Lignin and Lignin Derivatives to Promote Vegetative Growth and Metal Uptake in Mine Tailings

Bryan Tisch[1], Dr. Konstantin Volchek[2], Dr. Alexander Okonski[3], Dr. Mikhail Raskin[4], Cheryl Black[1]

[1]Natural Resources Canada, Mining and Mineral Sciences Laboratories, 555 Booth St., Ottawa, Ontario, Canada, K1A 0G1
[2]Scientific Applications International Corporation (SAIC), 3439 River Road, Gloucester, Ontario, Canada, K1A 0H3
[3]Elliot Lake Research Field Station, 75 Dieppe Ave., Elliot Lake, Ontario, Canada, P5A 2R8*
[4]Lignin Products Utilization LLC, 121 Erin Rd., Stoughton, MA 02072, USA

Abstract
Lignin and lignin derivatives (LD's), such as lignosulfonates, are major by-products of the pulp and paper industry. Lignin is currently used as a plant nutrient and soil agglomerator, and is known for its biodegradability, low cost and low toxicity. Lignin and LD's can be employed in either solid or liquid format, which provides excellent versatility in terms of its application, local site conditions, soil type and end goal. Previous work by Science Applications International Corporation (SAIC) Canada has shown that lignin, and especially lignin derivatives, effectively bind heavy metals and enhance their removal from soil and groundwater. As a result, the Mining and Mineral Sciences Laboratories of Natural Resources Canada, along with SAIC have initiated a laboratory research program to further investigate the applicability of lignin and LD's as an inexpensive method of both revegetating and detoxifying mine tailings and sludge ponds. The overall program will consist of both hydroponic and pot growth trials that examine the effect of lignin and LD's on biomass production and heavy metal content. The initial laboratory investigation has shown that the addition of lignin derivatives had a positive influence on plant biomass, when added to a low pH, high metal and sulphate solution.

Introduction

Lignins and lignin derivatives are non-toxic, natural by-products of the pulp and paper industry, and are well known for their ability to bind heavy metal ions (Varma *et* al., 1990; Volchek *et* al. 2000). Lignins are a group of phenolic polymers that confer strength and rigidity to the woody cell wall of plants (Lignin Institute, 2001). These compounds make up the second most abundant class of chemicals found in wood (cellulose is first). Commercial lignin is produced as a co-product of the paper industry, separated from trees by a chemical pulping process. Various processes are now available to remove and isolate lignin from these cells, and each process produces material with a different composition and properties.

Lignins were first used in leather tanning and dye baths in the 1880's. Today, they have found wide application, ranging from use as emulsifiers in animal feed, to fertilizers to the raw material in the production of vanillin and other foods (Lignin Institute, 1992).

With respect to fertilizers, lignins are capable of keeping micronutrients such as Cu, Co, Mn and Zn, in an available form under pH conditions that might normally cause insolubilization. Since they are more weakly bound than those made from other complexing agents, such as EDTA, they are more readily available to plants.

Raskin (1999) found that treating lignins with acid followed by oxidative degradation enhanced the complexing properties of lignins.

In general, plants less readily take up higher molecular weight lignins. It is possible, however, to separate lignins into different molecular weight fractions using ultrafiltration. In this way, lignin derivatives can be tailored to select properties that will enhance the uptake of micronutrients in nutrient deficient soils or

*Currently with Environment Canada, Existing Substances Branch, PVM, 351 St. Joseph Blvd., Hull, Quebec, K1A 0H3

decrease metal uptake where potentially toxic levels of metal exist.

There are two phytoremediation approaches that may be utilized in tailings or other contaminated soils: a) phytostabilization or phytorestoration, and b) phytoextraction (Vangronsveldl et al, 2000).

Phytostabilization involves metal inactivation as a result of the establishment of vegetation, with or without the use of chelating agents. This technique simply reduces the mobility of potentially toxic metals. Ideally, vegetative species would be chosen which limit the uptake of metals by roots and the subsequent translocation to the shoots. As indicated above, lignin derivatives may be tailored to play a role in reducing metal uptake. Phytoextraction, on the other hand, attempts to increase the removal of metals from soils and generally through the use of hyperaccumulating plant species.

Several plant physiological properties are important for phytoremediation, including potential metal accumulation, rate of metal translocation from roots to shoots, high biomass production and, high tolerance to specific metals (Landberg, 2000).

The Mining and Mineral Sciences Laboratory (MMSL) of Natural Resources Canada, along with Science Applications International Corporation (SAIC) have initiated a laboratory research program to investigate the applicability of lignin and lignin derivatives as an inexpensive method of both revegetating and detoxifying mine tailings and sludge ponds.

The current work is the first of a planned series of experiments, and was designed to evaluate the effect of lignin derivatives on the growth and metal uptake of several grass and legume species under typical conditions (low pH and elevated metals and sulphate) found within the rooting zone of metalliferous tailings. While it is recognized that none of the plant species chosen for study are regarded as hyperaccumulators, they all produce relatively high quantities of biomass. The intent was to examine the effect of lignin derivatives on biomass production and metal uptake by these species.

Methods and Materials

A hydroponic study was carried out over a 5-week growth period, to evaluate the effect of lignin derivatives on growth and metal uptake in the following six common (in Ontario) reclamation species.

Redtop (*Agrostis gigantea*)
Creeping Red Fescue (*Festuca rubra*)
Timothy (*Phleum pratense*)
Canada Bluegrass (*Poa compressa*)
Alsike Clover (*Trifolium hybridum*)
Birdsfoot Trefoil (*Lotus corniculatus*)

The experiment was carried out in triplicate, using 4" square pots containing pure silica sand (Ottawa sand) as an inert growth medium (to support the growing seedlings). Seeds were added to the pots at an equivalent rate of 75 kg/ha, placed in trays containing distilled water, and kept in the dark for one week to facilitate germination. After one week, the pots were placed into one of three separate trays containing one of the following treatment solutions:

1) ½ strength Hoaglands solution (nutrient control)
2) ½ strength Hoaglands solution + metals + lignin derivatives
3) ½ strength Hoaglands solution + metals

A modified Hoaglands solution was used as the nutrient solution control, by which to evaluate the effect of added metals, sulphate and lignin derivatives. Metals were added to the Hoaglands solution in the form of sulfates of Cu, Mn, Zn and Ni to yield the approximate constituents indicated in Table 1, generally representative of conditions within an oxidized tailings environment. The ½ strength Hoaglands solution was kept at its natural, near-neutral pH, while the metal treatment solutions were acidified with 10% sulphuric acid to a pH of approximately 4.5.

Table 1: Composition of Feed Solutions.

Component	Concentration (mg/L)
NO_3	233
PO_4	24
K	61
Cu	3
Mn	5
Ni	2
Zn	5
Fe	1.25
Ca	50
Mg	12
SO_4	1000

The trays were then placed into a growth chamber, which had been programmed to simulate conditions during a mid-summer day. An ebb and flow system was utilized, in which the pots were soaked for approximately 1 hour, four times daily. The pH and conductivity of the feed solutions were generally measured twice a week, and the feed solutions were periodically topped up. Dilute (10%) sulphuric acid was used to maintain the solution pH (solutions 2 and 3 only) near 4.5.

After four weeks, the shoots were tested for photosynthetic efficiency using an FMS-2 Fluorescence Monitoring System (Hansatech Instruments Ltd.). The pots were harvested by cutting the shoots at sand level, and then washing the sand from the roots with distilled water. The shoots and roots were dried in an oven at approximately 70 degrees Celsius, weighed, and then ground in a Wiley™ Mill in preparation for metal and nutrient analyses. Metal analyses had not been completed at the time of this publication; therefore no metal data are presented in this paper.

Results and Discussion

Utilizing NO_3^- as the nitrogen source resulted in a fair amount of difficulty in maintaining the pH of solutions 2 and 3 near 4.5. Most plants are able to utilize either NO_3^- or NH_4^+, and whereas plants grown in nutrient solution containing NO_3^- as the sole nitrogen source tend to increase solution pH, plants grown in solutions using NH_4^+ tend to lower the pH. A combination of NH_4^+ and NO_3^- can buffer against changes in pH, and will be used in all subsequent experiments.

Very poor germination and/or growth were achieved with creeping red fescue, alsike clover and birdsfoot trefoil. Therefore, these species are not discussed further.

As expected, shoot growth was greatest in the Hoaglands nutrient solution (Figure 1). Even at half strength this solution contains all the mineral elements needed for rapid plant growth, and in fact, are generally higher than what is typically available in soils. The addition of lignin derivatives to the metal enriched Hoaglands solution resulted in an increase in shoot growth of approximately 27%, 35%, and 16% for Redtop, Canada Blue Grass, and Timothy respectively (Figure 1). Without shoot metal data, it is difficult to specifically account for this increase, but it was likely due either to a decrease in the uptake of metals by plants grown in the solution containing the LD, or there was improved availability of nutrients with the additions of LD.

The same general trend was observed for root growth (Figure 2), although there was much more variability in the data.

Chlorophyll fluorescence can be used as a screening tool to provide an indication of photosynthetic efficiency in vegetation, and is dependent upon a range of factors including stresses caused by environmental conditions.

The addition of lignin derivatives improved the photosynthetic efficiency in Redtop and Timothy (Figure 3), to the level observed in the Hoaglands solution control. No effect was noted in Canada Blue Grass. The maximum photosynthetic efficiency (Fv/Fm) is always close to 0.8 in healthy leaves, irrespective of the plant species studied. A lower value indicates that some of the photosynthetic reaction centres are damaged. This is referred to as photoinhibition, and is common in plants under stress conditions.

Even though the photosynthetic efficiency was comparable between the Hoaglands control and with the addition of lignin derivatives, biomass production was lower in the solution containing the lignin derivatives. Plants grown in both solutions containing elevated metal levels at low pH were noticeably chlorotic (pale green). This indicates a nutrient deficiency/toxicity and is usually associated with nitrogen deficiency. Nitrate spot measurements in all three feed solutions were similar, at approximately 650 ppm. Therefore nitrogen was not in short supply. It is suspected that the low pH conditions in solutions 2 and 3 may have interfered with the uptake of nutrients, especially nitrogen. This hypothesis will be further exmined when metal and nutrient analysis on the roots and shoots is completed.

Conclusions

The addition of lignin derivatives to an acidic, metal-contaminated feed solution increased the quantity of shoot biomass produced by three of six species tested.

Chlorophyll fluorescence measurements indicated that photosynthetic efficiency was improved with the addition of lignin derivatives in Redtop and Timothy to levels similar to control plants.

Analysis of shoot and root metal levels will provide a clearer understanding of the mechanisms involved and the effect of the lignin derivatives.

Literature Cited

Landberg, T. 2000. Important Physiological Factors for Optimizing Phytoremediation. In: Proceedings of Phytoremediation 2000, April 6-8, 2000, Crete, Greece.

Lignin Institute. 1992. Lignins – Products with Many Uses. Lignin Institute Dialogue, Winter, 1992.

Lignin Institute. 2001. Lignin and its Properties. Lignin Institute Dialogue, Vol. 9, Number 1, July 2001.

Vangronsveldl, J., A. Ruttens, N. Spelmans1 and D. van der Lelie. 2000. Phytostabilization as a Working Technology. In: Proceedings of Phytoremediation 2000, April 6-8, 2000, Crete, Greece.

Raskin, M. 1999. Polyphenolic Derivative Chelating Agent. US Patent No. 5,880,271. March 9, 1999.

Varma, K.V.R., T. Swaminathan, and V.P.R. Subrahmanyam. 1990. Heavy Metal Removal with Lignin. Journal of Environmental Science and Health, Vol. A25, No. 3, pp.243-265.

Volchek, K., Raskin, M., Weber, M. 2000. Evaluation of Lignin Derivatives for Soil and Water Cleanup. In: Proceedings of the Seventeenth Technical Seminar on Chemical Spills (TSOCS), Environment Canada, Vancouver, BC, pp. 31-37.

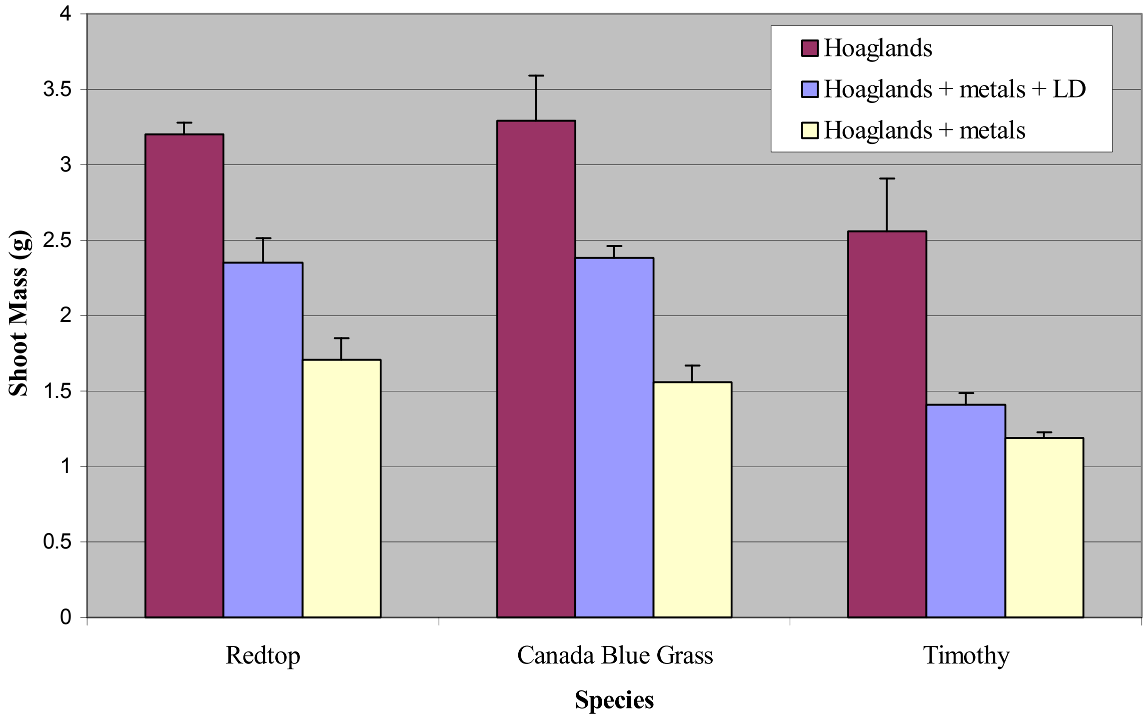

Figure 1: Effect of lignin derivatives on mean shoot biomass (+/- SE) after four weeks of growth. (n=3)

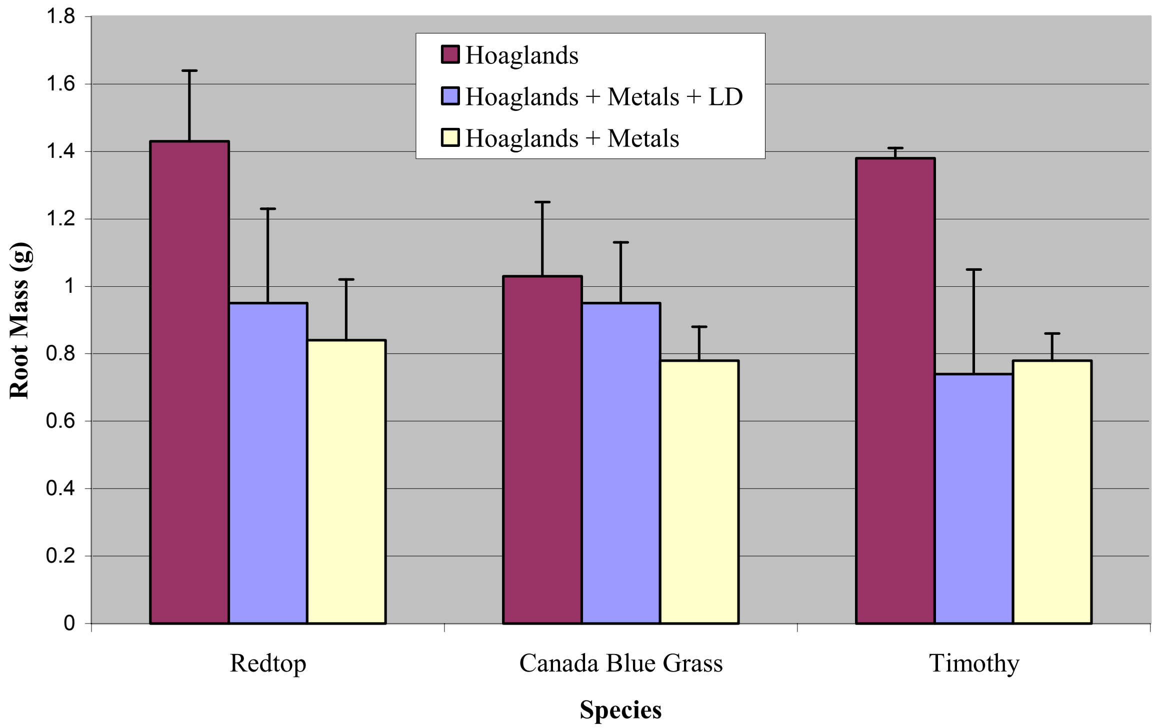

Figure 2: Effect of lignin derivatives on mean root biomass (+/- SE) after four weeks of growth. (n=3)

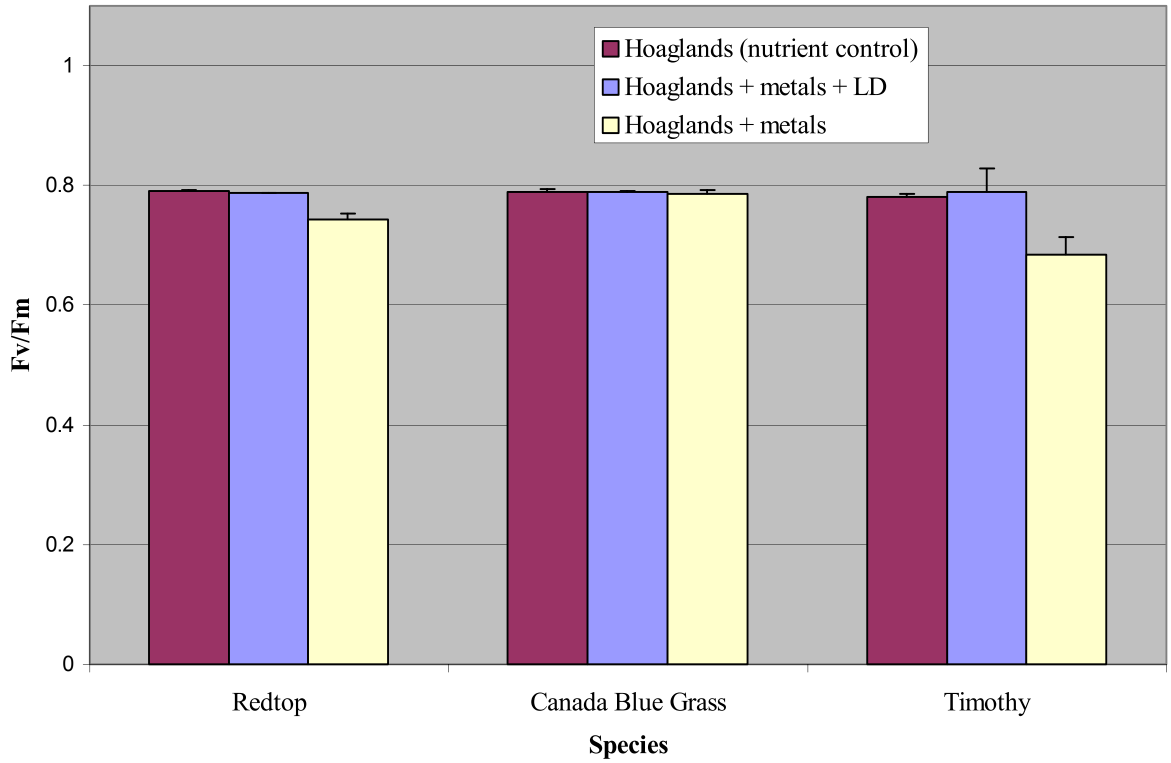

Figure 3: Effect of lignin derivatives on mean photosynthetic efficiency (+/- SE) after four weeks of growth. (n=15)