The relationship of selected plants and their vesicular-arbuscular mycorrhizae in a heavy metal environment

by Thomas William Ferns

A professional paper submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation
Montana State University

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ABSTRACT

In 1982-1984, work at Montana State University focused on searching for an "edaphic" VAM species which might have evolved with the heavy metal pollution characteristic of the Anaconda copper tailings ponds. The hypothesis was, that if heavy metal tolerant VAM existed at Anaconda, then protection for non-tolerant plants might be realized by infection with heavy metal tolerant VAM. VAM were found in the Anaconda tailings ponds associated with known metal tolerant plants, colonial bentgrass and tufted hairgrass. A greenhouse experiment designed to evaluate the "effectiveness" of the Anaconda VAM in a toxic metals environment was implemented. The experiment showed sudan grass, a non metal-tolerant species, was significantly inhibited by the Anaconda tailings microflora when compared with a "natural" Bozeman microflora and/or a sterile soil control. The conclusion was the Anaconda VAM were actually more "effective" at transporting toxic metals to the detriment of the non metal tolerant host, sudan grass.

INTRODUCTION

During the past decade, discoveries by scientists working with vesicular arbuscular mycorrhizae (VAM) fungi have generated an "explosion" of interest. Now that VAM are known to be nearly ubiquitous on most higher plants and significantly beneficial to their nutrition, a wider variety of research on VAM is being proposed by workers in many disciplines of the basic and applied sciences. Studies are wide ranging, but there is particular emphasis on nutrient uptake, host growth responses, water relations, host-pathogen-symbiont interactions, and lately, on commercial applications designed to conserve crop plant mineral nutrient resources (Nemec 1982).

In 1982-1984, work at Montana State University focused on searching for an "edaphic" VAM species which might have evolved with the heavy metal pollution characteristic of the Anaconda copper tailings ponds. The hypothesis was, that if heavy metal tolerant VAM existed at Anaconda, then protection for non-tolerant plants might be realized by infection with heavy metal tolerant VAM.

To test the hypothesis, several plant and soil samples were collected from the Anaconda tailings during the summer of 1982 and assayed for VAM infection. Soil conditions at Anaconda are exceptionally suited for this study with pH's as low as 1.8 and toxic concentrations of aluminum and zinc in most samples. The data showed a peripheral pattern for the distribution of VAM at the ponds. Vesicular arbuscular infections were occurring in pond perimeter plants, but not in the same species as sampling continued centripetally. The cause of this concentric distribution was explored via a controlled greenhouse experiment. The experiment was designed to test the VAM's "efficiency" in ameliorating metal toxicity. Growth of plants treated with one of three treatments; an Anaconda VAM population, a Bozeman VAM population, and a sterile control was compared. A successful greenhouse experiment would show whether Anaconda VAM contribute to their host's heavy metal tolerance, or alternatively if Anaconda VAM inhibit their host by more effectively absorbing heavy metals in a situation where concentrations of the heavy metals can become toxic.

LITERATURE REVIEW

Undoubtedly the largest environmental waste site in the State of Montana is the tailings ponds of the Butte copper mine. Located near the old Anaconda smelter site just east of Anaconda Montana, these tailings have been deposited in and around this site for seventy-five years (Stephenson pers. comm. 1983).

From their inception the ponds have been a source of heavy metal water pollution when wet, and noxious dust clouds when dry. The mineralogy of the Butte ore body (a copper mine) was a hydrothermal mineral deposit. This type of deposition normally includes sulfide minerals of gold, silver, copper, mercury, lead, zinc and other metals (Keller 1976). Many elements in the ore body are considered heavy metals. Heavy metals are loosely defined as a group of metallic elements with relatively high atomic weights capable of being toxic to biological systems at very low concentrations (Miller 1975). Because of the availability of toxic concentrations of heavy metals, the Anaconda ponds have been declared a hazardous waste site by the EPA (Lovell pers. comm. 1984). It is now important that the ponds be stabilized to contain these metals on site.

There are two options for stabilizing a site such as the Anaconda tailings. One, an engineering option, would be to mechanically drain the ponds and apply a top dressing of coarse rock simulating desert pavement. The other option would be to establish a cover of vegetation.

Stabilization of mining wastes by revegetation is the best economic long term solution to aesthetic and ancillary pollution problems (Jeffery et al. 1974). However, the metals present are usually phytotoxic and contribute to mineland revegetation failures (Bradshaw 1977). Nonetheless, rational revegetation procedures must ultimately be found to ameliorate the mining legacy. Derelict mining lands are a blight both aesthetically and environmentally.

A tailings pond from a sulfide ore deposit is probably the worst case scenario for heavy metal production. In the ore milling process up to one percent of the target ore, in this case copper, along with many of the secondary minerals not economically worth extraction, are flushed into the tailings ponds (Bradshaw 1971). The minerals lost in the smelting process are the source of inimical concentrations of heavy metals. Once in the tailings pond, the sulfide ores are exposed to an oxidation-reduction cycle accelerated by their small size and readily available moisture of the pond. The sulfides start producing acid, oxidizing to sulfates by various routes. The lowered pH increases the activity of the heavy metals in the soil solution (Dijkshoorn et al. 1981). Exacerbating the problem is the lack of organic matter or true clay particles with the cation exchange capacity (CEC) capable of immobilizing metals. An increase in the CEC would reduce the potential for metal toxicity (Bohn et al. 1979).

Succinctly stated, there are four prerequisites for increasing heavy metal mobility and the concomitant probability of toxicity (Bohn et al. 1979).

(1) Increasing the actual concentration of the metal in the soil solution.

(2) Decreasing the concentration of chelating soil organic matter.

(3) Decreasing the clay content (ie CEC) of the soil.

(4) Decreasing the soil pH (exceptions As, Cr and Se).

All four of the requisites are met at the Anaconda site.

Although there are many problems associated with a tailings pond, revegetation can be realized. Within the constraints of evolution, certain plant species, or more correctly edaphic varieties within a species, have shown an increased tolerance towards otherwise toxic levels of heavy metals (Bradshaw 1977). Two grasses, colonial bentgrass (Agrostis tenuis (Sibth.)) and tufted hairgrass (Deschampsia cespitosa (L.) Beauv.), known to be heavy metal tolerant, are dominant on revegetated portions of the Anaconda site (Surbrugg 1982). The basis for metal tolerance in higher plants is thought to be genetically controlled and specific for each metal (Bradshaw et al. 1965). An edaphic variety which shows tolerance for one metal may not be tolerant towards others (Jowett 1958). For example, in colonial bentgrass the basis for zinc tolerance appears to involve a number of genes with additive and dominant effects (Gartside and McN eilly 1974). Metal tolerant plants provide an effective avenue for successful revegetation.

Plant Responses to Heavy Metal Toxicity

The deleterious effects of heavy metals on plants can be expressed via several pathways:

(1) Competition with essential elements for root uptake or enzymatic sites (competitive inhibition).

(2) Inactivation of enzymes due to irreversible bonding or denaturation.

(3) Alteration of nucleic acid organization (teratogens).

(4) Alteration of membrane structure.

(5) Alteration of various macromolecular structures (e.g. spindle apparatus or mitochondria).

(6) Alteration of cytoplasmic water structure.

(7) Alteration of cytoplasmic colloid structure.

Those plant species existing in a heavy metal environment must obviously avoid these deleterious effects (Cahoon 1983).

There are two alternatives for plants living in a heavy metal environment and trying to mitigate the aforementioned hazards, tolerance and avoidance (Levitt 1980).

Tolerance of heavy metals is an internal amelioration of the heavy metal problem. There are several theoretical mechanisms which might account for the tolerance of edaphically adapted species. The following list of adaptations is from Cahoon (1983) and Popp (1983).

(1) Differential uptake of ions.

(2) Removal of ions via deposition.

(3) Removal of ions via extrusion.

(4) Chelation or complexation of ions by organic compounds.

(5) Selective translocation to non-vital areas of the plant.

(6) Alternate metabolic pathways which bypass inhibited enzymes.

(7) Increased production of inhibited enzyme(s).

(8) Production of organic antagonist.

(9) Decreased requirements for products of an inhibited system.

(10) Production of an altered enzyme insensitive to metal ion effects.

Compounds known to be involved in heavy metal tolerance are the organic acids-oxalate, malate, citrate; amino acids; and mustard oil glucosides. Because the plant families Caryophyllaceae, Polygonaceae and Oxalaceae typically exhibit free oxalate, and the Brassicaceae specialize in mustard oil gulcoside production, these families can be expected in areas of high metal content (Popp 1983). Tolerance of heavy metals once they have entered the plants roots seems to be the pervasive plant strategy.

The other alternative, avoidance or exclusion (Popp 1983), relies on external mechanisms which affect the adsorption and absorption of ions at the root surface. External mechanisms are chemical exudates which alter the rhizosphere chemistry controlling the availability of selected ions (Cahoon 1983). Foy et al. (1978) demonstrated that crop species can alter the rhizosphere pH, rendering aluminum ions insoluble and thus unavailable for uptake by the plant. However, Ernst (1976) stated that there was no evidence for higher plants excluding ions. Although the utilization of plant symbionts or other rhizosphere organisms to trap heavy metals before they enter the plant's root system may occur (Bradley et al 1981).

Mycorrhizae, a Plant Symbiont

Mycorrhizae are obligate fungal symbionts which share a close, stable association with higher plants. The mycorrhizae are divided into five subgroups 1) Ectomycorrhizae, 2) Ectendo or arbutoid mycorrhizae, 3) Endomycorrhizae or vesicular arbuscular mycorrhizae (VAM), 4) Ericoid mycorrhizae and 5) Orchid mycorrhizae. The division is based primarily upon morphology and host plant species. The majority of the mycorrhizae are either Ectomycorrhizae (trees and shrubs) or V A Mycorrhizae (most forbs and grasses).

V A Mycorrhizae are lumped into the Endogonales as a single family, the Endogonaceae. There are seven genera in the family. The genera are based on manner of spore formation and/or sporocarp morphology. Azygospore genera are Endogone, Acaulospora, Entrophospora, and Gigaspora. Chlamydospore genera include Glaziella, Glomus and Sclerocystis. Morphologically the VAM can be separated from the ectomycorrhizae by the lack of a hyphal mantle (Hartig net) and aseptate hyphae; and from the arbutoid, ericoid and orchid types also by the aseptate hyphae (Trappe and Schenck 1982). The VAM are termed vesicular arbuscular mycorrhizae because of structures they form in the host root's cortex. The vesicle represents a thick walled lipid storage organ, while the arbuscule resembles a modified haustorium. Arbuscules differ from haustoria in that they represent a site of bidirectional flow of carbon and nutrients between the host plant and the VAM (Brown and King 1982). Mycorrhizae as a whole have been associated with improving their host's mineral nutrition, water supply, hormone balance and preventing disease (Nemec 1982).

The role of VAM as a mediator of mineral nutrition is the best documented. Vesicular arbuscular mycorrhizae (VAM) are known to enhance phosphate uptake as well as uptake of copper and zinc in phosphorus deficient soils (Lambert et al. 1979). Alternately, phosphorus fertilization has been found to suppress metal uptake by mycorrhizal plants (Ross 1971). There is some evidence that metal uptake is reduced because phosphorus fertilization tends to inhibit mycorrhizal development (Mosse 1973). However, others (Baker 1978), contend that a change in the degree of mycorrhizal infection would affect the phosphorus nutrition of the plant, and in turn interact with the heavy metal uptake and/or tolerance of the plant. The fact that mycorrhizae can stimulate plant metal uptake in soils where the metals are sparingly available suggests that the influence of mycorrhizae on metal uptake should be studied. Especially when the host plants are growing in soils containing potentially toxic levels of heavy metals (Killham and Firestone 1983).

In the study of plant succession certain plant families (e.g. Chenopodiaceae, Brassicacae, Asteraceae and Poaceae) are generally the first to invade disturbed areas. Reeves et al. (1979) studied the mycorrhizal factor of plant succession on disturbed rangeland in Colorado. They found primary succession to be dominated by nonmycorrhizal plants of the Chenopodiaceae and Brassicaceae families. Their conclusion was that after a severe site disturbance mycorrhizae propagules were reduced to a point where those plants which are non-mycorrhizal were at an advantage. They found less than 1% of colonizing plants on disturbed sites were mycorrhizal, whereas 99% of the existing plants on nearby undisturbed sites were mycorrhizal. Their conclusion was that mycorrhizal species are more effective in the competition for soil nutrients and water and, that the mycorrhizal species would eventually replace non-mycorrhizal species in an orderly succession. It seemed a coincidence worth investigation that of the four families prominent in primary succession, it was the Poaceae (colonial bentgrass and tufted hairgrass), a normally mycorrhizal family, which had successfully colonized the acid heavy metal tailings ponds at Anaconda.

Although there is a large body of literature covering the metal tolerance mechanisms of colonial bentgrass and tufted hairgrass (Bradshaw 1965, Antonovics 1968, Gregory and Bradshaw 1965, Cox and Hutchinson 1980, Walley et al. 1974), their rhizosphere associations are rarely mentioned (Sparling and Tinker 1978). Recent work in England on metaliferous soils has suggested that heavy metal tolerant strains of mycorrhizae may protect plants against the effects of heavy metal pollution (Gildon & Tinker, 1981; Bradley et. al, 1981; Bradley et. al, 1982; Gildon & Tinker, 1983).

Bradley et al. (1981) worked with Calluna vulgaris, one of the most successful colonizers of heavy metal polluted soils in northern Europe. Calluna vulgaris is a member of the Ericaceae and as such is associated with ericoid mycorrhizae. Ericoid mycorrhizae are known to differ from vesicular arbuscular mycorrhizae by a heavier cell infection and by augmenting the host's nitrogen supplies as opposed to phosphorous augmentation by VAM. Additionally the Ericaceae is a family of woody plants which depend upon mycorrhizae as a root hair supplement more than herbaceous plants might. Bradley et al. (1981) found that whereas non-mycorrhizal plants showed no tolerance at high levels of aluminum and zinc, mycorrhizal infection with Pezizella ericae provided a major degree of resistance to the metals. They found that infection led to significant reduction of the heavy metal content of the shoot. Bradley et al. (1981) explained that this protective action is a result of enhanced binding of the heavy metals in the roots at the extensive mycorrhizae hyphal complexes.

In another example of mycorrhizal heavy metal mediation, Gildon and Tinker (1981 and 1983) worked with the vesicular arbuscular mycorrhizal fungus Glomus mossae. Two strains of Glomus mossae, one from an "average" agricultural soil, and one from a metaliferous soil, were compared. Gildon and Tinker (1983) found that the VAM isolate from the metaliferous soil was much more tolerant of zinc and cadmium in the soil than the agricultural VAM and suggested that the mycorrhizal infection from the metaliferous site could protect plants against heavy metal additions. Although Bradley et al (1981) cautioned about speculation as to exactly how such a heavy metal protection mechanism might work.

The experiments of Bradley et al. (1981) and Gildon and Tinker (1981 and 1983) compared mycorrhizal strains in which the metal tolerant strain had evolved in a naturally metaliferous soil. In areas where metaliferous soil conditions have been introduced by man, such as soils downwind of a metal smelter, near major highways or in contact with smelter tailings piles, the mycorrhizae may not have had sufficient time to evolve effective tolerance mechanisms. The proclivity of mycorrhizae for metals may in fact harm their symbionts in these circumstances. Killham and Firestone (1983) in a metal smelter effluent study found that the mycorrhizal plants exhibited reduced growth and higher heavy metal contents than their non-mycorrhizal controls. They concluded that mycorrhizal enhancement of heavy metal uptake caused reduced growth in plants exposed to an acidic environment and heavy metal depositions.

In the proximity of the Anaconda smelter, Hartman (1976), while studying fungal populations, found that various combinations of heavy metal pollutants were the main limiting factors responsible for reduced fungal propagule counts in the area. He also concluded that the reduced fungal diversity and propagule density may result in the reduction of mycorrhizal fungi. Although the roots of native basin wildrye (Elymus cinereus Scribn. & Merr.) from around Anaconda appeared to be mycorrhizal, subsequent observations of conifer roots from the same area indicated a lack of ectomycorrhizae. Hartman (1976) stated that the failure of the mycorrhizal association may partially account for past failures of the Anaconda Company to revegetate the area.

METHODS AND MATERIALS

This research was directed to the resolution of two questions. One, do the plants, colonial bentgrass and tufted hairgrass growing on the Anaconda tailings ponds possess VAM? And two, does the mycorrhizal association contribute to the metal tolerance of the plants?

To accomplish the research goals, sampling sites were located on the tailings. At each site native plants were collected with their associated root zone materials. After laboratory analysis to discern those roots infected with VAM, a distribution map of the VAM and their host plant species was drawn to facilitate future VAM collection. Those plants identified as VAM hosts were collected as an inoculum source for a greenhouse experiment to determine whether Anaconda VAM help or hinder a non-tolerant plant species (sudan grass, <u>Sorghum vulgare</u> Pers.) in a toxic situation (Anaconda tailings). Field characterization of salt hazard (EC) and acidity (pH) are reported for the 0-3 cm surface materials.

Collection of Plant and Soil Materials

Two collection trips were made to Anaconda, and one to Bozeman. The first trip July 19, 1983, was a general survey of the plants on the Anaconda tailings ponds. These ponds were haphazardly constructed as the need arose and are labeled by an alphanumeric identifier (eg. A1), as a semblance of construction chronology. The ponds are subjected to seasonal desiccation and flooding. A minimum of four plants and their associated soils were collected from each of four ponds, the B2, B1, C2 and D2 (see Fig. 1). All plants were identified using the flora key, Plants of the Pacific Northwest (Hitchcock and Cronquist 1976) and their location noted on the map (see Fig. 1). Samples of plant roots and their associated soils were taken for further analyses in the laboratory.

Collection sites were chosen by various criteria.

1) If the site was on an Anaconda tailings pond.

2) If there was easy access from a dike road.

3) If there was a relatively healthy stand of both colonial bentgrass and tufted hairgrass at the site.

4) And if the sites were previously investigated by Cahoon (1983) and Surbrugg (1982).

Plants collected at the B2 pond (Fig. 1) northern cell (pH 3.1, EC 0.8)(Cahoon's 1983 and Surbrugg's 1982 collection site), were colonial bentgrass and tufted hairgrass.

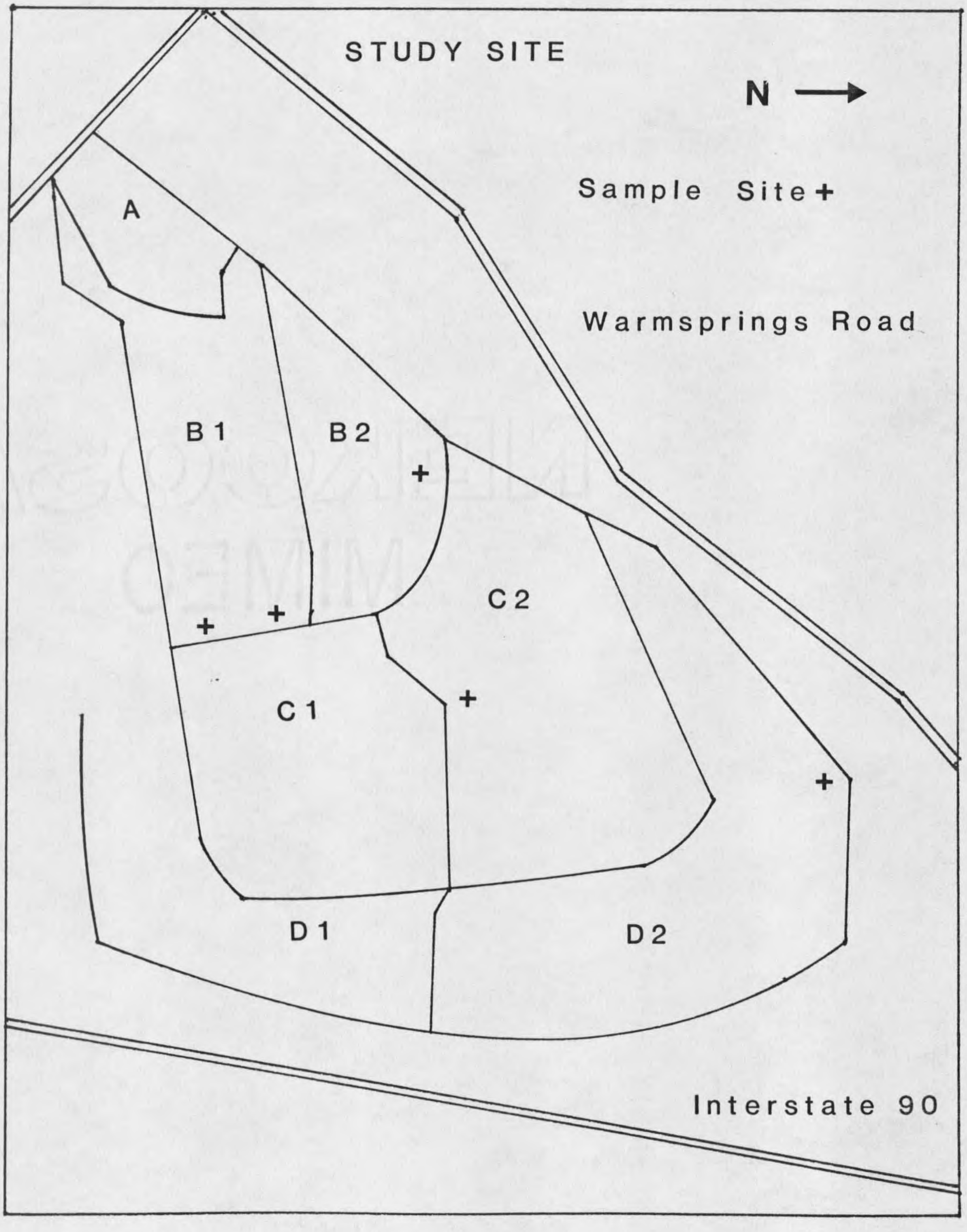

Figure 1. Collection Sites at the Anaconda Tailings.

The B2 pond was last used around 1930. Since that time water and sewage effluent from the town of Anaconda have been intermittently applied to its surface (Stephenson pers. comm. 1983).

At the B1 pond, northern cell, (pH 3.2 EC 1.0) colonial bentgrass and tufted hairgrass were again collected. The pond had not been used since 1930 (Stephenson pers. comm. 1983) but had received macronutrients as part of a fertilizer trial. Near the drainage bottom of the fertilizer trial area (pH 7.2), little meadow foxtail (Alopecurus aequalis Sobol.), Salix sp., Epilobium sp., were collected.

Those plants collected at the C2 pond, (pH 7.2 EC 2.5) were giant wildrye, growing on a pedestal of discarded bricks, and colonial bentgrass, tufted hairgrass and a Salix sp. from a nearby drainage way. The C2 collection site was near the head of a pond and the tailings were quite shallow. The willows observed in the area were thought to be growing in the original soil, beneath the pond. No willows were observed in deeper tailings. The C2 pond was used from 1950 to 1960 (Stephenson pers. comm. 1983).

At the northern end of the D2 pond, (pH 7.4 EC 3.3), colonial bentgrass and tufted hairgrass were again collected. This pond was the more recent of the ponds, last being used in the 1960's (Stephenson pers. comm.

1983). The D2 collection site was only 50 feet away from native vegetation (Fig. 1).

At Bozeman an agricultural soil (coarse loamy mixed Typic Haploboroll) was collected adjacent to MSU grain field trial plots near the corner of South 19th and Garfield. This soil was the source of VAM not adapted to toxic conditions such as those at Anaconda. Half of the Bozeman agricultural soil was autoclaved and used in the greenhouse experiment as a non-toxic non-VAM control. The Bozeman soil was collected on the MSU campus under a cover of mycorrhizal (Glomus sp.) smooth brome (Bromus inermis Leys.) and yellow lucerne (Medicago falcata L.).

After determining the plants, colonial bentgrass and tufted hairgrass from the D2 site were mycorrhizal, a second trip was taken to the Anaconda site. Ten VAM infected plants and their associated root zone materials were collected for further root analyses and as a VAM inocula source for the Bozeman greenhouse experiment. Tailings collected from the root zone of plants at the D2 site were used as the "toxic" soil in the greenhouse experiment. The toxicity of the tailings collected from the D2 site was confirmed by chemical analyses for heavy metals (Table 1).

Laboratory Analyses of Field Samples

The experimental soil materials were air dried, and then passed through a two millimeter sieve for uniformity. Chemical analyses were performed for basic fertility and selected heavy-metal ions. Nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, zinc, aluminum, cadmium, lead, and copper analyses were performed on both the Bozeman sandy loam and the Anaconda loamy sand tailings. In addition, nitrate nitrogen concentration of the autoclaved Bozeman soil was analysed to determine whether autoclaving released the organic nitrogen as nitrate nitrogen. Budget constraints precluded a complete analysis of metals. A list of methods for soils analyses is given in Appendix A.

Following field collection, plants were stored in open plastic bags at 4 C for a period of up to seven days. Before staining, the roots of the plants were soaked overnight in a 10% solution of calgon to assist in the removal of clay particles. After twelve hours the roots were washed until clean with tap water.

Clearing and staining of the roots for mycorrhizal development was based on the method of Kormanik and McGraw (1982). However, a 10% alkaline hydrogen peroxide wash was not used, and the 0.05% trypan blue in lacto-

Table 1. Chemical analyses of Bozeman soil and Anaconda tailings materials.

PARAMETER ANALYZED	BOZEMAN SOIL	ANACONDA TAILINGS
pH	6.9	2.9
EC mmhos	2.58	2.50
% Organic matter	3.7	0.24
Olsen Phosphorus ppm	34.1	8.0
Potassium ppm*	291.0	20.2
Calcium mg/L**	715.0	453.0
Magnesium mg/L	79.5	41.6
Sodium mg/L	10.9	6.5
Iron ppm	17.0	297.2
Zinc mg/L	0.43	99.7
Copper mg/L	0.206	5.92
Aluminum mg/L	0.5	23.9
Lead mg/L	<0.009	0.016
Cadmium mg/L	0.005	1.020
Nitrate kg/ha	2.2, 60.2***	<0.01
Texture s,si,c	59,26,15 sl	79,14,7 ls

* Cations reported in ppm are EDTA extracted.
** Cations reported in mg/L are from saturated paste.
*** Nitrate level is for the autoclaved Bozeman soil.

phenol stain was changed to 0.05% trypan blue in lactic acid to avoid the carcinogenic phenol fumes. By observation the resulting stain was equivalent to the stain of the lactophenol method.

A nonsystematic scanning of the roots under a 20X - 70X dissecting microscope was followed by confirmation of mycorrhizal structures with a 150X compound microscope. Kormanik and McGraw (1982) state that there is no strong evidence in the literature favoring the systematic or nonsystematic procedures for assaying stained roots. At the present level of mycorrhizal research, correlation between the degree of mycorrhizal infection and subsequent changes in plant nutrition can not be made. Therefore mycorrhizae were recorded as being either present (+) or absent (-) in the root system.

Where rapid VAM infection is desired (a research priority), soil inoculum is the preferred medium. This is because the greater number of infective propagules (external hyphae, mycorrhizal root fragments and spores) and the associated soil microflora can favor germination of spores (Ferguson and Woodhead 1982). Other methods (e.g. plating, funnel, etc.) may be more precise but, they are not as fast. The best method of estimating actual numbers of viable inocula in a soil medium is the most probable number technique (MPN) (Wilson and Trinick

1982). However because of time limitations, a faster albeit not as accurate method of estimating propagules, the plate count method was substituted (Daniels and Skipper 1982).

A VAM Greenhouse Experiment Contrasting VAM Metal Responses

After the D2 plants colonial bentgrass and tufted hairgrass had been identified as mycorrhizal, a greenhouse experiment was conducted to determine if the VAM found at Anaconda affected the growth response of a non metal-tolerant plant species, such as sudan grass. The experiment was based on the assumption that the Bozeman soil would have a non metal-tolerant VAM population, while the Anaconda soil would have a metal-tolerant VAM population. For statistical purposes the null hypothesis was; There are no differences in a non metal-tolerant plant's growth in a heavy metal toxic soil treated with, a metal-tolerant VAM, a non metal-tolerant VAM, or no VAM.

The Experimental Design

The greenhouse experiment was designed to test the effects of three VAM treatments on the autoclaved Anaconda tailings. There were six levels of treatment and five replications in a randomized complete block design for a total of ninety (3X6X5) experimental units. The experimental units were read nine times over the course of the experiment (Venator 1975). Therefore in order to evaluate the experiment as a whole, 810 (3x6x5x9) variables were analysed as a split plot in time experimental design.

The experimental unit was a sudan grass plant growing in a 15 cm X 2.5 cm ID plastic cone. Each plant container was plugged with cotton and filled with 40 g of a soil/tailings mix consisting of various combinations (8 g units) of VAM treatments and tailings.

Statistical analyses were completed with the computer program MSUSTAT (Lund 1983), utilizing the program AVMF (Analysis of Variance Multi Factors) split plot in time, with time being the main factor.

In an effort to reduce the number of uncontrolled variables in the greenhouse experiment, a common source of VAM inocula was used. Sudan grass was chosen as the common symbiont for VAM collected in both the control (Bozeman soil) and the response (Anaconda tailings). Sudan grass is often used as a VAM nurse crop symbiont because its fibrous root system provides a plethora of necessary sites for VAM infection (Ferguson and Woodhead 1982). It is not known to be metal tolerant, unlike the native Anaconda VAM hosts colonial bentgrass and tufted hairgrass (Cahoon 1983 and Surbrugg 1982).

Measurements of two response functions (leaf height and leaf numbers), were taken for a total of nine weeks after a three week waiting period for a total experimental time of twelve weeks. A third response function (VAM infection) was noted after the twelfth week's measurements by sacrificing the sudan plants and examining their root systems for vesicles with a dissecting microscope. Leaf height and leaf number were recorded because both described the overall effects of toxic tailings on sudan grass better than either response would have alone. Additionally, it is impossible to determine where a toxic effect might express itself as a plant response. Therefore increasing the number of response functions increased the probability of finding a significant result expressed by the treatments.

The Experimental Procedure

Four soils were used in the greenhouse experiment. The materials and their actual combinations were as follows.

1) The Bozeman sandy loam with its native VAM population.
2) The Bozeman sandy loam autoclaved and re-inoculated with a native Anaconda VAM population.
3) The Bozeman sandy loam autoclaved.
4) And the Anaconda tailings (loamy sand) autoclaved.

Three VAM treatments were used. Each of the three VAM treatments had six levels (0 g, 8 g, 16 g, 24 g, 32 g, 40 g) of VAM treatment in a factorial design.

1) Autoclaved Anaconda tailings diluted by six levels of the Bozeman soil with the Bozeman VAM inoculum.

2) Autoclaved Anaconda tailings diluted by six levels of Bozeman soil with the Anaconda VAM inoculum.

3) And autoclaved Anaconda tailings diluted by six levels of autoclaved Bozeman soils (a control).

The measured response functions were:

1) The growth of the sudan grass seedlings measured each week in centimeters from the soil surface.

2) The phenological growth of the sudan grass as measured each week by the total number of leaves.

3) And the presence (+) or absence (-) of mycorrhizae in the sudan grass roots at the termination of the Greenhouse experiment.

Four 46x30x10 cm, metal trays were used to produce the VAM inocula. Two trays contained native agricultural VAM from a Bozeman soil with smooth brome and yellow lucerne roots as the inoculum source. The other two trays contained VAM infected plants from the Anaconda tailings, either colonial bentgrass or tufted hairgrass. Sixteen surface sterilized (5.25% sodium hypochlorite) sudan grass seeds were placed among the live inocula plants in a seedbed of autoclaved Bozeman soil. When the sudan grass was approximately 15 centimeters high, the native plants being used for inoculum were killed by radically clipping their aerial shoots. This left just the sudan grass and the microflora associated with the native plant roots remaining in the trays.

The sudan grass was allowed to grow until December 1983 (3 to 4 months as recommended by Ferguson and Woodhead 1982). At this time the roots of the sudan grass were checked with a one centimeter cork borer for VAM infections and verified in all four trays. The plant tops were then removed, the soil dried and crushed by hand. Soils containing VAM inocula were stored in plastic bags in a cool (18 C) dry place until used five weeks later as soil VAM treatments.

RESULTS AND DISCUSSION

The complete analysis of variance tables for the leaf number response and for the leaf height response are found in Appendix B.

The response functions leaf height and leaf number were both significant (at the 5% level) for the main effects of weeks, treatments and levels; and the interaction level X treatments. Only the leaf height response gave a significant interaction (2.6%) for treatments X weeks. The week means were significantly different for each week of the leaf number response (Table 2), but not the leaf height response (Table 3). This can be interpreted as leaf additions were discernible on a weekly schedule while leaf growth was either subject to other factors, or on a non-weekly schedule. VAM treatment means were significantly different for each of the VAM treatments in all cases (Tables 4 & 5). The Anaconda VAM produced the least number of leaves and leaf growth. The sterile Bozeman soil produced the most sudan grass growth, and the Bozeman VAM exhibited an intermediate growth response.

Table 2. Least significant difference of leaf number data.

EXPERIMENT TIME WEEK	MEANS		VAM AMEMDMENT TYPE TREATMENT	MEANS		TOXIC TAILING'S LEVEL MEANS GRAMS VAM TREATMENT/ GRAMS TOXIC TAILINGS		
1	3.978	A	ANA VAM	5.581	A	0/40	5.793	A
2	4.833	B	BZN VAM	5.985	B	8/32	5.800	A
3	5.367	C	STERILE	6.835	C	16/24	5.993	B
4	5.756	D				24/16	6.052	B
5	5.989	E				32/8	6.059	B
6	6.298	F				40/0	6.207	C
7	6.756	G						
8	7.244	H						
9	7.644	I						

Means followed by same letter are not different at 95% CI.

Table 3. Least significant difference of leaf height (cm) data.

EXPERIMENT TIME WEEK	MEANS		VAM AMENDMENT TYPE TREATMENT	MEANS		TOXIC TAILING'S LEVEL MEANS GRAMS VAM TREATMENT/ GRAMS TOXIC TAILINGS		
1	8.033	A	ANA VAM	13.18	A	0/40	9.800	A
2	12.13	B	BZN VAM	15.50	B	8/32	15.48	B
3	15.33	C	STERILE	20.50	C	24/16	16.41	C
4	16.19	D				16/24	17.79	D
5	17.47	E				32/8	18.39	D
6	17.68	E				40/0	20.49	E
7	19.57	F						
8	20.31	G						
9	20.83	G						

Means followed by same letter are not different at 95% CI.

The significant interaction of levels X treatments (see Tables 7 & 8 Appendix B) shows that not all three VAM treatments behaved the same over all levels of tailings treatment. Tables 6 and 7 demonstrate that for the VAM treatments Bozeman VAM and sterile control, growth decreases with soil toxicity--as expected. However with the Anaconda VAM, growth increases (leaf number data Table 4) or lacks a significant trend (leaf height data Table 5) with increasing tailings levels. The different response trends of the VAM treatments to toxic tailings levels cause the significant interaction. Why the Anaconda inoculum caused a different result is very significant to the experiment.

If the leaf number response data is examined, a trend of decreasing leaf number with increasing increments of Anaconda VAM inoculum is apparent (Table 4). The fact that the number of leaves started by sudan grass at all levels of the Anaconda VAM inoculum are below the lowest number of leaves (Table 4) in sterile tailings control treatments seems to indicate that the Anaconda VAM actually contained an inimical component more inhibitory to sudan grass than the most toxic tailings level.

The third response function, mycorrhizal infection, was recorded as either present (+) or absent (-). The following table, Table 6, gives the results.

Table 4. Least significant difference of leaf number means for each VAM treatment by treatment level.

BOZEMAN VAM TREATMENT MEAN	LEVEL		ANACONDA VAM TREATMENT MEAN	LEVEL		STERILE BOZEMAN NO VAM TREATMENT MEAN	LEVEL	
5.7	8/32	A	5.3	16/24	A	5.8	0/40	A
5.8	0/40	AB	5.49	32/8	AB	5.96	8/32	A
5.9	32/8	BC	5.51	40/0	AB	6.51	16/24	B
5.9	24/16	BC	5.62	24/16	BC	6.58	24/16	B
6.1	16/24	C	5.76	8/32	C	6.73	32/8	B
6.38	40/0	D	5.78	0/40	C	6.73	40/0	B

Levels represent grams VAM treatment/grams toxic tailings.
Means followed by same letter are not different at 95% CI.

Table 5. Least significant difference of leaf height (cm) means for each VAM treatment by treatment level.

BOZEMAN VAM TREATMENT MEAN	LEVEL		ANACONDA VAM TREATMENT MEAN	LEVEL		STERILE BOZEMAN NO VAM TREATMENT MEAN	LEVEL	
9.8	0/40	A	9.8	0/40	A	9.8	0/40	A
14.13	8/32	B	11.6	16/24	B	20.33	16/24	B
14.47	16/24	B	11.69	24/16	B	20.40	8/32	B
15.47	32/8	B	14.71	8/32	C	22.87	24/16	C
18.82	24/16	C	15.0	40/0	CD	23.40	32/8	C
20.27	40/0	D	16.29	32/8	D	26.20	40/0	D

Levels represent grams VAM treatment/grams toxic tailings.
Means followed by same letter are not different at 95% CI.

Table 6. Occurrence of VAM infection in sudan grass roots.

TREATMENT LEVELS	BOZEMAN VAM TREATMENT	ANACONDA VAM TREATMENT	STERILE SOIL TREATMENT
40/0	+	+	-
32/8	+	+	-
24/16	+	+	-
16/24	+	+	-
8/32	+	+	-
0/40	-	-	-

Levels represent grams VAM treatment/grams toxic tailings.

The VAM infection data assures that the VAM treatments were viable at each level of inoculum. However the VAM infection data cannot assure that there was a significant difference in plant growth based on VAM infection levels alone as contended by Kormanik and McGraw (1982).

SUMMARY

The greenhouse experiment showed that the three treatments, Bozeman VAM grown in Bozeman soil, Anaconda VAM grown in Bozeman soil and sterile Bozeman soil, were significantly different over all levels combined and for all times combined. Furthermore the results of the experiment can be shown to fit into a hypothesis that VAM do indeed inhibit the growth of non adapted grass in a toxic metal environment as proposed by Killham and Firestone (1983).

A common strategy of metal tolerant plants is to translocate excess metals to non-vital areas in the plant. The Anaconda VAM may have adapted to life at Anaconda by translocating its metal problems to the metal tolerant hosts colonial bentgrass and tufted hairgrass. However when given a non metal-tolerant host such as sudan grass, the strategy would create a situation supported by the Anaconda VAM data. In this case a VAM adapted to the toxic environment would translocate the excess metals to the plant host. Sudan grass, a non metal-tolerant host, would show increased stress as the level of Anaconda VAM inoculum increased.

The Bozeman VAM would show a medium amount of translocating toxic metals to the sudan grass because the Bozeman VAM has not had to become adapted to a toxic environment and would thereby lack the systems necessary to manage the metals. The lack of adaptation to life in a toxic soil would weaken the Bozeman VAM itself, thereby reducing the efficiency of metal translocation to the sudan grass by the Bozeman VAM.

The best growth of the sudan grass was in the sterile Bozeman soil. Using the hypothesis that VAM translocate metals to their host, then a non-VAM soil (i.e. sterile Bozeman soil) would present the least metal hazard to the sudan grass. The lack of metal translocation by a VAM would result in the best growth of the sudan grass as observed.

Caveats

The data seem to fit the theory of Killham and Firestone (1983). However, exactly what was in the Anaconda and Bozeman experimental inocula is subject to debate. Although only VAM was the intended inocula, other organisms were undoubtedly passed in the root inocula media. Baker and Nash (1965) have shown that bacterial populations of the rhizosphere can have a profound influence on the nutrition and general health of a plant. What part these bacteria might have played in the greenhouse experiment is open to debate. Additionally the increased growth of the sudan grass in the sterile Bozeman soil might easily be explained by the increased amount of available nitrogen after soil sterilization.

An alternative hypothesis for the inimical qualities of the Anaconda VAM inoculum is that a fungal disease was carried in the inoculum roots from Anaconda. This hypothesis is based on the following.

1) In general fungi grow better in acid environments than bacteria.

2) Hartman (1976) found many pathogenic and facultative pathogenic fungi at Anaconda during his survey.

3) Plants growing in toxic environments are usually stressed.

4) Stressed plants are readily attacked by pathogens.

However the author tends to discount the alternative hypothesis. There was a difference in the size of the vesicle structures found formed from Anaconda medium (about 60% of the size of the Bozeman vesicles) versus the Bozeman medium. This would indicate at least two morphologically different VAM were involved in the study. The peripheral pattern of VAM at the Anaconda ponds indicates an evolving edaphic VAM at the site. And the VAM distribution data does tend to support the hypothesis that the Anaconda VAM is evolving mechanisms to deal with the toxic metal levels in the tailings ponds.

Albeit the question of, if the disease and nitrate level possibilities are masking the true response, or are additive with the true response, or perhaps even synergistic with the true response, is still valid.

CONCLUSIONS

There was a significant difference between the three VAM treatments. The Anaconda VAM caused the poorest performance of the non metal-tolerant sudan grass in a metal toxic situation. Therefore, based on this research, the successful vegetative stabilization of the Anaconda tailings ponds will depend upon the metal tolerance systems inherent in the main tailings colonizers, colonial bentgrass and tufted hairgrass, and not on the metal tolerance systems of their associated mycorrhizae.

LITERATURE CITED