PHYTOSTABILIZATION OF METAL MINE TAILINGS USING TALL FESCUE


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ABSTRACT

Mined areas near the city of Galena, Kansas, provide a source of heavy metal contamination, particularly cadmium (Cd), lead (Pb) and zinc (Zn). The metals move off-site in response to erosion by wind and running water, spreading the contamination beyond its original extent. Phytostabilization of the mine tailings could limit the spread of these heavy metals. The purpose of this study was to attempt field-scale inoculation of tall fescue (Festuca arundinacea Schreb) with mycorrhizal fungi to enhance growth and survival on a metal-contaminated mine spoil, and to study the influence of cattle manure as a soil amendment on basic soil chemical properties and on the chemical fractionation of Cd, Pb, and Zn. No evidence of successful infection by mycorrhizal fungi was found. After the first growing season, vegetative cover reached 71% but then steadily declined to 29% over the next two growing seasons. Extractable soil phosphorus (P) levels remained constant throughout the study and were sufficient for normal plant growth. Extractable potassium (K) levels declined throughout the study and were not sufficient for normal plant growth after the third year. Soil pH decreased from an average of 7.9 early in the study to 6.5 at the end. Plant tissue Zn concentrations suggested Zn phytotoxicity that became worse over the course of the study. Manure increased the amount of organically bound metals and generally decreased exchangeable levels. Between the first and third years of the study, exchangeable forms of metals generally increased while residual forms decreased. These changes are likely the result of the soil acidification that occurred during this same period, and may have contributed to the decline in fescue productivity because of increased problems with Zn phytotoxicity.
INTRODUCTION

In the United States, approximately 65% of Superfund sites that have reached the point of having a Record of Decision include metals among their contaminants, and 16% have metals as their only contaminant (US EPA, 1997). Lead is the most common metal found in Superfund sites, Cd is the fourth, and Zn is the sixth most common (McClean and Bledsoe, 1992); these metals are often found together at mining and smelting sites because galena (PbS, the most important Pb ore) and sphalerite (ZnS, the most important Zn ore) are frequently found in the same ore deposit (Berry and Mason, 1959; Maynard, 1983), and Cd may substitute for Zn in sphalerite (Fleischer, 1955). Deposits of galena and sphalerite were found in the Tri-State Mining District of southeastern Kansas, southwestern Missouri, and northwestern Oklahoma in 1848, and this region of the United States was one of the most prolific Pb and Zn mining areas in the world for the 100 years lasting from 1870 to 1970 (McKnight and Fischer, 1970; Spruill, 1987). Although the peak of mining operations was in the 1950s, and the last mine closed in 1970, mine waste (locally called chat) remains on the surface and is a source of heavy metal contamination. Lead, Zn and Cd contamination from mining and other operations caused the USEPA in 1985 to establish the Cherokee County, Kansas, Superfund site and the Galena, Kansas, Superfund subsite (Norland, 1991), shown in Figure 1.

Heavy metals still present in the chat material have created a legacy of risk to human and animal health and to the environment. The adverse effects of Pb on human health are well known (Madhava et al., 1989; Martin, 1991; Landrigan and Curran, 1992), and wind-blown dust has been shown to be an exposure pathway for Pb at mining sites (Gulson et al., 1994). Cadmium, like Pb, has no value as a nutrient, and is highly toxic to plants and animals (Yasumura, et al., 1980, Alloway, 1990). Despite being an essential plant nutrient, excessive soil Zn is harmful to plants (Chaney and Giordano, 1977; Weatherly et al., 1980; and Kiekens, 1990). Few soils can
naturally achieve phytotoxic levels of Zn, and most Zn-contaminated soils result as by-products of human activities such as mining and the creation of mine wastes (Chaney, 1993).

Even decades after mining activity has ceased, vegetation has established poorly or not at all on the piles of chat. Numerous factors can limit plant growth on contaminated soils and mine spoils including poor soil chemical and physical properties, and phytotoxicity from metals such as Zn, nickel (Ni) and copper (Cu). Soil amendments can be used to correct problems with soil chemical and physical properties. Residual materials such as animal manures, biosolids, and composts will add plant nutrients, improve soil water holding capacity and may help reduce phytotoxicity problems (Pierzynski and Schwab, 1993; Li et al., 2000). Several studies have shown that the use of commercial inorganic fertilizers to add plant nutrients will not improve plant growth, while the addition of animal wastes such as cattle manure greatly increases plant growth and survival (Hetrick et al., 1994; Levy et al., 1999). Further, Hetrick et al (1994) showed that inoculation of tall fescue with arbuscular mycorrhizal fungi (Glomus sp.) greatly enhanced biomass production in a high Zn chat material under greenhouse conditions. Suggested mechanisms included altered root architecture and rhizosphere microflora, enhanced ability to withstand stress, and sequestration of Zn in fungal hyphae. This work provided the basis for the treatments used in the study described in this paper.

Darmer (1992) has shown that revegetation reduces the off-site movement of mine waste, and Green et al. (1997) wrote that this would also reduce the spread of heavy metals in the Galena area because most of the metals there are adsorbed onto the soil matrix. Mathematical modeling by Green et al. (1997) indicated that grass buffers could reduce sediment loss by 18% to 25% over that of bare soil, and that sediment loss would be reduced by nearly 70% in the case of total grass cover, which would greatly reduce the off-site transport of Pb and Zn from the chat. This particular type of phytoremediation has been called *phytostabilization* by Chase (1995), to
distinguish it from other remedial methods involving plants. Schnoor (1997) stated that, since
metals do not ultimately degrade, using phytostabilization to capture them in situ is sometimes
the best alternative over sites that cover a large area. The Galena Superfund subsite covers
approximately 324 hectares (approximately 801 acres).

The objectives of this study were to attempt field-scale inoculation of tall fescue with
mycorrhizal fungi to enhance growth and survivability on a metal-contaminated mine spoil, and
to study the influence of cattle manure as a soil amendment on basic soil chemical properties and
on the chemical fractionation of Cd, Pb, and Zn.

MATERIALS AND METHODS

At a test site within the city limits of Galena, Kansas, five treatments with four
replications were imposed on 20 plots on chat material. Soil samples collected from the area
prior to treatment applications showed the chat to contain average total Zn, Cd, and Pb
concentrations of 22690, 53, and 2050 mg kg\(^{-1}\), respectively, and had a pH (1:1 soil-to-water) of
7.7. The chat at this location had an average of 81% sand-sized, 13% silt-sized, and 6% clay-
sized by weight. The chat consists primarily of chert, a silicified carbonate which typically is
alkaline in nature. The site was graded to a uniform 5% slope prior to establishing the
treatments.

The treatments included a manure-amended and seeded control (SC); manure-amended,
seeded and inoculated with mycorrhizal fungi (IN); and a manure-amended, seeded treatment on
which benomyl fungicide was applied (BF). The inoculum was commercially prepared and
contained primarily *Glomus etunicatum* Becker & Gerde. and *Glomus clarum* Nicol. & Shenck.
It was spread on the plots as a mixture of peat and sand and incorporated by hand. The benomyl
treatment was made as 300 g of active ingredient per plot applied approximately monthly from
April to September the first two growing seasons. This treatment was utilized to prevent natural infection of tall fescue by indigenous mycorrhizal fungi. Also included were an unseeded and manure-amended control (UMC), and an unseeded control, which was not manure-amended (UC). These final two treatments were included to allow the differentiation of the effects of manure amendment and plant growth on soil chemical properties. The manure additions were equivalent to 90 Mg/ha. The manure was spread on the plots by hand and incorporated with a roto-tiller. Treatments not receiving manure were also roto-tilled to ensure equal disturbance of all plots. The SC, IN and BF treatments were seeded and became vegetated with tall fescue, variety KY-31 (endophyte infected).

The plots were initially seeded in Fall 1995, representing an optimum time for establishing tall fescue in southeast Kansas. A very dry fall and winter followed and little germination occurred. The plots were seeded again in February 1996, which resulted in successful establishment of tall fescue. Growth was assessed over the 1996, 1997, and 1998 growing seasons.

Basal (ground) cover and vegetative (canopy) cover measurements were taken in October 1996 and May and October of 1997 and 1998 in the vegetated plots using the method of Owensby (1973). Soil samples were collected from the top 10 cm of each plot in spring 1996, fall 1997, and fall 1998. Ten subsamples were composited from each plot. All samples were analyzed for pH (1:1 soil-to-water). Samples collected spring 1996 and fall 1998 were analyzed for Bray-1 extractable phosphorus (P) and 1 M ammonium acetate extractable potassium (K) to assess plant nutrient availability (North Central Regional Publication No. 221, 1998). In addition, the Pb, Zn, and Cd in the chat collected spring 1996 and fall 1997 were sequentially extracted according to the methodology of Tessier et al. (1979), which operationally defines metals in exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic-bound and residual
factions. The exchangeable fraction is considered to be bioavailable (that is, available for uptake by organisms), and the residual fraction is considered to be non-bioavailable. The carbonate-bound, Fe/Mn oxide-bound, and the organic-bound fractions are progressively less bioavailable.

Root samples were stained with Trypan Blue (Phillips and Hayman, 1970) and examined microscopically at 6, 12, and 18 months following reseeding. To further distinguish the presence of mycorrhizal colonization within the roots, specifically by fungal species used as inoculum, Polymerase Chain Reaction (PCR) primers were developed for *G. etunicatum* and *G. clarum* using the technique of Abbas et al. (1996).

Statistical analyses were performed using SAS for Windows Version 6.12 (SAS Institute, 1985), using least significant differences (LSD) for mean separations at P<0.05.

**RESULTS AND DISCUSSION**

Trypan blue staining techniques failed to reveal clear evidence of mycorrhizal colonization of Fescue root samples at any of the three sampling times. Evidence of *G. etunicatum* and *G. clarum* colonization was found using DNA primers, however it was sporadic throughout the study and did not correspond to any of the treatments. For this reason, we concluded that the inoculation was not successful and that any differences in fescue growth and tissue composition could not be attributed to the influence of mycorrhizae and these data are, therefore, not presented here.

Figure 2 shows vegetative (canopy) cover (VC) and basal (ground) cover (BC) for the vegetated treatments over the period from October 1996 to October 1998. There were no significant treatment effects on VC or BC at any time during the study, which is consistent with the findings on mycorrhizal fungi infection. The vegetative cover for October 1998 (28.5%) was lower than previous point counts taken in May 1997, October 1997, and May 1998 (71.7%);
but was not as low as that reported in the initial vegetative cover point count following planting of the tall fescue (26.7% in October 1996). The October 1998 basal cover (10.4%) was the lowest recorded since the tall fescue was planted, when compared with previous point counts from October 1996 (11.8%), May 1997 (35.7%), October 1997 (23.6%), and May 1998 (36.9%). However, the values in October 1998 were not significantly lower than those in October 1996. The cyclic nature of the basal cover measurements reflects the production of new tillers each spring and the corresponding loss of some tillers over each growing season. The decrease in vegetative cover over time may indicate that the manure amendment was no longer supplying nutrients to the fescue, or that the manure is no longer helping to alleviate problems with zinc phytotoxicity on the chat. Green et al. (1997) modeled the effect of vegetative cover on sediment loss from the watershed in which this experiment was located. Sediment loads were decreased by 33, 55, 68, and 71% compared to a barren condition as vegetative cover increased from 0 to 100% by 25% increments. The modeling work suggests that the declines in vegetative cover observed in this study would have a significant negative impact on sediment transport and, consequently, metal losses from the watershed if vegetative cover were not maintained.

Soil pH was significantly lower in the UC treatment compared to all treatments receiving manure the spring following establishment of the treatments (Table 1). No such treatment effects were seen fall 1997 or 1998. Cattle manure has been shown to increase soil pH, in part because CaCO₃ is often added to cattle rations (Eghball, 1998). Overall, soil pH consistently declined over the course of the study, regardless of treatment. The exact cause for this is not known, however, it does suggest that the grading of the plots performed prior to treatment applications may have exposed sulfide minerals to oxidizing conditions, which then lowered soil pH over time. Further, the decrease in soil pH may have contributed to the decline in the fescue stand.
over the course of the study as the phytoavailability of cationic metals such as Zn increases as soil pH decreases.

Concentrations of Cd, Pb, and Zn in fescue were not influenced by treatments at any point in the study (Table 2), which is also consistent with the lack of mycorrhizal fungi infection noted above. Cadmium concentrations were generally less than 7 mg/kg while Pb concentrations were less than 12 mg/kg. The Cd concentrations exceed maximum tolerable levels for use as forage for cattle (NRC, 1984). Zinc plant tissue concentrations of >500 are thought to be phytotoxic (Chaney, 1993). Zinc concentrations in samples collected spring 1996 are approximately 500 mg/kg and those in samples collected spring 1997 are consistently >500 mg/kg, suggesting possible Zn phytotoxicity. Further, Zn concentrations are higher spring 1997 than spring 1996, which is consistent with the decrease in soil pH, vegetative cover, and basal cover that occurred during this same time period. Levy et al. (1999) saw no evidence of Zn phytotoxicity when they grew switchgrass and big bluestem in chat collected from the Tri-State mining district in southwestern Missouri. However, they based this conclusion on the lack of visual symptoms of Zn phytotoxicity, and growth comparable to an unfertile control with low water holding capacity.

The sum of the metal fractions for the sequential extraction scheme should approximately equal the total metal concentration in the soil as determined in an independent analysis. The average total metal concentrations, as calculated by the sum of the fractions, are 81 mg/kg for Cd, 2079 mg/kg for Pb, and 20680 mg/kg for Zn, with little difference between the spring 1996 and fall 1997 samples. These totals are similar to the 2050 and 22690 mg/kg values determined for Pb and Zn, respectively, in an independent analysis. The total for Cd is considerably higher than the 53 mg/kg determined in a separate analysis, and may reflect a compounded analytical error associated with measuring low concentrations and summing the five fractions.
The treatments having vegetation had significantly lower exchangeable Cd compared to the JC treatment for the spring 1996 sampling, and two of the three vegetated treatments had significantly lower exchangeable Cd compared to the UMC treatment (Table 3). By fall 1997, no such treatment effects were found. This illustrates the influence of plant growth on the partitioning of metals in contaminated soils. The addition of manure significantly increased organic-bound Cd for both the spring 1996 and fall 1997 samplings, illustrating the influence of the organic matrix of the manure on the fractionation of the Cd. Also, exchangeable Cd was approximately two times higher in the fall 1997 samples compared to the spring 1996 samples. This likely reflects the influence of soil acidification on metal fractionation.

Treatments having manure amendments generally had significantly lower exchangeable and carbonate-bound Pb and Zn, and significantly higher organic Pb and Zn for both sampling dates (Tables 4 and 5) compared to the UC treatment. Residual Zn was higher for the UC treatment compared to any other treatment receiving manure with the spring 1996 samples, which is a desirable consequence of soil amendment if one assumes the residual fraction is less bioavailable. This same effect was present fall 1997, but it was not as consistent. Similar to Cd, exchangeable Pb and Zn were higher for the fall 1997 samples compared to spring 1996. Further, residual Zn was lower for the fall 1997 samples compared to the spring 1996 samples. Early in the study it appeared that the manure was effective in reducing bioavailable forms of Zn by converting them to residual Zn, which likely helped establish vegetation, but the beneficial effect of the manure was not as evident by the end of the study. Again, this is likely due to the soil acidification that was observed.

Relatively few studies in the literature report on the influence of soil amendments on the chemical fractionation of soil metals. Pierzynski and Schwab (1993) showed consistent reductions in exchangeable Zn upon addition of various organic by-products (cattle manure, turkey litter, alkaline stabilized biosolids) to a metal contaminated soil with corresponding reductions in Zn.
phytotoxicity problems with soybean. Ma and Rao (1997) reported a 10 to 95% reduction in nonresidual forms of Pb in soils amended with rock phosphate.

Nutrient deficiencies can limit plant productivity on mine spoil materials. The addition of manure significantly increased extractable P and K in both the spring 1996 and fall 1998 soil samples, with the exception of the SC treatment in 1996 (Table 6). Extractable P concentrations remained constant over the course of the experiment and were at levels sufficient for forage production while K levels decreased and were at levels insufficient for forage production by the end of the study (Whitney, 1990). Thus, low soil K levels could have also limited fescue production. The reason for this decrease is not known, however, given the coarse texture of the chat and the high rainfall environment, leaching is a probably cause. Nitrate and ammonium levels were low overall, with few treatment effects. The UMC treatment did have significantly higher extractable NH₄-N concentrations compared to all other treatments for the spring 1996 samples. This reflects the manure additions without plant uptake of N. Organic C levels were significantly higher in fall 1998 for treatments receiving manure compared to the treatment that did not receive manure. However, there was no significant effect of vegetation. Organic C would help to retain water and plant nutrients in the soil. Ideally, in a sustainable phytostabilization, soil organic C levels would need to be maintained or enhanced by the presence of vegetation. Otherwise, soil physical and chemical properties would likely revert to those present initially, which would make it difficult for vegetation to survive over long periods of time.

CONCLUSIONS

No evidence was found to indicate that inoculation of tall fescue by mycorrhizal fungi was successful, either by direct examination of root samples, DNA amplification, or by differences in growth or composition of the fescue. The addition of manure did initially promote
the growth of fescue with VC values as high as 71%. However, VC declined consistently over time, reaching a low of 28% at the end of the study. A variety of factors may have contributed to this decline. Plant tissue analysis suggested Zn phytotoxicity, which would make it less likely that the fescue would survive typical environmental stresses such as drought and cold temperatures. Soil pH decreased from an average of 7.9 early in the study to 6.5 at the end, which may be attributed to the oxidation of sulfide minerals. Chemical fractionation of metals in the soil indicated that manure applications generally decreased exchangeable forms of the metals and increased organic forms. Between the first and third years of the study, exchangeable forms of Pb and Zn generally increased while residual forms decreased. These changes are likely the result of the soil acidification that occurred during this same period, and may have contributed to the decline in fescue productivity because of increased problems with Zn phytotoxicity.

Additional study is needed on the use of soil amendments and fescue for phytostabilization of metal mine tailings. The potential benefits of more frequent manure applications clearly needs to be investigated along with additional methods or amendments for alleviating Zn phytotoxicities. Investigations on alternative plant species would also be appropriate.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of a grant from the Great Plains – Rocky Mountain Hazardous Substance Research Center.
Figure 1. Location of the Tri-State Mining District of southeastern Kansas and the city of Galena (after Lambert et al., 2000).
Figure 2. Basal or ground cover (BC - dashed lines), and vegetative or canopy cover (VC - solid lines) of vegetated plots over time. SC = manure-amended and seeded control; IN = manure-amended, seeded, and inoculated with mycorrhizal fungi, and BF = manure-amended, seeded, and treated with benomyl fungicide.
Table 1. Soil pH over time. All treatments but UC received manure. Means within a column followed by the same letter are not significantly different at P<0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spring 1996</th>
<th>Fall 1997</th>
<th>Fall 1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeded control (SC)</td>
<td>8.0a</td>
<td>6.8ab</td>
<td>6.5a</td>
</tr>
<tr>
<td>Inoculated (IN)</td>
<td>8.1a</td>
<td>6.9a</td>
<td>6.6a</td>
</tr>
<tr>
<td>Benomyl Fungicide (BF)</td>
<td>8.2a</td>
<td>6.7b</td>
<td>6.5a</td>
</tr>
<tr>
<td>Unseeded, manured control (UMC)</td>
<td>8.0a</td>
<td>6.7b</td>
<td>6.4a</td>
</tr>
<tr>
<td>Unseeded, unmanured control (UC)</td>
<td>7.4c</td>
<td>6.7b</td>
<td>6.6a</td>
</tr>
</tbody>
</table>
Table 2. Cadmium, lead, and zinc concentrations in fescue collected spring 1996 and 1997. Means within a column followed by the same letter are not significantly different at P<0.05.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cd</th>
<th>Pb</th>
<th>Zn</th>
<th>Cd</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeded control (SC)</td>
<td>4.7a</td>
<td>11.1a</td>
<td>498a</td>
<td>3.5a</td>
<td>3.7a</td>
<td>641a</td>
</tr>
<tr>
<td>Inoculated (IN)</td>
<td>5.6a</td>
<td>11.3a</td>
<td>494a</td>
<td>4.7a</td>
<td>5.4a</td>
<td>704a</td>
</tr>
<tr>
<td>Benomyl Fungicide (BF)</td>
<td>5.7a</td>
<td>9.4a</td>
<td>455a</td>
<td>5.0a</td>
<td>6.7a</td>
<td>594a</td>
</tr>
</tbody>
</table>
Table 3. Fractionation of soil Cd into exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic and residual fractions. Means within a column followed by the same letter are not significantly different at P<0.05.

<table>
<thead>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SC #</td>
<td>6.9c</td>
<td>5.7c</td>
<td>2.3b</td>
<td>19a</td>
<td>37cd</td>
<td>12a</td>
<td>4.2b</td>
<td>3.3a</td>
<td>9.5a</td>
<td>47a</td>
</tr>
<tr>
<td>IN</td>
<td>6.6c</td>
<td>6.4ab</td>
<td>2.4b</td>
<td>15bc</td>
<td>35cd</td>
<td>17a</td>
<td>5.4ab</td>
<td>3.8a</td>
<td>7.4a</td>
<td>35a</td>
</tr>
<tr>
<td>BF</td>
<td>8.5bc</td>
<td>10.4a</td>
<td>3.5ab</td>
<td>16b</td>
<td>44bc</td>
<td>16a</td>
<td>6.0b</td>
<td>4.6a</td>
<td>7.2a</td>
<td>43a</td>
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<td>UMC</td>
<td>12.4ab</td>
<td>10.3a</td>
<td>3.6ab</td>
<td>14c</td>
<td>53ab</td>
<td>23a</td>
<td>6.3ab</td>
<td>5.4a</td>
<td>7.0ab</td>
<td>53a</td>
</tr>
<tr>
<td>UC</td>
<td>12.7a</td>
<td>10.3a</td>
<td>4.0a</td>
<td>10d</td>
<td>64a</td>
<td>25a</td>
<td>10.5a</td>
<td>6.9a</td>
<td>5.0b</td>
<td>44a</td>
</tr>
</tbody>
</table>

@exchangeable (Exch.), carbonate (Carb.), Fe/Mn oxide (Fe/Mn).

#seeded control (SC), inoculated (IN), benomyl fungicide (BF), unseeded manured control (UMC), unseeded control (UC).
Table 4. Fractionation of soil Pb into exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic and residual fractions. Means within a column followed by the same letter are not significantly different at P<0.05.

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<tbody>
<tr>
<td>SC#</td>
<td>71b</td>
<td>593b</td>
<td>344a</td>
<td>17a</td>
<td>1174a</td>
<td>147b</td>
<td>486b</td>
<td>733a</td>
<td>21a</td>
<td>887a</td>
</tr>
<tr>
<td>IN</td>
<td>67b</td>
<td>432c</td>
<td>257b</td>
<td>13a</td>
<td>778b</td>
<td>99b</td>
<td>474b</td>
<td>707a</td>
<td>19a</td>
<td>766a</td>
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<td>BF</td>
<td>49b</td>
<td>531bc</td>
<td>296ab</td>
<td>21a</td>
<td>932b</td>
<td>123b</td>
<td>486b</td>
<td>736a</td>
<td>24a</td>
<td>891a</td>
</tr>
<tr>
<td>UMC</td>
<td>48b</td>
<td>516bc</td>
<td>313ab</td>
<td>19a</td>
<td>980ab</td>
<td>96b</td>
<td>406b</td>
<td>658a</td>
<td>18a</td>
<td>801a</td>
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<tr>
<td>UC</td>
<td>167a</td>
<td>776a</td>
<td>329ab</td>
<td>10b</td>
<td>1172a</td>
<td>240a</td>
<td>776a</td>
<td>879a</td>
<td>3.4b</td>
<td>773a</td>
</tr>
</tbody>
</table>

* Exchangeable (Exch.), carbonate (Carb.), Fe/Mn oxide (Fe/Mn).

† Seeded control (SC), inoculated (IN), benomyl fungicide (BF), unseeded manured control (UMC), unseeded control (UC).
Table 5. Fractionation of soil Zn into exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic and residual fractions. Means within a column followed by the same letter are not significantly different at P<0.05.

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<tr>
<th>Treatment</th>
<th>Exch.@</th>
<th>Carb.</th>
<th>Fe/Mn</th>
<th>Organic</th>
<th>Residual</th>
<th>Exch.</th>
<th>Carb.</th>
<th>Fe/Mn</th>
<th>Organic</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
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<td>8068bc</td>
<td>1557ab</td>
<td>788a</td>
<td>7499bcd</td>
<td>677ab</td>
<td>6767a</td>
<td>5852a</td>
<td>1970a</td>
<td>3676c</td>
</tr>
<tr>
<td>IN</td>
<td>194bc</td>
<td>6491c</td>
<td>1130b</td>
<td>661ab</td>
<td>6984cd</td>
<td>556bc</td>
<td>7570a</td>
<td>5836a</td>
<td>1574a</td>
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</tr>
<tr>
<td>BF</td>
<td>174c</td>
<td>9512b</td>
<td>2112a</td>
<td>669ab</td>
<td>8253b</td>
<td>712ab</td>
<td>8935a</td>
<td>6874a</td>
<td>1789a</td>
<td>4687ab</td>
</tr>
<tr>
<td>UMC</td>
<td>242b</td>
<td>10194ab</td>
<td>2182a</td>
<td>548b</td>
<td>9331b</td>
<td>831a</td>
<td>8923a</td>
<td>7605a</td>
<td>1677a</td>
<td>4807ab</td>
</tr>
<tr>
<td>UC</td>
<td>338a</td>
<td>12500a</td>
<td>2060a</td>
<td>324c</td>
<td>12725a</td>
<td>357c</td>
<td>7247a</td>
<td>9398a</td>
<td>813b</td>
<td>6498a</td>
</tr>
</tbody>
</table>

@exchangeable (Exch.), carbonate (Carb.), Fe/Mn oxide (Fe/Mn).
#seeded control (SC), inoculated (IN), benomyl fungicide (BF), unseeded manured control (UMC), unseeded control (UC).
Table 6. Extractable nitrates, ammonium, phosphorus and potassium for soil samples collected spring 1996 and fall 1998, and total organic carbon concentrations for samples collected fall 1998. Means within a column followed by the same letter are not significantly different at P<0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NO$_3$-N</th>
<th>NH$_4$-N</th>
<th>P</th>
<th>K</th>
<th>NO$_3$-N</th>
<th>NH$_4$-N</th>
<th>P</th>
<th>K</th>
<th>Organic C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>SC#</td>
<td>2.8ab</td>
<td>3.8b</td>
<td>95a</td>
<td>223bc</td>
<td>4.2a</td>
<td>2.4a</td>
<td>105a</td>
<td>41a</td>
<td>1.7a</td>
</tr>
<tr>
<td>IN</td>
<td>2.2bc</td>
<td>4.2b</td>
<td>88a</td>
<td>318ab</td>
<td>2.7a</td>
<td>2.1a</td>
<td>134a</td>
<td>44a</td>
<td>1.8a</td>
</tr>
<tr>
<td>BF</td>
<td>3.0a</td>
<td>6.5b</td>
<td>96a</td>
<td>388a</td>
<td>5.0a</td>
<td>2.3a</td>
<td>108a</td>
<td>48a</td>
<td>2.1a</td>
</tr>
<tr>
<td>UMC</td>
<td>2.1cd</td>
<td>20.3a</td>
<td>81a</td>
<td>373ab</td>
<td>2.8a</td>
<td>2.5a</td>
<td>121a</td>
<td>41a</td>
<td>1.7a</td>
</tr>
<tr>
<td>UC</td>
<td>1.5d</td>
<td>3.7b</td>
<td>18b</td>
<td>153c</td>
<td>3.4a</td>
<td>2.2a</td>
<td>8b</td>
<td>23b</td>
<td>0.6b</td>
</tr>
</tbody>
</table>

#seeded control (SC), inoculated (IN), benomyl fungicide (BF), unseeded manured control (UMC), unseeded control (UC).
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