage and inflorescences, oven-dried at 70 C, chopped, digested in nitric-perchloric acid, and analyzed with ICP (Havlin and Soltanpour 1980). Inflorescences make up a small portion of saltgrass production in most years.

TABLE 2. Profile description for soil near copper smelter at Anaconda, Montana.

Horizon	Depth (cm)	Texture	Structure	Consistency	Roots	pH <sup>a</sup>	EC <sup>a</sup> (mmhos/cm)	SAR <sup>a</sup>
A	0-8	very fine sandy loam	massive	soft very friable slightly sticky, slightly plastic	few stems, rhizomes	7.4-8.0	0.6-0.8	0.2
Ab	8-25	gravelly very fine sandy loam	moderate medium angular blocky	soft very friable slightly sticky, slightly plastic	few fine and very fine roots	7.2-7.8	0.3-1.1	0.1-0.2
Bw	24-25+	gravelly very fine sandy loam	massive	soft very friable nonsticky, nonplastic	common fine and very fine roots	7.5-8.1	0.4-0.9	0.2

<sup>a</sup>Ranges based on three field subsamples of each horizon.

TABLE 3. Species composition of two *Distichlis spicata* var. *stricta* communities.

Species	Streambank tailings site		Copper smelter site	
	Canopy coverage	Frequency	Canopy coverage	Frequency
	----- % -----			
<i>Agrostis</i> sp.	NP <sup>a</sup>		0.1	2
<i>Distichlis spicata</i>	32.6	92	32.8	95
<i>Elymus cinereus</i>	NP		17.0	60
<i>Juncus balticus</i>	1.0	4	NP	
<i>Muhlenbergia asperifolia</i>	NP		0.1	2
<i>Tetradymia canescens</i>	NP		1.9	5
Litter	39.7	100	45.3	100
Bare soil	49.3	100	21.6	95
Surficial rock fragments <sup>b</sup>	NP		3.8	52

<sup>a</sup>NP = not present.

<sup>b</sup>>2 mm.

Plant community compositions are summarized in Table 3. *Distichlis spicata* is the sole dominant species in the community located on the streambank tailings. At the smelter site, the much taller *Elymus cinereus* is subdominant in coverage, and several other species are present. *Elymus cinereus*, *Muhlenbergia asperifolia*, and *Tetradymia canescens* are more abundant at other sites near the smelter than is *D. spicata*.

## RESULTS AND DISCUSSION

### Metal Concentrations of Soils

Tables 4 and 5 present mean concentrations of Cu, Zn, and Mn by soil depth at each site. Subsample concentrations of metals for each depth increment varied, thus the rather large variance. Three replicates of one soil sample were analyzed concurrently with other total

and extractable metal determinations. The average coefficients of variation for extractable and total metal determinations were 3.0% and 3.4%, respectively, demonstrating good precision for laboratory techniques. Therefore, the high variability in metal concentrations from proximate soil samples appears to be due to variations in alluvial deposits rather than imprecise analysis.

At the smelter site, total and available metal concentrations decrease with depth as expected for pollutants deposited on the surface (Table 4). Root distribution was difficult to quantify due to the presence of 20-35% rock fragments, but roots were scarce below .5 m. Flagstones often terminated root penetration and resulted in concentrations of roots immediately above the flagstones. The A horizon contained mostly stems and some rhizomes, so roots were most abundant in the Ab and Bw horizons (8-45+ cm).

TABLE 4. Heavy metal concentrations in copper smelter soil supporting *Distichlis spicata* (means and standard deviations).

Depth (cm)		Cu	Zn	Mn
		----- $\mu\text{g/g soil}$ -----		
0-8	Total	3600 $\pm$ 980	2600 $\pm$ 520	1200 $\pm$ 130
	DTPA	410 $\pm$ 52	180 $\pm$ 47	2.8 $\pm$ 2.1
8-25	Total	1900 $\pm$ 650	1400 $\pm$ 480	780 $\pm$ 160
	DTPA	320 $\pm$ 150	200 $\pm$ 37	11 $\pm$ 14
25-45+	Total	410 $\pm$ 180	500 $\pm$ 170	570 $\pm$ 69
	DTPA	190 $\pm$ 110	200 $\pm$ 50	13 $\pm$ 17

TABLE 5. Heavy metal concentrations in alluvial tailings soil supporting *Distichlis spicata* (means and standard deviations).

Depth (cm)		Cu	Zn	Mn
		----- $\mu\text{g/g soil}$ -----		
0-13	Total	3700 $\pm$ 1200	1700 $\pm$ 480	1700 $\pm$ 510
	DTPA	800 $\pm$ 220	240 $\pm$ 72	170 $\pm$ 76
13-33	Total	13,000 $\pm$ 3600	5700 $\pm$ 1700	5300 $\pm$ 1200
	DTPA	690 $\pm$ 190	130 $\pm$ 80	68 $\pm$ 42
33-46	Total	9200 $\pm$ 7300	6200 $\pm$ 4800	4900 $\pm$ 6400
	DTPA	440 $\pm$ 290	86 $\pm$ 50	41 $\pm$ 35
46-100	Total	340 $\pm$ 250	410 $\pm$ 420	710 $\pm$ 380
	DTPA	43 $\pm$ 30	26 $\pm$ 27	31 $\pm$ 13
100-127	Total	120 $\pm$ 73	67 $\pm$ 40	310 $\pm$ 200
	DTPA	15 $\pm$ 30	3.9 $\pm$ 2.5	11 $\pm$ 6.1

At the streambank tailings site, the depositional sequence of varied tailings materials and subsequent migration of soluble metals have resulted in the highest metal concentrations in the zone where roots are most abundant (13-46 cm depth). Metal concentrations were lower in the surface horizon (0-13 cm) where underground stems and rhizomes were well represented but few roots occurred. Metal concentrations were lowest in the 46-127-cm-depth interval, where roots were less common than higher in the profile.

Total Cu concentrations in the upper 46 cm at the streambank tailings site and upper 8 cm at the smelter site were 100 or more times higher than "normal" soils. Total Cu concentrations in soils summarized by Adriano (1986) averaged 30  $\mu\text{g Cu/g soil}$ , ranging from 2 to 250  $\mu\text{g Cu/g soil}$ . Total Cu concentrations in samples of soils in Japan ranged from 4 to 176  $\mu\text{g Cu/g soil}$  (Iimura 1981). In Japan the legal limit of Cu in agricultural soils is 125 ppm (based on 0.1 N HCl extraction), as stipulated in the Agricultural Land Soil Pollution Prevention Law (Chino 1981).

Total Zn concentrations in the upper horizons of the studied saltgrass soils were roughly 15 to 70 times higher than average. Adriano (1986) reported total Zn concentrations ranging from 1 to 900  $\mu\text{g Zn/g soil}$ ; the average was 90  $\mu\text{g Zn/g soil}$ . Soil samples from Japan contained 10 to 662  $\mu\text{g Zn/g soil}$  (Iimura 1981).

Total Mn concentrations reported in Tables 4 and 5 range from normal to about 10 times higher than usual. Total Mn concentration in soils reported by Shacklette et al. (1971) averaged 560  $\mu\text{g Mn/g soil}$ , with values ranging from 1 to 7,000  $\mu\text{g Mn/g soil}$ . In another summary, the mean total Mn content of surface soils ranged from 80 to 1,315  $\mu\text{g Mn/g soil}$  (Kabata-Pendias and Pendias 1984).

In a different study conducted near East Helena, Montana, soils were sampled and mapped on the basis of metal contamination from a smelter located in East Helena, which is about 89 km northeast of the study sites reported here. Soils that presumably were unaffected by smelter pollution had geometric mean DTPA-extractable concentrations in the upper 38 cm of soil of approximately

TABLE 6. Heavy metal concentrations in foliage of *Distichlis spicata* and *Elymus cinereus* grown on soils described in Tables 1, 2, 4, and 5.

Site	Species	Cu	Zn	Mn
		----- μg/g tissue -----		
Tailings	<i>D. spicata</i>			
	Inflorescence	490	490	430
	Foliage	130	230	150
Smelter	<i>D. spicata</i>			
	Inflorescence	260	190	45
	Foliage	94	120	25
	<i>E. cinereus</i>			
	Foliage	87	91	39

1 mg/kg Cu, 15 mg/kg Mn, and <0.1 mg/kg Zn (CH2M Hill 1987). The soils reported here contain far more Cu and Zn than these background concentrations.

In summary, comparisons of our data with those reported in the literature demonstrate that the upper .5 m of soil at the streambank tailings site had very high concentrations of all three metals. The smelter soil had very high Cu and Zn concentrations, but not Mn.

#### Metal Concentrations of Foliage

Foliar metal concentrations of *Distichlis spicata* and *Elymus cinereus* were similar; however, *D. spicata* inflorescences accumulated higher metal concentrations, particularly Cu, than did foliage (Table 6).

Plant shoots usually do not accumulate more than 200 μg Cu/g foliage (dry weight), a concentration often considered the threshold value above which plant injury may occur (Kabata-Pendias and Pendias 1984). Maximum tolerable levels of Cu in forage (total diet) for cattle, sheep, and horses are 100, 25, and 800 μg/g of forage, respectively (NAS 1980). These values are based on residues in human food as well as animal health. Foliar concentrations reported in Table 6 are unacceptable for sheep, marginal for cattle, and acceptable for horses, since horses are not generally considered a meat source in the United States.

The maximum tolerable diet concentration of Mn for cattle and sheep is 1000 μg Mn/g forage (NAS 1980). Many plant species are affected by Mn concentrations around 500 μg Mn/g tissue (dry weight), but resistant species or races can tolerate concentrations above 1000 μg/g. The foliar Mn concentration of saltgrass (Table 6) is acceptable for livestock.

Animals are tolerant of high Zn levels in their diet (National Research Council 1979). Maximum tolerable diet concentrations of Zn are 500 μg Zn/g forage for cattle and 300 μg Zn/g forage for sheep (NAS 1980). Cereal grains and vegetables usually contain less than 50 μg Zn/g tissue (Kabata-Pendias and Pendias 1984). While elevated, the foliar Zn concentration of saltgrass is acceptable for livestock grazing.

Considering the very high metal concentrations in the soil, *D. spicata* appears largely to exclude these metals from foliage. Only the Cu concentrations are near or above levels that might pose a problem if humans consumed livestock that grazed solely on foliage grown on heavily contaminated soils from the affected areas. However, livestock ingestion of contaminated soils could greatly increase livestock intake of heavy metals, particularly when a short-grass such as inland saltgrass is grazed. For this reason, livestock grazing of heavily contaminated sites is not recommended. Under open-range grazing conditions, unpalatable species are desirable for revegetation of metal-contaminated sites.

The preadaptation of saltgrass for metal-rich sites, its low palatability, and its soil-binding characteristics make *D. spicata* an attractive grass for revegetation of appropriate sites. Special metal-tolerant varieties could be developed from genotypes present on sites such as those reported here. In current revegetation practices, *D. spicata* is usually propagated from rhizomes due to problems with germination and seedling emergence; however, seeding is much cheaper and more convenient. Stratification and scarification treatments can increase naturally low germination (Cluff et al. 1983). Metal-tolerant races

of inland saltgrass have potential for revegetation of metal-contaminated areas in the West if establishment from seed proves to be practical.

## LITERATURE CITED

- ADRIANO, D. 1986. Trace elements in the terrestrial environment. Springer-Verlag, New York. 533 pp.
- CHANG, A., A. PAGE, AND J. WARNEKE. 1987. Long-term sludge applications on cadmium and zinc accumulations in Swiss chard and radish. *Journal of Environmental Quality* 16: 217-221.
- CH2M HILL. 1987. Remedial investigation of soils, vegetation, and livestock for East Helena site (ASARCO), East Helena, MT. EPA Work Assignment No. 68-8L30.0. 436 pp.
- . 1989. Silver Bow Creek flood modeling study—Nov. 30, 1989. Document BOI20840. 85 pp.
- CHINO, M. 1981. Metal stress in rice plants. Pages 65-80 in *Heavy metal pollution in soils of Japan*. Japan Scientific Society Press, Tokyo. 302 pp.
- CLUFF, G., G. EVANS, AND J. YOUNG. 1983. Desert saltgrass seed germination and seedbed ecology. *Journal of Range Management* 36: 419-422.
- DAINES, R., AND A. GOULD. 1985. The cellular basis of salt tolerance studies with tissue cultures of the halophytic grass *Distichlis spicata*. *Journal of Plant Physiology* 199: 269-280.
- DAUBENMIRE, R. 1959. A canopy-coverage method of vegetational analysis. *Northwest Science* 33: 43-64.
- . 1974. *Plants and environment*. 3rd ed. John Wiley & Sons, New York. 422 pp.
- DAY, D. 1965. Particle-size fractionation and particle-size analysis. Pages 545-566 in *Methods of soil analysis, part 1. Physical properties and mineralogical properties, including statistics of measurement and sampling*. American Society of Agronomy, Madison, Wisconsin.
- DODD, J., AND R. COUPLAND. 1966a. Vegetation of saline areas in Saskatchewan. *Ecology* 47: 958-968.
- . 1966b. Osmotic pressures of native plants of saline soils in Saskatchewan, Canada. *Journal of Plant Science* 46: 479-485.
- DODD, R., D. RENNIE, AND R. COUPLAND. 1964. The nature and distribution of salts in uncultivated saline soils in Saskatchewan. *Canadian Journal of Science* 44: 165-175.
- HAVLIN, J. L., AND P. N. SOLTANPOUR. 1980. A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* 11: 969-980.
- IMURA, K. 1981. Background content of heavy metals in Japanese soils. Pages 19-26 in *Heavy metal pollution in soils of Japan*. Japan Scientific Society Press, Tokyo. 302 pp.
- KABATA-PENDIAS, A., AND H. PENDIAS. 1984. *Trace elements in soils and plants*. CRC Press, Boca Raton, Florida. 315 pp.
- LINDSAY, W., AND W. NORVELL. 1969. Development of a DTPA micronutrient soil test. *Agronomy Abstracts* 69: 84.
- LUDWIG, J., AND W. MCGINNIES. 1978. Revegetation trials on a saltgrass meadow. *Journal of Range Management* 31: 308-311.
- NATIONAL ACADEMY OF SCIENCES (NAS). 1980. *Mineral tolerance of domestic animals*. Washington, D.C. 577 pp.
- NATIONAL RESEARCH COUNCIL. 1979. *Zinc. Medical and biological effects of environmental pollutants*. University Park Press, Baltimore, Maryland. 471 pp.
- POOLE, B. 1980. Biota of the northern Rockies: a review of *Distichlis spicata*. *Proceedings of the Montana Academy of Science* 39: 89-96.
- PRODCERS, R. 1978. Circle West vegetation and baseline study. Montana DNRC Technical Report No. 1. 115 pp.
- SHACKLETTE, H., J. HAMILTON, J. BOERNGEN, AND J. BOWLES. 1971. *Elemental composition of surficial materials in the conterminous United States*. USGS Professional Paper 574-D. Washington, D.C. 71 pp.
- SOLTANPOUR, P., A. KHAN, AND W. LINDSAY. 1976. Factors affecting DTPA-extractable Zn, Fe, Mn, and Cu from soils. *Communications in Soil Science and Plant Analysis* 7: 797-821.
- UNGAR, I. 1969. Plant communities of saline soils at Lincoln, NE. *American Midland Naturalist* 78: 98-120.
- . 1974. Inland halophytes of the United States. In: W. H. Queen, ed., *Ecology of halophytes*. Academic Press Inc. 605 pp.
- WEAVER, J. 1954. *North American prairie*. Johnsen Publication Company, Lincoln, Nebraska. 348 pp.

Received 6 February 1991

Revised 12 July 1991

Accepted 15 July 1991