

**A Literature Review of the
Use of Native Northern Plants for the Re-
Vegetation of Arctic Mine Tailings and
Mine Waste**

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Summary

This work examines the difficulties in using native species for revegetation projects with particular emphasis on heavily contaminated northern metalliferous sites. Starting with a review of the effects of toxicity on plants, the work progresses into the topics of phytoremediation and the unique properties required for a plant to survive on the extreme climatic and nutrient conditions of a former mine site. Finally specific plant literature is reviewed with the conclusion being a recommendation for AEL to pursue the evolving field of bryophyte restoration in conjuncture with the contemporary methods.

Introduction

Metal mine and mill wastes are the most difficult and highly visible reclamation problems facing land rehabilitation specialists today (Munshower 1994, Neuman et al. 1993). At many mine sites combinations milling, smelting, and refining operations result in large volumes of wastes, debris, and contaminated soil. Re-entrainment of these wastes continues to be a problem. Release mechanisms include air dispersion, surface water runoff, and wind erosion, resulting in secondary contaminant sources in surface and subsurface soils, sediments, surface water, and fugitive dust.

As global populations grow, the demand for natural resources accelerates. New mining and mineral extraction technology also has spurred resurgence in the mining industry. Such developments can create severe disturbances that potentially threaten the integrity of watersheds including water quality, wildlife habitat, aesthetic resources, and other environmental concerns. These severe disturbances often suspend succession and thus intensify contamination problems. The resulting loss of vegetation decreases soil stability, soil shading, organic matter, nutrient cycling, and in turn, degrades wildlife habitat.

The cold climates of northern Canada increases the time required for disturbances to rehabilitate naturally. Depending on soil conditions (root zone characteristics), drastic disturbances may require hundreds or thousands of years to achieve functional plant communities. Hardrock mine lands pose additional problems for revegetation, primarily: steep slopes; unfertile soil media (low cation exchange capacity, low water holding capacity, low organic matter); extreme moisture, temperature, and wind fluctuations; acidic soils; and heavy metal contamination. To accelerate rehabilitation, proper plant selection on these harsh sites is crucial.

Numerous scientists and regulatory agencies recognizing the importance of adaptation and biological diversity, frequently require the re-establishment of a permanent vegetative cover of the same variety native to the area (Roundy et al. 1997). Scientists contend it is essential that indigenous native plant species be selected that are

evolutionary products of that environment. Native indigenous species have a long history of genetic sorting and natural selection by the local environment. Over the long-term, these plants are often better able to survive, grow and reproduce under the environmental extremes of the local area than introduced plants originating in other environments (Brown 1997, Munshower 1994).

The utilization of tolerant races, even on amended or top soiled sites, is suggested because the subsoil is often a major portion of the root zone materials. Species exhibiting acid/heavy metal tolerances may also reduce the need for lime and are a precaution against poor mixing of amendments with acidic wastes. Populations of acid and metal tolerant vegetation have been successfully selected, propagated and established on abandoned mine sites in Europe and Africa. Tolerant populations have been identified in North America but none have been propagated for seed production.

Improving the metal tolerance of plants is of interest to scientists internationally. Many papers have recently been published on developing transgenic metal tolerance (Hasegawa et al. 1997, Manual de la Fuente et al. 1997, Vatamaniuk et al. 1999). However, the biology is complex, poorly understood, and the technology needed to transfer genes to wild plants is not well developed. Ethical and biological questions related to releasing genetically engineered organisms into the environment are also pertinent.

Traditional plant breeding (plant selection) seems to be the best approach at present.

Except when extensive amelioration has been employed, reclamation efforts on hard rock mine sites in North America have met with limited success. The majority of native seed currently being used on metal mine reclamation projects in North America were developed for coal strip-mine reclamation and range renovation outside of the climatic conditions of northern Canada. The following work reviews the difficulties with establishing plants on mine waste, with particular emphasis on metalliferous sites in northern Canada.

Ionic Toxicity to Plants

Chemical concentrations that control plant growth are classified into three levels. There is a concentration range at which no effects are observed on any species, a range at which all species are susceptible, and in between the area where a few adapted or resistant species are able to survive. Classic examples are the halophytes of saline soils, calcifuges on acid soils, flood tolerant plants on waterlogged soils, and plants which have evolved resistance to air pollution.

Ionic toxicity can be classified on the basis of two distinctions:

- 1) The concentration at which the toxicity occurs;
- 2) On a presence or absence basis i.e. whether or not the element is essential to plant growth, or can a deficiency occur.

If an ion is toxic only at high concentrations, usually the problem for the plant is low soil water potential; making it impossible for the plant to take up water, even if it has the capacity to exclude the toxic compound from itself. Similarly, if an ion is both an essential micro nutrient and yet toxic at very low concentrations (Zn^{2+} , Cu^{2+}), a plant may be excluding an essential element unless it has some mechanism where uptake can be very precisely controlled.

I ECOLOGICAL SITUATIONS WHERE TOXICITY OCCUR:

A. Saline and Sodic Soils

Soils affected by high levels of sodium salts develop in two ways. Coastal soils that are annually inundated by sea water, are ionically dominated by Na^+ and Cl^- . Alternatively, in dry inland areas where evaporation exceeds precipitation, rain water containing dissolved salts at very low concentrations can cause massive accumulation of salt in the upper soil layers. In these soils Na^+ and SO_4^{2-} predominate. Sometimes soils rich in Na_2CO_3 , are also produced volcanically. Salt deserts are among the most stable ecological communities and cover huge areas of the world.

Plants that grow on soils with high salt concentrations are termed *halophytes*. To survive they must overcome the following problems;

- 1) Osmotic effects----The solute potential of seawater is -20 bar, and even, 5% NaCl has potential of -4.2 bar. To take water from such solutions a plant must achieve an even lower intracellular osmotic potential.
- 2) Specific ion effects----- high concentrations of Na^+ and Cl^- are toxic to most cells and the associated high concentrations of Mg^{2+} , SO_4^{2-} can also be lethal.
- 3) Habitat effects---- soils affected by salt tend to be extreme areas in other respects (soils acting like cement) This occurs because high Na^+ content causes deflocculation of soil clay particles and loss of air-filled pore space and the soil behaves like cement.

B. Calcareous and Acid Soils

Field botanists are aware of the importance of limestone as a determinant of vegetation. Soils formed over parent materials containing a high proportion of CaCO_3 (limestones, chalks, and a plethora of rocks and glacial deposits) have high pH levels. Limestone soils are well buffered by a reservoir of CaCO_3 and only in extreme conditions can one lower the pH of these sites below 5. On the other hand soils formed on parent material such as granite, or base poor sands are typically dominated by Al^{3+} as the major cation. These soils are strongly acid, their pH controlled by a complex hydrated aluminum ion buffer system. Natural Al-based soils seldom have pH values below 3.5. Lower pH values are encountered, but usually in soils with very little aluminum - e.g. ombrotrophic peat - or where soil sulphides are oxidized to sulphate, producing in effect sulphuric acid.

The differences between calcareous and acidic soils are not just those of pH, nor only of Ca^{2+} and Al^{3+} ion concentrations. H^+ ions are toxic to most plants at pH values below 3, and below pH 4.0 mineral soils contain so much soluble Al^{3+} as to be severely toxic. However, pH also controls the solubility of Mn^{2+} , Fe^{3+} , and many other cations. Both Mn and Fe are essential nutrient elements, which may be present at toxic concentrations in acid soils and below deficiency levels in calcareous areas. Of the other major nutrients, K^+ is displaced from exchange sites at low pH, and lost by leaching. P availability varies in a complex fashion with pH, but tends to be deficient at high and low pH levels. Nitrogen availability is very low in acid soils because of impaired microbiological activity. In summary, plants of calcareous soils (*calcicoles*) must contend with deficiencies and plants of acidic soils (*calcifuges*) with toxicities, though there are important exceptions to this generalization. Table 1 summarizes these relationships.

C. Metal-contaminated Soils

Numerous reports exist of naturally occurring toxic levels of heavy metals. For our purposes the most important are copper (Cu), zinc (Zn) and lead (Pb), and more rarely cadmium (Cd), chromium (Cr), cobalt (Co), and nickel (Ni).

Although natural contamination provides fascinating problems for plants, the type of metal-affected soil that has attracted most attention is where the soil is artificially contaminated - polluted by metals. Typically, this occurs where waste been dumped from mining or smelting operations, air pollution or even from such improbable sources as galvanized fences.

Table 1: Ionic relationships of calcareous and acid soils.

<i>Ion</i>	<i>Calcifuges on acid soils</i> (pH<5)	<i>Calcicoles on calcareous soils</i> (pH>6.5)
H^+	High: may become toxic to non-adapted plants	Low: not required
OH^-	Low: not required	High: may compete with anions for uptake
HCO_3^-	Low: not required	High: may compete with anions for uptake
Ca^{2+}	Low: if very deficient, may disrupt membrane function	High: may cause phosphate precipitation at root surface, and compete with other cations for uptake
Al^{3+}	High: not required and may cause precipitation of	Low: not required

		ions (e.g. H_2PO_4^-) at root surface inhibition of Ca^{2+} uptake and transport, and interfere with DNA metabolism, <i>inter alia</i>	
Fe^{3+}	High:	acts similarly to Al^{3+} in phosphate precipitation	Low: deficiency a major problem
Mn^{2+}	High:	toxicity relatively unimportant in relation to Al^{3+}	Low: deficiency common in agricultural conditions
MoO_4^{n-}	Low:	deficiency may interfere with N fixation	High: occasionally produces toxicity symptoms
$\text{NO}_3^-/\text{NH}_4^+$	Balance in favour of NH_4^+ ;	nitrification inhibited	Balance in favour of NO_3^- ;
$\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$	H_2PO_4^- - dominant species	absorbed by Fe and Al	nitrifying bacteria active
K^+	Displaced from exchange sites by H^+ ; leached		HPO_4^{2-} increases; absorbed by Ca
			Ca^{2+} ions may interfere with uptake

Where mineral deposits are the source of contamination, release of the metal ions is usually facilitated by low pH. Copper, for example, occurs as the sulphide or carbonate ores, the latter being more labile in acid conditions. Copper sulphide reacts with ferric sulphate formed by the natural oxidation of ferric sulphide to release soluble copper and sulphate ions (Peterson and Nielsen, 1978). The activity of oxidizing bacteria, species *Thiobacillus* and *Ferrobacillus* acting upon iron sulphide can indirectly release copper, nickel, zinc, arsenic and even molybdenum from their native minerals (Firth, 1978). The exception to the greater availability of toxic heavy metals in acid soils is molybdenum, which can become toxic on calcareous soils.

Table 2: A simple classification of the effects of toxins on plants.

<i>A. Effects on the ability to acquire resources</i>			
(i)	Acquisition of water:	(a)	osmotic effects arising from excess solute concentrations
		(b)	inhibition of cell division, reducing root growth
(ii)	Acquisition of nutrients:	(a)	competition between ions
		(b)	damage to membranes

- | | | |
|-------|---|---|
| | (c) | effects on symbionts |
| | (d) | inhibition of cell division |
| (iii) | Acquisition of CO ₂
and light energy: | (a) stromatal malfunction caused by
gases ^a
(b) chlorophyll bleaching ^a |
- B. *Effects on the ability to utilize resources*
- (i) inhibition of enzyme action
 - (ii) inhibition of cell division
 - (iii) loss of respiratory substrates; O₂ deficiency^a
-

II. EFFECTS OF TOXINS ON PLANTS

The range of substances capable of adversely affecting plant growth is enormous, and the specific effects of these toxins are too numerous to document. For instance, Foy *et al.* (1978) suggest that aluminum alone may fix phosphate on root surfaces and decrease root respiration, cell division, cell wall rigidity and the uptake and utilization of Ca, Mg, P, K and H₂O. It is possible to classify these effects according to whether they exert their influence on the acquisition of resources by the plant or on the utilization of those resources (Table 2).

A. Acquisition of Resources

1. Water

Only two ions commonly reach solution concentrations sufficient to cause plants osmotic problems. They are chlorides, (usually in wet saline soils) and sulphate (in dry sodic soils) both typically associated with sodium as the cation, although magnesium and calcium may also be involved.

It was long considered that this was the major cause of salt toxicity. However, recent evidence shows that this is not the case. Low water potentials are, as a result of pollution are not normally damaging even to non-halophytes. To summarize this, pollution as commonly believed does not normally kill plants by preventing them from taking up water.

2. Nutrients

Plant roots absorb ions from a complex medium containing the dozen or more essential nutrient ions, but also a range of non-essential ions and organic compounds. If severe imbalances arise in this supply, the plant may not be able to take up nutrients efficiently, because of direct effects of toxic ions on root metabolism and function. As a result, even essential ions can become toxic. (e.g. magnesium, see below) Plant species show great differences in the extent to which they can tolerate variation in ionic ratios.

The simplest effects are those where interactions occur outside the root. The process of transpiration causes accumulation of some ions at the root surface, which are diffusely absorbed by the plant. If accumulation exceeds uptake, on calcareous sites, calcium salts bind to the roots. Since many calcium salts are insoluble, this inhibits the diffusive supply of nutrients to the plant. In acid soils, aluminum may accumulate particularly aluminum phosphate.

Damage to the plasmalemma (the roots nutrient selective boundary) can also occur. Calcium, as a plant nutrient, is largely involved in maintaining membrane function (Burstrom, 1968; van Steveninck, 1965), and one of the effects of aluminum toxicity is a reduction in Ca uptake (Clarkson and Sanderson, 1971).

More common effects, are where ions interfere directly with each other's uptake. Such interactions are competitive, where closely related ions compete for the same uptake sites, or non-competitive, where the toxic ion simply inactivates the uptake mechanism.

Competitive and non-competitive inhibition can be distinguished by their effect on uptake kinetics. In non-competitive cases, the actual uptake mechanism is incapacitated and so the maximum rate attainable is reduced. Competitive inhibition depends on the relative concentrations of the two ions. Competition occurs usually between chemically related ions, such as the alkali metals (Na^+ , K^+ , Rb^+) or alkali earths (Ca^{2+} , Mg^{2+} , Sr^{2+}). Thus, saline soils are toxic due to competitive inhibition of K^+ uptake. Serpentine soils are toxic as Mg^{2+} inhibits Ca^{2+} .

It should also be remembered that nutrient supply in soil is heavily dependent on microbial symbionts, N-fixing bacteria and P-supplying mycorrhizas. Any adverse effects on these symbionts will severely reduce the nutrient supply. Most *Rhizobium* strains are more or less inactive below pH 5, although *Mycrica gale* (bog myrtle. Potential use for Arctic), with an actinomycete symbiont can continue to utilize atmosphere N at a pH as low as 3.3 (Bond, 1951).

III. RESISTANCE TO TOXICITY

Plants, which grow in soils that contain levels of toxic ions lethal to other species, utilize four main mechanisms:

- (i) Phenological escape - where the stress is seasonal, the plant may adjust its life cycle to grow and reproduce in the most favorable season.
- (ii) Exclusion - the plant is able to recognize the toxic ion and prevent its uptake
- (iii) Amelioration - the plant absorbs the ion but minimizes its effects. This may involve chelation, dilution, localization or excretion.
- (iv) Tolerance - the plant evolves a metabolic system, which can function at toxic concentrations

The most resistant species employ more than one such mechanism. However, the adoption of any one or any combination imposes important physiological and ecological constraints.

A. Escape Mechanisms

1. Phenology

Seasonal stresses permit the adjustment of the plant life cycle so that growth occurs at the most favorable time. There is evidence of seasonality of toxic ion concentrations in a variety of soils. Where bacterial transformations of heavy metal ores are involved, spring levels are low, since low winter temperatures inhibit bacterial activity and high rainfall promotes leaching.

Soil pH fluctuates seasonally in many soils, notably on waterlogged soils as redox potentials change. Gupta and Rorison (1975) found changes of the order of 1 pH unit in a podzol and regular seasonal changes of 2.0 to 2.5 units occur in waterlogged peat (Fitter *et al.*, 1980). In mineral soils, such changes have dramatic effects on Al^{3+} concentrations, resulting in mature plants growing in Al^{3+} concentrations that are lethal to seedlings (Rorison *et al.*, 1958). These species exist as their seedling germination window has adjusted to the seasonally lower levels. Natural examples include the early spring growth of herbs in temperate deciduous forests, and in the rain-triggered growth of desert ephemerals.

2. Direct Environmental Modification

Rarely plants alter the environment by reducing the toxicity. The classic example is rice plants oxidizing Fe^{2+} to Fe^{3+} by O_2 excretion from the roots to avoid Fe^{2+} -toxicity and effectively raising the pH. Rice to my knowledge has not been used in mine site restoration.

B. Exclusion

Prima facie, exclusion of a toxic ion is the ideal resistance mechanism. Numerous examples exist of plaquing where the plant physically excludes the toxin after uptake. Our classic examples are cattails and some horsetails.

1. Recognition

Plants typically have highly selective uptake systems, capable of distinguishing chemically similar ions. However, a number of ion pairs cause problems, particularly K^+ and Na^+ , and Ca^{2+} and Mg^{2+} . Excess Mg^{2+} ions disrupt the ability of plants to distinguish calcium. Such conditions exist on many serpentine (ultra-basic) soils, and may account for the general paucity of vegetation there. Plants growing these soils, adaptive ecotypes of *Agrostis stolonifera* or *A. canina* (grasses), appear to take up the two ions more in proportion to the external concentrations than do susceptible ecotypes of the same species.

2. External Structures

Even if the plant can recognize the toxic ion and exclude it, there are still metabolically active structures, which cannot be protected. Clearly, all cell membranes in contrast with the external solution are potentially at risk, and if the ion can move in the apoplast, this may include all cells as far in as the endodermis. As mentioned, copper ions in contrast to zinc ions cause leakage of potassium from cells, presumably by membrane damage. Wainwright and Woolhouse (1975) showed that a Cu-resistant race of *Agrostis tenuis* was only half as susceptible to such K^+ leakage as a Zn-resistant or a normal race.

In addition, all plant roots have surface enzymes, of which the best known and studied are the acid phosphatases. The function of these enzymes is not clear; but it is widely assumed that they are involved with the breakdown of organic phosphate in soil. However, there is no evidence that plants are able to utilize directly soil organic P (Abeyakoon and Pigott, 1975). Nevertheless, acid phosphates are widespread and are inhibited by toxic ions in soil. Wainwright and Woolhouse (1975) found differential responses to Al^{3+} and Cu^{2+} of enzymes from different ecotypes of *A. tenuis*. In the case of Cu, kinetic analysis indicates a non-competitive inhibition, with adaptive differences in inhibitor constant (k_i) between the two ecotypes. The k_i for copper-resistant plants is 1.50 mM Cu^{2+} , and for the susceptible enzyme 0.54 mM Cu^{2+} ; the smaller value indicates that the susceptible enzyme has a greater affinity for copper, which presumably forms an ineffective complex with it.

Such results suggest a change in the molecular properties of the enzyme as a basis for resistance to toxicity. However, the preparation used is of cell-wall fragments rather than purified enzyme, which leaves open the possibility that the enzyme is in some way protected from free copper ions in the resistant ecotype. This is certainly more consistent with work on other systems exhibiting enzyme “tolerance”. Nevertheless, cell walls, both inside and outside the plant, are liable to experience higher concentrations of toxic ions than other parts of the plant. There are many active molecules within or adjacent to these walls, most conspicuously the uptake systems for other ions. Whether or not these show a direct resistance to ionic toxicity is not yet known.

3. Deficiencies: Ionic Imbalance

As a resistance mechanism, exclusion provides problems where the toxic ion is metabolically essential at low concentrations, such as Cu^{2+} , Zn^{2+} or Fe^{2+} . Is resistance to these ions less often achieved by exclusion than is the case for Pb^{2+} , Cd^{2+} , Cr^{2+} and other non-essential toxins? The evidence is far from clear. Although it was possible for Antonovics *et al.* (1971) to state that “nowhere...is there any evidence for (tolerant) plants having an exclusion mechanism,” Mathys (1973) demonstrated exclusion of zinc in *Agrostis tenuis*, whereas Wu and Antonovics (1975) found accumulation of Zn^{2+} in resistant ecotypes of *Agrostis stolonifera*.

At present, it is not possible to make firm generalizations on this point. However, it is likely that the stimulatory effects on growth and root elongation produced by low concentrations of Al^{3+} in calcifuges species (Clarkson, 1967; Fig. 6.6) are due to the

presence of Al-binding sites on the root surface. When these are unoccupied by Al^{3+} ions they tend to bind the similar Fe^{3+} ions, and may thus cause Fe deficiency. Grime and Hodgson (1969) showed that growth responses to Al tend to be diphasic, with an initial decline at very low concentrations, due to the toxic effect of Al on cell division, and a peak at slightly higher concentrations, resulting from the liberation of Fe from binding sites on the cell wall. Finally, at high Al concentrations, growth falls off again. The position of the peak of growth varied according to the pH-tolerance of the species or ecotype, approximately 20-30 μM Al^{3+} in calcifuges such as *Nardus stricta* and *Urtica europaea*, and approximately 5 μM in calcicoles (*Bromus erectus*, *Scabiosa columbaria*).

In those experiments, the position of the initial growth depression also altered - from around 1 μM in calcicoles to as high as 10 μM in calcifuges. There is also evidence (Foy *et al.*, 1978) that some Al resistance may be due to an ability to prevent Al^{3+} migrating through the free space to the meristem, thus protecting cell division. Hemming (quoted by Foy *et al.*, 1978) found a resistant wheat variety could withstand a hundred-fold increase in external Al concentration before Al enters the root meristem, compared to a sensitive variety. This again suggests a binding mechanism in the cell wall.

4. Osmotic Imbalance

There is little evidence that low water potentials are inherently damaging, even to mesophytes, until extremely low values (less than -20 bar) are reached. As long as the xylem water potential is lower than that of the soil water, uptake will continue. If such osmotic adjustment does not take place, water stress will occur. Tal (1971) found that cultivated tomatoes (*Lycopersicon esculentum*) took up less Na and Cl when subjected to high salinity than the wild *L. peruvianum* with adverse effects on both relative water content and growth rate. In contrast, Greenway (1962) using barley and Gates *et al.* (1970) with soybean varieties found precisely the opposite. It was the sensitive varieties that took up most salt. Presumably their osmotic adjustment was achieved at the expense of a toxic effect of the salt itself.

Generally, agricultural species achieve resistance by exclusion (Greenway, 1973) and the same is true for a number of wild plants. Tiku and Snaydon (1971) found that salt-marsh populations of *Agrostis stolonifera* had lower leaf Na concentrations than normal plants at the same salinity. It is dangerous to draw conclusions from leaf analyses. However, this conforms with the observations of Ahmad and Wainwright (1976) that the leaves of the salt-marsh *A. stolonifera* were less wettable and retained only 1/16th as much NaCl after immersion in sea water as leaves of inland plants: apparently the exclusion mechanism here is in part physical.

Cultivation plants, however, rarely experience severe salt stress and appear to be able to make good the osmotic deficit created by salt exclusion by internal synthesis of solutes. Arid saline habitats, in contrast, present much lower soil water potentials and halophytes there appear to carry out much of their osmotic adjustment by NaCl uptake. When this occurs, other problems arise as salt is generally highly toxic at such concentrations.

C. Amelioration

For a variety of reasons, it may not be possible for a plant to exclude a toxic ion. If high internal concentrations must be withstood, the ions will either have to be removed from circulation in some way, or tolerated within the cytoplasm. The former process is here termed amelioration. Four approaches are apparent:

- (i) Localization, either intra- or extra cellularly, and usually in the roots;
- (ii) Excretion, either actively through glands on shoots or by the roots; or passively by accumulation in old leaves followed by abscission;
- (iii) Dilution, which is primarily significant in relation to salinity;
- (iv) Chemical inactivation, where the ion is present in a combined form of reduced toxicity.

1. Localization

Analyses of both roots and shoots of a plant are rarely carried out, but data compiled by Tyler (1976) and summarized in Table 3 suggest that for *Anemone nemorosa* metals can be divided into four groups:

- (i) Those more or less equally distributed throughout the plant, including the essential major nutrients, K^+ , Ca^{2+} and Mg^{2+} . Rb^{2+} behaves as K^+ in most plants.
- (ii) Those showing some accumulation in the root, including the three important micro-nutrients Cu^{2+} , Zn^{2+} and Mn^{2+} .
- (iii) Non-essential toxic ions such as Al^{2+} and Cd^{2+} , which are primarily stored in the root. Fe, a micronutrient, also falls into this class, presumably because of its high availability in soil, in contrast to Cu^{2+} , Zn^{2+} and Mn^{2+} .
- (iv) Pb is clearly exceptional in that the root content is grossly higher than that of the shoots. It is possibly significant, however, that its actual concentration in the shoots is the same as that for Cd^{2+} .

Accumulation of toxic ions by roots is a widespread phenomenon. In all but one of the examples quoted in Table 3. Concentrations in the roots were higher than in the shoots. Even if roots have an inherently higher tolerance to toxins than shoots, this clearly implies some form of localization.

Table 3: Shoot concentrations and ratios of root concentration to shoot concentration for 12 elements in *Anemone nemorosa* (from Tyler, 1976)

<i>Shoot concentration</i> <i>(p.p.m.)</i>	<i>root:shoot</i> <i>ratio</i>

Widely distributed	Ca ²⁺	7180	0.8
	Mg ²⁺	2970	1.3
	K ⁺	11,400	1.3
	Rb ²⁺	35	1.6
Slight root storage	Mn ²⁺	405	2.3
	Cu ²⁺	10.5	2.4
	Zn ²⁺	113	3.6
Major root storage	Al ²⁺	260	10.7
	Cd ²⁺	1.24	11.7
	Fe ²⁺	217	12.4
	Na ²⁺	242	16.7
Shoot exclusion	Pb ²⁺	1.04	62.6

Turner (1969) showed that zinc resistance in *Agrostis tenuis* was associated with the accumulation of the element in cell wall fractions. In both resistant and normal plants, 70-80% of Zn²⁺ in the root cell walls was in the pectic fraction. The distinction lay in the proportion of root Zn²⁺ that was in the cell walls, as opposed to the soluble fraction - two-thirds in the resistant, one-third in the sensitive plants (Peterson, 1969). Recently, a very specific association has been shown between Pb²⁺ ions and the pectinic acid fraction of the cell wall (Lane *et al.*, 1978). The pectic fractions of the cell wall have a considerable cation exchange capacity and the ability to form cross-linked structures - hence one of the major roles of Ca²⁺ in plant metabolism - and are well suited to the binding of polyvalent cations. However, several limitations must be borne in mind. First, the toxicity due to NaCl will not be amenable to this solution, as both ions are mono-valent. Secondly, cell walls are not inert but contain active enzymes. Thirdly, any such system is likely to be saturable and have threshold effectiveness.

There are two possible escapes from the last problem. It is possible that binding sites could be continually synthesized within each cell wall. However, this would require enzyme action, and these enzymes would be exposed to the toxic ion. Alternatively, continued growth could provide new sites for chelation. In the latter case, the maintenance of a healthy meristem is critical, particularly in the case of Al²⁺. Therefore, it seems probable that the ability to protect the meristem from damage may be central to the resistance mechanism.

(a) *Intracellular.* If much higher concentrations of ion are found in the roots than the shoots, it is strong evidence for extracellular localization, possibly binding to the pectic fractions of the cell walls. Once ions have crossed the plasmalemma, there is no general reason for their restriction to the roots. If the ion is to be kept out of general circulation, it must be accumulated in a compartment within the cell. Lead appears to accumulate in dictyosome vesicles in the corn *Zea*, with mitochondria, plastids and nuclei remaining

Pb-free (Malone *et al.*, 1974). Isolated mitochondria are capable of accumulating Pb^{2+} *in vitro*, so that clearly some specific affinity is exhibited by the dictyosomes.

The intracellular distribution of NaCl is more complex. The chloroplasts of the halophyte *Limonium vulgare* appear to accumulate Cl^- (Larkum, 1968). More generally, however, salt accumulated by plants to maintain osmotic integrity appears largely in the vacuole. Direct evidence for this is limited, although Hall *et al.* (1974) showed Rb^+ accumulation there. However, convincing indirect evidence comes from the lack of specific tolerance to toxic ions exhibited by higher plant enzymes active within the cell.

Enzymes from halophytic bacteria, which have extremely limited intracellular compartmentation, can operate *in vitro* at very high NaCl concentrations (Ingram, 1957). In those eukaryotes that have been examined from fungi (e.g. *Dunaliella parva*, Heimer (1973)) to higher plants, no intracellular enzymes have been shown convincingly to exhibit any resistance to high concentrations of NaCl. Occasionally, stimulations of enzyme activity are reported at low NaCl concentrations, usually less than 100 μM . However, these can be found equally in glycophytes and halophytes.

The explanation appears to be that NaCl is isolated in the vacuole and so is not in the same compartment as the enzymes. However, this implies an osmotic imbalance between cytoplasm and vacuole. The concentration of Cl^- , for example, in the vacuoles of the algae *Tolypella intricata* was five times more than in the cytoplasm (Larkum, 1968). The cytoplasm must be at the same water potential as the vacuole, and this is achieved using organic solutes. Thus, the halophilic fungus *Dunaliella parva* contains large quantities of glycerol (Ben-Amotz and Avron, 1973). Its nitrate reductase is unaffected *in vitro* by glycerol concentrations greater than 3 M. Although it can grow in 2 M NaCl (4 times sea water), nitrate reductase activity is reduced by 50% in 0.4 M NaCl. The alga *Ochromonas malhamensis* also uses glycerol for osmotic balancing (Kraus, 1969).

It appears to be a general observation that the enzymes and organelles of halophytes are not tolerant of high NaCl concentrations. However, they can withstand the low water potentials of isosmotic solutions of organic solutes. Von Willert (1974) found that malate dehydrogenase in several halophytes was more inhibited by sucrose than by NaCl. However, Stewart and Lee (1974) have found evidence that the amino acid proline is used by many halophytes for osmotic balance. In a wide range of plant species, they found proline to comprise on average 54.6% of the amino-acid pool in halophytes compared to 2.9, 2.4 and 4.0%, respectively in calcicoles, calcifuges and ruderals. Proline accumulation was directly related to the salinity of the medium. It was found to have no effect on the activity of nine enzymes extracted from *Triglochin maritima*, at concentrations where NaCl was extremely inhibitory. Since the concentrations used (up to 700 mM) were comparable to measure internal NaCl levels, and since Treichel (1975) found that the ratio of proline to Na^+ and Cl^- in the vacuole was maintained constant in three halophytes, the suggested role for proline is very attractive. Stewart and Lee were also able to show that inland races of *Armeria maritima* had a much lesser tendency to accumulate proline than maritime ones when subjected to NaCl concentrations above 100 mM.

Of the eleven halophytes tested by Stewart and Lee, *Plantago maritima* showed no proline accumulation. Presumably, it achieved osmo-regulation by other means. Indeed, Story *et al.* (1977) have found that another amino-acid, glycine-betaine, is of greater significance than proline in many species. Furthermore, it is established that different, though analogous, mechanisms operate in relation to heavy metals. Here, there is no osmotic problem and zinc for example may be localized in the vacuole. The problem here is the electrochemical balance. This is solved by complexing the Zn^{2+} ions with malate to avoid disturbance of the electrochemical equilibrium (Mathys, 1977).

As with halophytes, there is no evidence for actual tolerance by intracellular enzymes to high concentrations of ions. The resistant ecotypes maintain full enzyme function *in vitro* by means of compartmentation. Indeed, zinc-resistant *Silene vulgaris* appears to have an increased zinc requirement, presumably because of an active chelating system. The activity of nitrate reductase in its leaves is increased by growing the plant in 0.4 mM Zn^{2+} , whereas even 0.1 mM is inhibitory to the non-resistant ecotype (Mathys, 1975; Fig. 6.8).

(b) *Chemical inactivation.* Zinc resistance is a multiple phenomenon, partly involving exclusion in the root cell walls and localization in the cell vacuoles, where the ion is complexed with malate. Such complexes are probably widespread. For example, copper is normally translocated chelated with polyamino-polycarboxylic acids (Tiffin, 1972) and may even allow the plant to retain the toxic ion in the metabolic compartment, but in an inactive form.

When interest in the calcicole-calcifuge problem first appeared in experimental form, much stress was laid on Ca^{2+} levels. It is now generally accepted that the effects found on strongly acid or calcareous soils are better explained in terms of Al^{3+} toxicity and Fe^{2+} deficiency, among others. Nevertheless, Ca^{2+} is one of the most variable elements in different soils, and its significance in less extreme conditions has been overlooked. Horak and Kinzel (1971) have attempted to classify plants in respect of their K:Ca ratios and the form in which Ca^{2+} occurs in the plant. Using standard diagrams they suggest the existence of three distinct forms of cation nutrition:

(i) oxalate plants, which take up Ca but remove it from circulation as oxalate such as Polygonaceae, Chenopodiaceae, most Caryophyllaceae and Violaceae (e.g. *Silene inflata*);

(ii) calcitrophes, which require high concentrations of free calcium such as the Crassulaceae and most of the Cruciferae and Leguminosae (e.g. *Coronilla vaginalis*);

(iii) potassium plants, which have large amounts of K^+ and little free Ca^{2+} and are found in the Umbelliferae, Campanulaceae and Compositae (e.g. *Achillea clavennae*).

The ecological significance of this metabolic diversity is unclear since the ability to tolerate calcareous or acid soils follows no such clear taxonomic pattern. It may

influence the ability of plants to colonize unusual habitats such as serpentine soils with high Mg:Ca ratios. Serpentine-adapted plants do not generally exclude Mg^{2+} (Proctor, 1971). However, it may bind it as oxalate (e.g. *Petrorhagia saxifraga*, Caryophyllaceae - Ritter-Studnicka, 1971). Calciotrophe species simply allow it to accumulate in the cell sap (*Sedum album*, *Biscutella laevigata* - Horak and Kinzel, 1971).

It is necessary to gain a wider perspective on cation utilization. Austenfeld (1974b) subjected *Salicornia europaea*, a characteristic salt-marsh plant, to a range of salinities and found that the Na^+ content of the plant responded in a simple fashion to a range of NaCl concentrations from 0 to 250 mM. The bulk of the Na^+ was water soluble, whereas the level of water-soluble oxalate increased dramatically. Possibly *Salicornia* uses oxalate to inactivate Ca^{2+} when grown at low salinity, and Na^+ at high salinities. If *Halimione portulacoides*, another salt-marsh plant, is grown at high NaCl levels, water-soluble oxalate increases. At high $CaCl_2$ concentrations, it is the water-soluble fraction that accumulates.

2. Excretion

Animals faced with excessive ion loads typically excrete them. Plants generally rely on controls in uptake - simpler because their food is taken up in its component parts, not as a package. However, plants are capable of excretion. A major disadvantage of excretion for plants is that they are stationary. The excreted substances will remain in the root zone and may eventually lead to a build-up of toxin. This does not apply to plants growing in water.

The simplest form of excretion is the loss of an organ, which has become saturated with the toxin. It is generally true that old leaves have much higher salt or heavy metal contents than young leaves and buds. Old leaves of tea may contain up to 30,000 p.p.m. (3%) Al^{2+} , most of which is in the epidermis (Matsumoto *et al.*, 1976). Plants, which accumulate Al in the shoots in this fashion, are generally found in more primitive woody families (Chenery and Sporne, 1976), suggesting that this technique was adopted early in Angiosperm evolution. It is noticeable that localization may also occur within a leaf. Yellow margins of mustard leaves contained 2300 p.p.m. Mn^{2+} , while the green parts had only 570 p.p.m. (Williams *et al.*, 1971). What appears at first sight to be a toxicity symptom may be part of the plant's resistance mechanism.

The relationship between toxin accumulation and abscission may be complex. High salt levels may hasten senescence (Prisco and O'Leary, 1972). The same is almost certainly true of toxic heavy metals. Copper induces leaf chlorosis even in very resistant *Becium homblei* (Reilly and Reilly, 1973). In halophytes, abscission is most significant in rosette plants, which continually produce new leaves as the old ones senesce (Albert, 1975).

More active excretion also occurs, at least for salt. Salt is apparently actively withdrawn from the xylem back into the xylem parenchyma (Yeo, *et al.*, 1977) and possibly extruded from the roots back into the medium. Certainly, a potassium-stimulated Na^+ efflux can be shown to occur across root plasmalemmas (Jeschke, 1973). the most important route, however, is through the glands found on leaves of mangroves, *Atriplex*

and *Halimione portulacoides*, among others. In *Halimione* the glands are grown in high salt media, and the glands always contain much higher concentrations of Na^+ and Cl^- than the sap from the leaves (Baumeister and Kloos, 1974). The glands are highly selective, K^+ , Na^+ , Cl^- and HCO_3^- being secreted against a concentration gradient, and Ca^{2+} , NO_3^- , SO_4^- and H_2PO_4^- being retained against a concentration gradient.

The activity of these glands, at least in *Limonium vulgare*, is not constitutive but is induced by growth in NaCl (Hill and Hill, 1973). A Cl^- -stimulated ATPase in these glands shows a 300% increase in activity if incubated *in vitro* with NaCl, compared with water or even Na_2SO_4 . Such a flexible system could be of great significance in environments showing wide fluctuations. A further advantage of inducibility is in the metabolic cost of an energy-consuming process such as salt excretion. In *Tamarix ramosissima* 65% of leaf Na^+ , 82% of leaf Cl^- (and strikingly 90% of leaf Al^{3+} and 88% of leaf Si) could be removed by washing (Kleinkopf and Wallace, 1974). No other elemental losses exceeded 40%. Although net photosynthesis was actually stimulated by up to 200 meq litre⁻¹ of NaCl, growth declined even at 10 meq litre⁻¹ and was reduced to 32% of the control at 200 meq litre⁻¹. The growth increase was apparently due to energy losses through increased respiration maintaining salt excretion.

3. Dilution

Toxic metals such as zinc and copper have specific affinities for particular biochemical groups. Thus, copper reacts with sulphhydryl groups. Nitrate reductase, an SH-enzyme, is particularly susceptible to Cu-poisoning. Zinc reacts primarily with carboxyl groups. For these toxins, dilution by increasing cell water content is not a practical resistance mechanism. Against salinity, with its osmotic and non-specific toxic effects, dilution is widespread and effective.

In many halophytes succulence, NaCl specifically stimulates an increase in water content per unit dry weight. Both Na^+ and Cl^- ions can produce succulence and there is an intriguing link between succulence and CAM metabolism. In *Mesembryanthemum crystallinum*, high salt levels induce both succulence and CAM. The malate produced by CAM appears to have an additional role in balancing charge discrepancy between Na^+ and Cl^- .

D. Tolerance

Resistant prokaryotes and some simple eukaryotes such as yeasts show true tolerance of their metabolic systems. Enzymes extracted from them operate *in vitro* in the presence of toxin concentrations lethal to the enzymes of eukaryotes. The membranes of halophilic bacteria are astonishingly permeable. *Halobacterium* admits molecules up to a molecular weight of 40,000 (Ginzburg, 1969).

Eukaryotes have much more complex intracellular compartmentation and rely on this to ensure that their metabolic systems do not experience high concentrations of toxins. Nevertheless, such stress may induce enzymatic changes. Salt induces the synthesis of a

new isozyme of malate dehydrogenase in peas (Hassan-Porath and Poljakoff-Mayber, 1979), which may be better equipped to operate at low potentials.

If one examines plants under mild stress, it is apparent that enzyme adaptation can occur. Cultivated oat is a calcifuge and has an Mg^{2+} -activated ATPase system in the roots. Under low-Ca conditions, it operates adequately. By contrast, the calcicolous wheat has a Ca^{2+} -activated ATPase inhibited by Mg^{2+} . If grown under low salt conditions, ATPase activity is equally stimulated by Ca^{2+} and Mg^{2+} (Kylin and Kahr, 1973). Clearly, some enzymic changes have occurred.

There are some enzymes, however, which cannot be protected by compartmentalization - those in the plasmalemmas and cell walls. In some cases, cell wall enzymes, particularly acid phosphatases, have been shown to be tolerant of much higher levels of toxic ions (Cu^{2+} , Zn^{2+}) resistant in normal plants (Wainwright and Woolhouse, 1975). Unfortunately, little is known of the function of these enzymes.

IV. THE ORIGIN OF RESISTANCE

Some forms of resistance to toxic ions are ancient. Saline habitats are as old as the Angiosperms. A high proportion of halophytes, in particular the Chenopodiaceae, including the genera *Chenopodium*, *Atriplex*, *Halimione*, *Salicornia*, *Suaeda* and *Salsola*, is strongly indicative of an ancient origin. Similarly, Al accumulators, plants containing more than 1000 p.p.m. Al in their shoots, tend to be primitive on taxonomic grounds (Chenery and Sporne, 1968), suggesting that this physiologically crude method of excretion is also in evolutionary terms a first attempt.

It seems likely that the more sophisticated mechanisms have evolved particularly the combination of resistance mechanisms that characterize most specialist toxicity-resistant species. In many cases, resistance is found to be multi-layered, an exclusion mechanism at the root reducing the intensity and the ions that penetrate being localized, inactivated or excreted.

Table 4: Selection for Cu and Zn resistance in *Argrostis tenuis* (Walley *et al.*, 1974). Plants were grown for four months in soil, Copper-mine waste, or Zinc-mine waste and the resistance of survivors measured by rooting tillers in $0.5 \text{ g litre}^{-1} \text{ Ca (NO}_3)_2$, containing the appropriate metal. Resistance is measured on a 0-100 scale with 100 representing insensitivity to the toxin.

Population	Treatment	Mean and (maximum) tolerance of survivors to	
		Zn	Cu
Pasture	Direct measurement	0.6 (1.7)	2.0 (8.6)
Commercial	Grown on soil	N.D.	5.6 (8.5)

	Grown on Cu-waste	N.D.	48.0 (77.5)
	Grown on Zn-waste	31.8 (41.3)	0.3 (0.9)
Copper mine	Direct measurement	0.8 (0.8)	79.0 (87.5)
	Grown on soil	17.6 (20.2)	85.3 (93.5)
	Grown on Zn-waste	36.4 (47.8)	52.1 (66.9)
Zinc mine	Direct measurement	93.0 (93.0)	3.8 (3.8)

In the case of heavy metal resistance, it has been demonstrated by Bradshaw and others that individuals with a measure of resistance exist in normal populations not previously exposed to toxicity (Walley *et al.*, 1974). Table 4 shows that plant of *Agrostis tenuis* growing on zinc- or copper-waste from old mines have high resistance to the appropriate metals and to that metal only. It is possible, however, to grow commercial seed on contaminated soil to select a few individuals (1-2%), which have Cu-resistance indices as high as naturally occurring populations. Zinc-resistance appears to be more complex since the most resistant individual selected was only about half as resistant as the mine population.

If such effects can be produced in a population in one generation, one would naturally expect to observe them occurring in the wild. Zinc-resistance has been observed under a galvanized fence after 30 years (Bradshaw *et al.*, 1965). Wu and Bradshaw (1972) also found that Cu-resistance progressively increases in lawns established at different times around a copper refinery.

The dramatic effects of heavy metal toxicity on plants and the consequential massive selection pressures have highlighted the ease with which physiological attributes of plants can be altered. Recently, it has been shown that the adaptation of plants to many other environmental variables exhibits a similar small-scale pattern. *Anthoxanthum odoratum* grows in many of the Park Grass plots at Rothamsted which have received different fertilizer treatments for over 120 years. Plants from closely adjacent plots are clearly distinct in their responses to Ca^{2+} , Al^{2+} , P, and Mg^{2+} (see Davies and Snaydon, 1974).

Typically, such adaptation involves ecotypic differentiation, that is the evolution of distinctly adapted genotypes. More rarely, resistance is explained by phenotypic plasticity, and appears to be true for the resistance of *Typha latifolia* to zinc, although possibly *Typha* is physiologically inherently Zn-resistant (MacNaughton *et al.*, 1974). Nevertheless, it seems likely that investigation of plastic responses to mild toxicity would be amply repaid.

Phytoremediation

The term phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated

environments (Cunningham et al. 1997; Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation (Ensley, 2000). Although the term, phytoremediation, is relatively recent, it has been practiced for decades (Cunningham et al. 1997; Brooks, 1998a). Research using semi-aquatic plants for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era (Timofeev-Resovsky et al. 1962; Salt et al. 1995a). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of metals in their tissues without exhibiting symptoms of toxicity (Reeves and Brooks, 1983; Baker and Brooks, 1989; Baker et al. 1991; Entry et al. 1999).

Chaney, 1983 was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal polluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal (Cunningham et al. 1995; Comis, 1996; Ebbs et al. 1997). By definition, a hyperaccumulator must accumulate at least 100 mg g⁻¹ (0.01% dry wt.), Cd, As and some other trace metals, 1000 mg g⁻¹ (0.1 dry wt.) Co, Cu, Cr, Ni and Pb and 10,000 mg g⁻¹ (1 % dry wt.) Mn and Ni (Reeves and Baker, 2000; Watanabe, 1997).

Phytoremediation consists of four different plant-based technologies each having a different mechanism of action for the remediation of metal-polluted soil, sediment, or water. These include: rhizofiltration, which involves the use of plants to clean various aquatic environments; phytostabilization, where plants are used to stabilize rather than clean contaminated soil; phytovolatilization, which involves the use of plants to extract certain metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants absorb metals from soil and translocate them to the harvestable shoots where they accumulate. Although plants show some ability to reduce the hazards of organic pollutants (Cunningham et al. 1995; Gordon et al. 1997; Carman et al. 1998), the greatest progress in phytoremediation has been made with metals (Salt et al. 1995a; Watanabe, 1997; Blaylock and Huang, 2000).

Phytoremediative technologies, which are soil-focused, are suitable for large areas that have been contaminated with low to moderate levels of contaminants. Sites, which are heavily contaminated, cannot be cleaned through phytoremediative means because the harsh conditions will not support plant growth. The depth of soil, which can be cleaned or stabilized, is restricted to the root zone of the plants being used. Depending on the plant, this depth can range from a few inches to several meters (Schnoor et al. 1995). Phytoremediation should be viewed as a long-term remediation solution because many cropping cycles may be needed over several years to reduce metals to acceptable regulatory levels. This new remediation technology is competitive, and may be superior to existing conventional technologies at sites where phytoremediation is applicable.

Rhizofiltration

Metal pollutants in industrial-process water and in groundwater are commonly removed by precipitation or flocculation, followed by sedimentation and disposal of the resulting

sludge (Ensley, 2000). A promising alternative to this conventional method is rhizofiltration, a phytoremediative technique designed for the removal of metals in aquatic environments. The process involves raising plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov et al. 1995; Salt et al. 1995a; Flathman and Lanza, 1998; Zhu et al. 1999b). Root exudates and changes in rhizosphere pH also may cause metals to precipitate onto root surfaces. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal (Flathman and Lanza, 1998; Zhu et al. 1999b). Most researchers believe that plants for phytoremediation should accumulate metals only in the roots (Dushenkov et al. 1995; Salt et al. 1995a; Flathman and Lanza, 1998). Dushenkov et al. 1995 explains that the translocation of metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of contaminated plant residue needing disposal. In contrast, Zhu et al. 1999b suggest that the efficiency of the process can be increased by using plants which have a heightened ability to absorb and translocate metals within the plant. Despite this difference in opinion, it is apparent that proper plant selection is the key to ensuring the success of rhizofiltration as a water cleanup strategy.

Dushenkov and Kapulnik, 2000 describe the characteristics of the ideal plant for rhizofiltration. Plants should be able to accumulate and tolerate significant amounts of the target metals in conjunction with easy handling, low maintenance cost, and a minimum of secondary waste requiring disposal. It is also desirable plants to produce significant amounts of root biomass or root surface area. Several aquatic species have the ability to remove heavy metals from water, including water hyacinth (*Eichhornia crassipes* (Mart.) Solms; Kay et al. 1984; Zhu et al. 1999b), pennywort (*Hydrocotyle umbellata* L.; Dierberg et al. 1987), and duckweed (*Lemna minor* L.; Mo et al. 1989). However, these plants have limited potential for rhizofiltration, because they are not efficient at metal removal, a result of their small, slow-growing roots (Dushenkov et al. 1995).

These authors also point out that the high water content of aquatic plants complicates their drying, composting, or incineration. Despite limitations, Zhu et al. 1999b indicated that water hyacinth is effective in removing trace elements in waste streams. Terrestrial plants are thought to be more suitable for rhizofiltration because they produce longer, more substantial, often fibrous root systems with large surface areas for metal sorption. Sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrial candidates for metal removal in water. The roots of Indian mustard are effective in the removal of Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov et al. 1995), and sunflower removes Pb (Dushenkov et al. 1995), U (Dushenkov et al. 1997a), ¹³⁷Cs, and ⁹⁰Sr (Dushenkov et al. 1997b) from hydroponic solutions.

Rhizofiltration is a cost-competitive technology in the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr, Pb, and Zn (Kumar et al. 1995b; Ensley, 2000). The commercialization of this technology is driven by economics as well as by such technical advantages as applicability to many problem metals, ability to treat high volumes, lesser need for toxic chemicals, reduced volume of secondary waste, possibility of recycling, and the likelihood of regulatory and

public acceptance (Dushenkov et al. 1995; Kumar et al. 1995b). However, the application of this plant-based technology may be more challenging and susceptible to failure than other methods of similar cost. The production of hydroponically grown transplants and the maintenance of successful hydroponic systems in the field will require the expertise of qualified personnel, and the facilities and specialized equipment required can increase overhead costs. Perhaps the greatest benefit of this remediation method is related to positive public perception. The use of plants at a site where contamination exists conveys the idea of cleanliness and progress to the public in an area that would have normally been perceived as polluted.

Phytostabilization

Sometimes there is no immediate effort to clean metal polluted sites, either because the responsible companies no longer exist or because the sites are not of high priority on a remediation agenda (Berti and Cunningham, 2000). The traditional means by which metal toxicity is reduced at these sites is by in-place inactivation, a remediation technique that employs the use of soil amendments to immobilize or fix metals in soil. Although metal migration is minimized, soils are often subject to erosion and still pose an exposure risk to humans and other animals.

Phytostabilization, also known as phytorestoration, is a plant-based remediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion; provides hydraulic control, which suppresses the vertical migration of contaminants into groundwater; and physically and chemically immobilizes contaminants by root sorption and by chemical fixation with various soil amendments (Cunningham et al. 1995; Salt et al. 1995a; Flathman and Lanza, 1998; Berti and Cunningham, 2000; Schnoor, 2000). This technique is actually a modified version of the in-place inactivation method in which the function of plants is secondary to the role of soil amendments. Unlike other phytoremediative techniques, the goal of phytostabilization is not to remove metal contaminants from a site, but rather to stabilize them and reduce the risk to human health and the environment.

The most comprehensive and up-to-date explanation of the phytostabilization process is offered by Berti and Cunningham, 2000. Before planting, the contaminated soil is plowed to prepare a seed bed and to incorporate lime, fertilizer, or other amendments for inactivating metal contaminants. Soil amendments should fix metals rapidly following incorporation, and the chemical alterations should be long lasting if not permanent. The most promising soil amendments are phosphate fertilizers, organic matter or bio-solids, iron or manganese oxyhydroxides, natural or artificial clay minerals, or mixtures of these amendments. Plants chosen for phytostabilization should be poor translocators of metal contaminants to aboveground plant tissues that could be **consumed** by humans or animals. The lack of appreciable metals in shoot tissue also eliminates the necessity of treating harvested shoot residue as hazardous waste (Flathman and Lanza, 1998). Selected plants should be easy to establish and care for, grow quickly, have dense

canopies and root systems, and be tolerant of metal contaminants and other site conditions which may limit plant growth.

The research of Smith and Bradshaw, 1992, led to the development of two cultivars of *Agrostis tenuis* Sibth and one of *Festuca rubra* L which are now commercially available for the phytostabilization of Pb-, Zn-, and Cu contaminated soils. Phytostabilization is most effective at sites having fine-textured soils with high organic-matter content but is suitable for treating a wide range of sites where large areas of surface contamination exist (Cunningham et al. 1995; Berti and Cunningham, 2000). However, some highly contaminated sites are not suitable for phytostabilization, because plant growth and survival is not a possibility (Berti and Cunningham, 2000). At sites which support plant growth, site managers must be concerned with the migration of contaminated plant residue off site (Schnoor, 2000) or disease and insect problems which limit the longevity of the plants. Phytostabilization has advantages over other soil-remediation practices in that it is less expensive, less environmentally evasive, easy to implement, and offers aesthetic value (Berti and Cunningham, 2000; Schnoor, 2000). When decontamination strategies are impractical because of the size of the contaminated area or the lack of remediation funds, phytostabilization is advantageous (Berti and Cunningham, 2000). It may also serve as an interim strategy to reduce risk at sites where complications delay the selection of the most appropriate technique for the site.

Phytovolatilization

Some metal contaminants such as As, Hg, and Se may exist as gaseous species in environment. In recent years, researchers have searched for naturally occurring or genetically modified plants that are capable of absorbing elemental forms of these metals from the soil, biologically converting them to gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization, the most controversial of all phytoremediation technologies. Mercury and Se are toxic (Wilber, 1980; Suszcynsky and Shann, 1995), and there is doubt about whether the volatilization of these elements into the atmosphere is safe (Watanabe, 1997). Selenium phytovolatilization has been given the most attention to date (Lewis et al. 1966; Terry et al. 1992; Bañuelos et al. 1993; McGrath, 1998), because this element is a serious problem in many parts of the world where there are areas of Se-rich soil (Brooks, 1998b). However, there has been a considerable effort in recent years to insert bacterial Hg ion reductase genes into plants for the purpose of Hg phytovolatilization (Rugh et al. 1996; Heaton et al. 1998; Rugh et al. 1998; Bizily et al. 1999).

Although there have been no efforts to genetically engineer plants, which volatilize As, it is likely that researchers will pursue this possibility in the future. According to Brooks, 1998b, the release of volatile Se compounds from higher plants was first reported by Lewis et al. 1966. Terry et al. 1992 report that members of the Brassicaceae are capable of releasing up to 40 g Se ha⁻¹ day⁻¹ as various gaseous compounds. Some aquatic plants, such as cattail (*Typha latifolia* L.), are also good for Se phytoremediation (Pilon-Smits et al. 1999a). Unlike plants that are being used for Se volatilization, those, which volatilize Hg, are genetically modified organisms. *Arabidopsis thaliana* L. and tobacco

(*Nicotiana tabacum* L.) have been genetically modified with bacterial organomercurial lyase (*MerB*) and mercuric reductase (*MerA*) genes (Heaton et al. 1998; Rugh et al. 1998). These plants absorb elemental Hg(II) and methyl mercury (MeHg) from the soil and release volatile Hg(O) from the leaves into the atmosphere (Heaton et al. 1998). The phytovolatilization of Se and Hg into the atmosphere has several advantages. Volatile Se compounds, such as dimethylselenide, are 1/600 to 1/500 as toxic as inorganic forms of Se found in the soil (DeSouza et al. 2000). The volatilization of Se and Hg is also a permanent site solution, because the inorganic forms of these elements are removed and the gaseous species are not likely to be redeposited at or near the site (Atkinson et al. 1990; Heaton et al. 1998).

Furthermore, sites that utilize this technology may not require much management after the original planting. This remediation method has the added benefits of minimal site disturbance, less erosion, and no need to dispose of contaminated plant material (Heaton et al. 1998; Rugh et al. 2000). Heaton et al. 1998 suggest that the addition of Hg(O) into the atmosphere would not contribute significantly to the atmospheric pool. However, those who support this technique also agree that phytovolatilization would not be wise for sites near population centers or at places with unique meteorological conditions that promote the rapid deposition of volatile compounds (Heaton et al. 1998; Rugh et al. 2000). Unlike other remediation techniques, once contaminants have been removed via volatilization, there is a loss of control over their migration to other areas. Despite the controversy surrounding phytovolatilization, this technique is a promising tool for the remediation of Se and Hg contaminated soils.

Phytoextraction

Phytoextraction is the most commonly recognized of all phytoremediation technologies. The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept, while phytoextraction is a specific cleanup technology. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix (Kumar et al. 1995a). In practice, metal accumulating plants are seeded or transplanted into metal polluted soil and are cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the aboveground shoots where they accumulate. If metal availability in the soil is not adequate for sufficient plant uptake, chelates or acidifying agents may be used to liberate them into the soil solution (Huang and Cunningham, 1996; Huang et al. 1997a; Lasat et al. 1998).

After sufficient plant growth and metal accumulation, the aboveground portions of the plant are harvested and removed, resulting in the permanent removal of metals from the site. As with soil excavation, the disposal of contaminated material is a concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal (Kumar et al. 1995a). In some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation (Comis, 1996; Cunningham and Ow, 1996). Phytoextraction should be viewed as a long-term remediation effort, requiring many

cropping cycles to reduce metal concentrations (Kumar et al. 1995a) to acceptable levels. The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years (Kumar et al. 1995a; Blaylock and Huang, 2000). This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Kumar et al. 1995a; Blaylock and Huang, 2000). Many factors determine the effectiveness of phytoextraction in remediating metal-polluted sites (Blaylock and Huang, 2000). The selection of a site that is conducive to this remediation technology is of primary importance. Phytoextraction is applicable only to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. Soil metals should also be bioavailable, or subject to absorption by plant roots.

As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characters include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Kumar et al. 1995a; Cunningham and Ow, 1996; Blaylock et al. 1997; McGrath, 1998). It is the combination of high metal accumulation and high biomass production that results in the most metal removal. Ebbs et al. 1997 reported that *B. juncea*, while having one-third the concentration of Zn in its tissue, is more effective at Zn removal from soil than *T. caerulescens*, a known hyperaccumulator of Zn. This advantage is due primarily to the fact that *B. juncea* produces ten-times more biomass than *T. caerulescens*. Plants being considered for phytoextraction must be tolerant of the targeted metal, or metals, and be efficient at translocating them from roots to the harvestable above-ground portions of the plant (Blaylock and Huang, 2000). Other desirable plant characteristics include the ability to tolerate difficult soil conditions (*i.e.*, soil pH, salinity, soil structure, water content), the production of a dense root system, ease of care and establishment, and few disease and insect problems. Although some plants show promise for phytoextraction, there is no plant, which possesses all of these desirable traits. Finding the perfect plant continues to

Arctic and SubArctic Plant Ecology

Reproduction and Growth

Seed Germination.

Most arctic/subarctic plants disperse seed at the end of the growing season in August and September. The major exceptions are willows, aspen, and balsam poplar, which disperse seeds in May and June. Seed dormancy patterns for the species used in revegetation can be grouped into four types:

1. Non-dormant seeds. Non-dormant seeds can germinate over a wide range of temperatures as soon as they are dispersed.

2. Conditionally dormant seeds. Most species in the arctic/subarctic have seeds that are dormant under certain conditions. These seeds can germinate at high temperatures in the light but not at the lower soil temperatures, which occur, when the seeds are dispersed at the end of the growing season. Physiological changes occur in the seed as it overwinters, and the seeds germinate at low soil temperatures in the spring. Many species have seeds, which will not germinate without light, even in the spring.
2. Deep dormant. Some arctic/subarctic plants have seeds, which are completely dormant, when they are dispersed. These seeds will not germinate at any temperature until they have overwintered.
4. Hard seeded. Some of the legumes, including *Oxytropis* sp., are hard seeded, that is, they have seed coats which keep water out of the seed. This seed coat must be broken or decompose before the seed can germinate. Additional information on the germination requirements of arctic/subarctic species is presented in Densmore (1974, 1979, 1997) and Densmore and Zasada (1977, 1983), and these papers and other literature on germination of arctic/subarctic species are summarized in Baskin and Baskin (1998).

Seedling Establishment.

Arctic/subarctic plants generally have smaller and lighter seeds than temperate plants. Most seeds are in the weight range of 0.3-0.15 mg per seed. This means that newly germinated seedlings are very small and do not have reserves to rapidly produce roots. For this reason, most plants require mineral soil for seedling establishment. Mineral soil is moister and cooler than the soil organic layer, which dries out quickly and can heat to 50 °C in the sun. Thus, the following should be taken into consideration during revegetation:

- Native plant seeds that are dispersed onto mulches and erosion control mats usually do not produce established seedlings.
- When topsoil is salvaged and re-spread, at least part of the surface of the re-spread soil should be firm mineral soil. The salvaged organic layer does not make a suitable seedbed.

Seedling Growth Rate.

Almost all arctic/subarctic plants are perennials with seedlings that grow very slowly. Even relatively fast-growing herbs, which colonize disturbed areas, such as fireweed (*Epilobium angustifolium*), do not reach mature size until the second or third year of growth. Colonizing trees and shrubs such as alder, aspen, paper birch, and willow usually grow quite slowly for several years, then grow more rapidly to mature size. Species, which dominate the later stages of succession, such as spruce, dwarf birch, alpine blueberry, and labrador tea, remain small and relatively inconspicuous for about 10 years and continue to grow relatively slowly throughout life. This is partly because arctic/subarctic plants have high root to shoot ratios (see Root to Shoot section), and most of the early growth goes into the root system. The following should be taken into consideration during revegetation:

- Natural revegetation from seed or assisted revegetation with direct seeding of native plants will not provide surface erosion control for 1 to 10 years.
- Revegetation from seed will not improve visual quality for 1 to 10 years.
- Seedlings are too small during their first year to use much fertilizer. Standard rapid-release fertilizers applied at the time of seeding usually leach out of the soil before the plants are large enough to utilize the fertilizer.

Root to Shoot Ratio.

Arctic/subarctic plants, particularly tundra plants, have a high root to shoot ratio. This means the biomass of roots is much larger (sometimes as much as six times larger) than the aboveground biomass of stems and leaves. The large root mass is needed to obtain nutrients and water at low soil temperatures. Consider the following:

- Plants, which are fertilized in the greenhouse or in the field with rapid-release fertilizer, will tend to develop a low root to shoot ratio. Once the fertilizer is gone, aboveground growth will stagnate for a long period of time while the plants expand the root system.
- Container-grown plants should have less fertilizer (especially nitrogen) than is usually applied to horticultural plants or top growth will be stimulated at the expense of roots.
- Slow-release fertilizer should be used in all field seeding and transplanting.

Shape of the Root System.

Arctic/subarctic plants have shallow root systems, which spread horizontally, with most of the roots occurring in the top 40-50 cm of the soil. This is primarily because soil temperatures are too low or the soil is frozen at lower depths. Because of these shallow root systems, the following should be considered:

- Standard transplanting methods for trees and shrubs need to be modified to accommodate a root “saucer” instead of a root “ball.” Tree spades are impractical because of the spreading root systems and rocky soils. When salvaging topsoil and/or vegetation, only the shallow rooting zone needs to be salvaged, and the salvaged vegetation should be in large blocks.

Disturbance and Plant Succession in the Arctic/subarctic

Natural disturbances include:

- Wildfire—in interior Alaska, most white spruce forests burn before trees reach 250 years of age, but tree line stands burn less frequently. Black spruce forest has an average interval of 80 years between fires.
- River erosion and deposition—rivers and streams erode banks and deposit gravel, sand, and silt to form new floodplains.
- Glacial outwash—glacial meltwater deposits gravel, sand, and silt.
- Glacial recession—receding glaciers expose new areas for plant colonization.
- Mass wasting—landslides are very common

The development of plant communities on disturbed sites is referred to as plant

succession. Primary succession describes the establishment of plants on substrates that previously had not supported vegetation. Examples of natural disturbances that create new substrates for primary succession include river erosion and deposition and glacial outwash (Vioreck 1966, 1970; Walker et al. 1986).

Secondary succession is the reestablishment of vegetation on sites where it once existed, such as revegetation after wildfire or revegetation after the impacts of human trampling. Secondary succession often proceeds much faster than primary succession, as nutrients, organics, and propagules may remain in the soil after the disturbance and increase recovery rates. Some natural disturbances can result in both primary and secondary succession. An example of this kind of disturbance is a landslide, where the upper portion may expose new substrate for primary succession, while the mixture of soil and vegetation at the bottom of the slide undergoes secondary succession.

Succession Following Wildfire.

Although succession is complex and difficult to predict, there are general trends for succession following different types of natural disturbance. Succession after a fire, for example, typically follows several stages (Van Cleve et al. 1986). In the first stage, light-seeded plants such as fireweed, willow, balsam poplar, and paper birch arrive and establish on microsites where mineral soil has been exposed. At the same time, many plants already present on the site, such as fireweed, bluejoint (*Calamagrostis canadensis*), rose, alpine blueberry, willow, paper birch, or aspen, will sprout from stumps, roots, or rhizomes. Spruce seedlings establish and grow slowly. During the second stage, the maturing shrubs and deciduous tree saplings may dominate, with spruce forming a low understory beneath them. Next, the deciduous hardwoods may form a dense canopy and shade the understory, leading to the invasion of shade-tolerant and soil-cooling moss. Heavy litter fall may temporarily inhibit moss invasion, but once shade-tolerant moss has established, conditions change. The soil cools and moss inhibits hardwood regeneration and the establishment of shrubby species. Spruce continues to grow as the hardwoods die out and fail to regenerate. During the third stage, after about 200 years, patches of hardwood remnants and a spruce forest are apparent.

Riparian Succession.

The general pattern of riparian (e.g., along rivers and streams) succession in forested areas is the establishment of herbs and willows (primarily felt leaf willow) as soon as the surface is sufficiently stable. A vigorous willow stand develops by 10 years, followed by increasing importance of alder and balsam poplar from 20 to 50 years (Vioreck 1970; Walker et al. 1986). Balsam poplar dominates from 50 to 100 years, after which the understory of white spruce gradually gains dominance. All of the tree and tall shrub species, which dominate different successional stages, may establish together early in succession, but the slower growing, long-lived species gradually replace the fast-growing, short-lived species (Walker et al. 1986). On tundra riparian areas, legumes (primarily *Astragalus*, *Hedysarum*, and *Oxytropis* sp.) and *Shepherdia canadensis* occur with or replace alder at the highest elevations, and the willow and alder stage is followed

by low willows and dwarf birch, with an eventual shift to herbaceous tundra (Bliss and Cantlon 1957; Moore 1982). The nitrogen-fixing plants such as alder, legumes, and *Shepherdia canadensis* establish at early stages of succession and add nitrogen to the soil (Vioreck 1966).

Human-Caused Disturbance.

In this century humans have introduced new disturbances to North. In the arctic/subarctic, an environment already characterized by poor growing conditions, human-caused disturbances can make plant establishment and growth more difficult. Types of human-caused disturbance include:

- Trampling and social trails.
- Road construction and maintenance.
- Construction of facilities.
- Abandoned roads and gravel pits.
- Mining activities.

Each of these disturbances changes the natural environment in different ways. Trampling impacts are usually not severe, and an area can be restored relatively easily by, for example, closing it to further foot traffic. Construction disturbances are usually relatively easy to restore if revegetation was part of the original plan and soils and plants were actively salvaged. Abandoned roads, gravel pits, and placer mining disturbances limit plant establishment and growth the most and often present the greatest challenge to revegetate.

Gene Pool Conservation

One of the most important and least recognized resources is the gene pool represented by undisturbed plant communities have not been invaded by nonnative plants. Most of the nonnative plants are lawn and garden plants and agricultural weeds, which have not spread beyond the disturbed sites where they were introduced.

Nonnative plants, which can easily be inadvertently spread to revegetation sites, include the conspicuous and abundant dandelions (*Taraxacum officinale*).

Conserving the gene pool requires not only keeping nonnative plant species from establishing but also requires that the native plants used on a site be genetically similar to the plants in adjacent undisturbed areas. The following general rules for collecting seeds and cuttings and transplanting sod or individual plants are recommended:

- The collection site should be within a 6-km radius of the disturbed site.
- The collection site should be within the same general broad vegetation type. Species that occur over a wide range of habitat types often have habitat specific ecotypes which are genetically distinct.
- The collection site should be free of nonnative species.

These recommendations are based on the fact that the species used in revegetation have the potential for long distance dispersal of their genes. They have wind-dispersed pollen,

wind-dispersed seeds, or animal dispersed seeds.

be the focus of many plant-breeding and genetic-engineering research efforts.

Properties of Tailings and Mine Waste

Soil is by definition consists of the following components: 1) a mineral component derived from the breakdown of the parent material; 2) an organic mass, and 3) a living component including microorganisms (Weston, 1973). Unfortunately, not only is mine waste often lacking these components, it also has many properties which inhibit plant growth. Moreover, each site has its own unique physical and chemical characteristics, which will affect revegetation.

Chemical Properties of Mine Waste

Characteristic elevated metal levels, coupled with acidity and deficiency of macro-nutrients are major factors to be overcome in a successful revegetative program. Tailings and other wastes from metalliferous ores contain some metals (i.e. Al, Mn, Fe, Cr, Cu, Ni, Pb, Cd, Zn). These metal concentrations are much greater than those normally found in soils (Bowen, 1966, Baker 1991, McGrath, 1998). Elevated levels of heavy metals occur because of inefficient extraction techniques in the milling process, or an unwillingness to extract non-commercial elements. E.g. old, abandoned lead-zinc tailings in Wales contained as much as 4 to 8% Pb and 6 to 9% Zn (Smith and Bradshaw, 1974; Bradshaw et al., 1978). In such cases as gold mining, the desired element (gold) may be in very low quantities compared with undesirable and toxic elements such as As. As there is no market for the As it is simply left in the tailings. Finally, many elements in tailings are by-products of the milling process. In sulfide ores, Cu is removed from chalcopyrite, Zn from sphalerite and Pb from galena. Pyritic tailings from Cu-Ni operations contained over 25% Fe and up to 27% sulfur (Orgram and Fraser, 1978); asbestos tailings contained 23% Mg, 0.3% Cu and 0.2% Ni from serpentine rock surrounding asbestos veins (Moore and Zimmerman, 1977).

Heavy metal ions can be phytotoxic, even at very low concentrations in solution (Bowen, 1966). The solubility of many metals (e.g. Al, Mn, Fe) increases significantly with increasing acidity ($\text{pH} < 4.0$), (Coleman and Thomas, 1967; Buckman and Brady, 1970, Neuman 1993). The availability of heavy metals for plant uptake is thus greater on sites with a $\text{pH} < 4.0$, than, with neutral or basic pH (Peterson and Nielson, 1973, Stanley et. al 2000). However, in non-acid tailings, total concentrations of various heavy metals may attain such high levels that the soluble portion is sufficient to be toxic to most plants. Also, salts of a few elements such as Mo, B, As, and Se may increase in solubility at higher pH.

Heavy metals (e.g. Zn, Cu, Ni) inhibit root elongation and cause Fe-induced chlorosis because they interfere with iron mobility and metabolism in roots preventing iron being utilized in leaves (e.g. Hewitt, 1948; Wilkins, 1957; see next chapter). There is also evidence of poor xylem formation and absence of root hairs (see next chapter)).

On sulfide tailings containing iron pyrite (FeS_2), acidity is a problem as a result of pyrite oxidation where pH's of less than 2.0 can be attained (Peterson and Nielson, 1973; King, et al., 1973; Andrews, 1977). It is now known that the oxidation of iron pyrite is catalyzed by chemoautotrophic bacteria; e.g. *Thiobacillus ferro-oxidans* (Ivarson, 1973; Anderson, 1978).

In addition to acid generation, a hard 'ferruginous cap' impervious to root penetration, often forms on pyritic tailing. This is because surface evaporation and capillary action concentrate hydrated ferric oxides, ferric sulphates, and other metal salts at the tailing surface (Michelutti, 1974). Pyrrhotite (FeS) behaves similarly, but it is 80 times more reactive, and under some conditions, spontaneous combustion can occur (Michelutti, 1974). The heat produced by the reaction will be sufficient to inhibit growth of any vegetation. Consequently, pyrrhotite tailings are often kept submerged in water.

Most soils range in pH from 4.5 to 8.0 (Buckman and Brady, 1970). Plant species differ in their sensitivity to high acidity, but few vascular plants can tolerate a pH less than 4.0 (Jackson, 1967). The high concentration of free H^+ ions generated in the oxidation of sulfide tailings, can injure plants and physiologically impair the absorption of Ca, Mg and P (Rorison, 1977). Sulfate ions produced by oxidation generally do not have any influence on plant growth in the inorganic form; however, high concentrations of SO_4 in pyritic tailings could interfere with nitrate or phosphate ion uptake by affecting osmosis at root surfaces (Rorison, 1977).

Macronutrients N and P are particularly low in tailings and other mine wastes (Peterson and Nielson, 1973). Nitrogen is taken up by plants as ammonium (NH_4^+) and nitrate (NO_3^-) ions, and is a vital constituent of amino acids in proteins and nucleic acids (Bidwell, 1974). Since N is completely absent in tailings or wastes these ions are at notoriously low levels.. The autotrophic microorganisms, *Nitrosomonas* and *Nitrobacter*, which are largely responsible for nitrification of ammonia to nitrite and nitrite to nitrate, function very poorly at the low pH's which occur in acid tailings (Rorison, 1977). Many strains of *Rhizobia* (nitrogen fixing bacteria) cannot survive at $\text{pH} < 4.0$ (Jackson, 1967, Salt et. al 1995).

Nitrate ions are held weakly by positively charged particles and are easily leached from tailings and other low pH sources. Ammonia ions are held strongly by negative charged clay particles but are easily lost in coarse textured substrates of low cation exchange (Gemmell, 1977b). Strong bases can react with ammonia salts and liberate N as gaseous ammonia which is lost to the atmosphere (Gemmell, 1977b).

Phosphorus is often in limited supply in soils at both low and high pHs. Plants absorb phosphorus as inorganic phosphate ions. In acid wastes Fe, Zn, and Al ions react with P to form insoluble phosphate precipitates which interfere uptake by plants. Phosphorus can be immobilized as highly insoluble forms of calcium phosphate, or, absorbed by ferric hydroxide in alkaline waste (Gemmell, 1977b).

Potassium, required by plants in large amounts, is retained in material with a high cation exchange capacity, but is easily leached in acidic substrates with low cation exchange capacity. Potassium maintains the ionic balance in cells, is important in photosynthesis, and has a catalytic role in protein synthesis.

Calcium and magnesium are both essential for plants, but are very soluble at low pH, thus are easily leached from acid substrates. Calcium is important in the synthesis of pectin in cell walls and formation of nuclei and mitochondria in cells whereas magnesium is involved in numerous enzymic reactions and in chlorophyll synthesis (Sutcliffe and Baker, 1978, Baker and Brooks, 1989).

Generally, sulfur accounts for 0.2-0.7% of the biomass (dry weight) of plants and has a specialized role in formation of the amino acids (Anderson, 1978). Although sulfur is present at very high concentrations in pyritic tailings, sulfide and elemental sulfur are highly insoluble and not available to plants (Bidwell, 1974). However, sulfate ions are readily leached from tailings. Sulfate levels of five thousand to seven thousand ppm have been-shown to reduce plant growth by as much as 40 to 50% in some cases (Chapman, 1966).

Physical Properties of Tailings

The physical characteristics of tailings and other mine wastes can inhibit the establishment of vegetation, i.e. instability, texture, lack of organic matter, susceptibility to drought, high surface temperatures, surface evaporation, etc. (Bradshaw et al., 1976). Slipping of unstable tailings on steep slopes can cause root tearing (Goodman et al., 1973), wind erosion of open tailings causes abrasive damage to stems and leaves of plants, while gullyng by water erosion exposes roots (Gemmell, 1977b).

The texture of the waste is also a factor revegetation. Fine textured tailings of clay-like particles (<0.002 mm diameter) in tightly packed aggregates, result in low permeability and infiltration by water causing high water tables (e.g. Murray, 1971). Clay soils hold large amounts of water and dry out slowly, but the water that remains is held with great tenacity). Many tailings are of a sandy texture (>.05 mm diameter), porous, and have low moisture holding and cation exchange capacities resulting in nutrient leaching.(Knabe, 1965).

Another difficulty is the textural variability across a tailing site. This results from the segregation of particle sizes along horizontal gradients from slurry discharge points in the

pond (Murray, 1971). This compounds the problems for seedling establishment, which are associated with either fine or coarse textured materials.

All tailings lack organic matter (Weston, 1973). Revegetation therefore requires the addition of some form of organic matter. Because of their poor water retention and open exposure, tailings are characterized by drought even in regions where precipitation is fairly high (Michelutti, 1974). Tailings often contain high levels of inorganic salts responsible for high osmotic pressures in the soil solution (Peterson and Nielson, 1973). This results in low water potentials making it difficult for plant roots to take up moisture (Bidwell, 1974; Faiz and Weatherley, 1978).

Temperature extremes, caused by daytime solar heating, and radiation cooling at night, occur on tailings (Goodman et al., 1973). In particular, the tailings colour (often-dark) influence the severity of these effects. Bare mine spoils attained surface temperatures of 18 C higher daylight hours (Ott and Wood, 1978).

The high evaporation rates on tailings often results in metal surface concentrations which are many times higher than the original discharge rate (e.g. high concentrations of lead sulfate)(Pitcairn, 1969).

Biological Properties

Tailings usually lack the essential fungal root associates (i.e. mycorrhiza) which are advantageous to vascular plants by increasing the surface areas of roots for more efficient uptake of moisture and nutrients from soil (Powell, 1974; Marx, 1977; Fitter, 1977). Mycorrhizae are less common on sandy soils which, like tailings, are low in organic matter (Kruckelmann, 1974). Mycorrhizal fungi often fail to form symbiotic associations in high temperatures which commonly occur on mine wastes (Marx et al., 1970). Elevated levels of heavy metals and other elements (e.g. Cu, Ni, As) can be toxic to mycorrhizae (Trappe, 1973; Harris and Jurgensen, 1977). Sulfur, which is present in all sulfide tailings, is toxic to many fungi causing interference with aerobic respiration (Bowen, 1966).

Conventional Restoration Methods

Most tailing and mine waste revegetation consists of efforts to amend or alter the sites physical or chemical properties such that commercial species (often grasses) may grow. Few efforts have involved the use of naturally occurring metal tolerant plants. These conventional agricultural methods; e.g. liming, fertilizer application, mulching, hydro-seeding have been successfully utilized in the revegetation of the typically non-toxic disturbed sites such as pits and quarries, strip mined land and pipeline corridors (Murray, 1974; Peterson and Peterson, 1977; Marshall, 1979; CLRA, 1979).

However, when these methods have been applied to the typically toxic Canadian mine waste the results has been costly and unsatisfactory. (Peters, 1970; 1978; Leroy, 1973;

Murray, 1973; Weston, 1973; Michelutti, 1974,1978; Gullen, 1975; Keller and Leroy, 1975; Vivyurka, 1975; Courtin, 1976; Moore and Zimmermann, 1977; Ogram and Fraser, 1978; Watkin, 1978, Brown et. al 1997).

Acid (pyritic) tailings are usually limed to ameliorate low pH for and make heavy metals less available. Calcitic (CaCO_3) or dolomitic limestone, ($\text{CaMg}(\text{CO}_3)_2$), the latter having a high Mg content, are preferred over lime (CaOH) which is corrosive (Barber, 1967; MacLean and Dekker, 1976; Webber et al., 1977; Lundberg et al., 1977). Tailings often have very high liming requirements; e.g. 25 to 180 tons per hectare on pyritic Cu tailings (Watkin, 1978). Fertilizers are often applied to tailings at very high rates because of the low nutrient status of tailings (Laughlin, 1962, 1973; Laughlin et al., 1973). Expensive, slow release fertilizers are sometimes used to minimize nutrient loss by leaching in coarse textured tailings (Klock et al., 1975; Sheard, 1976). Applications of sewage sludge has been used to increase nutrients as well as to provide organic matter for higher moisture holding and cation exchange capacities (Courtin, 1976; Berry and Marx, 1977).

Mulches, decrease extremes in diurnal temperature flux, reduce water loss by evaporation. Other materials (e.g. straw, hardwood, sawdust and burlap strips) have been used with varying success (Stover, 1973; Heede, 1975; Emanuel, 1976). Attempts to cover toxic tailings with a soil overburden (Michelutti, 1974; Jeffrey, 1975; Farmer et al., 1976; MacLean and Dekker, 1976; Day et al., 1977) often fail due to acidification of the soil layer, from the buried tailings (Pionke and Regowski, 1979). Soluble metals move by capillary action from the toxic substrate beneath the amended layer, into the rhizosphere of the vegetative cover (Smith and Bradshaw, 1972).

Conventional seed mixtures of perennial grasses are used to obtain a stabilizing cover (USDA, 1972; Murray, 1973; Cook et al., 1974; Lesko et al., 1975). Legumes have nitrogen-fixing bacteria (Rhizobia) associated with their roots, which can provide a self-sufficient nitrogen supply for the tailings. Herbaceous legumes are, however, susceptible to metal toxicity at low pH and metal tolerance in legumes is rare (Johnson et al., 1977). Lupines are found in quite acidic soil in nature. Cultivated lupines showed a degree of Al and Mn tolerance to water culture (Rorison, 1972). Legume seed germination and seedling establishment can be hampered by additions of nitrogen fertilizers (Bradshaw, 1977).

Sudden declines in growth often occur four or five years after initial treatments were made, due to a loss of fertility brought about by the depletion of nutrients and leaching (Johnson et al., 1977). Commercial species grow rapidly, and, because of their high nutrient demands, require re-application of fertilizers in order to maintain growth. Also, recycling of nutrients in tailings is low because of insufficient microbial activity necessary to decompose plant litter. (Gemmell, 1977b).

Commercial Species.

The following listing of adapted, commercially available Graminoid species and cultivars is produced by the USGS 2000 as a list of commercial cultivars available in Alaska. These are products of plant breeding programs often the work of the University of Alaska or the Canadian Government.

- a. 'Arctared' Red Fescue, *Festuca rubra*, was released in 1965 as a revegetation species showing extreme hardiness throughout Alaska (Hodgson, 1978). The overly aggressive, sod-forming nature of this species often makes this cultivar unacceptable in reclamation. However, in erosion control the cultivar is outstanding. Also, the aggressive nature of this sod forming species may be a way of preventing the invasion of native shrub species such as alder and willow. The University of Alaska Agricultural Experiment Station and the USDA cooperatively developed the cultivar.
- b. 'Boreal' Red Fescue, *Festuca rubra*, was developed by the Canadian Department of Agriculture Research Station, Beaverlodge, Alberta (USDA 1972). This very hardy cultivar is similar to Arctared in adaptation and potential use in Alaska. It is often substituted for Arctared and is less expensive.
- c. 'Pennlawn' Red Fescue, *Festuca rubra*, was released in 1954 by the Pennsylvania Agricultural Experiment Station (USDA 1972). The cultivar is not as hardy as Arctared or Boreal, but still has potential in milder areas of Alaska. This cultivar was selected for turf uses, and therefore, tends to be used for landscaping more than for revegetation.
- d. 'Egan' American Sloughgrass, *Beckmannia syzigachne*, was released by the Alaska Plant Materials Center in 1990 as a wetland rehabilitation cultivar (Wright, 1991a). This is the state's first cultivar developed solely for wetland restoration. Additionally, the species has wildlife benefits by providing forage and seed for waterfowl (Wright 1992).
- e. 'Alyeska' Polargrass, *Arctagrostis latifolia*, is a cultivar developed by the University of Alaska Agricultural Experiment Station. The prime purpose for this cultivar is revegetation in interior and western Alaska (Mitchell, 1979). The species is adapted to moderately wet areas (Wright 1992).
- f. 'Kenai' Polargrass, *Arctagrostis latifolia*, is a variety recommended for forage and revegetation in the central interior and southern portions of Alaska (Mitchell, 1987). This species has potential for revegetating wet areas. This cultivar was developed by the Alaska Agriculture and Forestry Experiment Station at Palmer, Alaska.
- g. 'Sourdough' Bluejoint, *Calamagrostis canadensis*, is a cultivar with a wide range of adaptability. The species occurs throughout Alaska on both dry and wet sites. The cultivar was developed by the University of Alaska Agricultural Experiment Station for revegetation in northern latitudes (Mitchell, 1979). Commercial availability is erratic, and when it is available, the seed is expensive (Wright 1992).
- h. 'Norcoast' Bering Hairgrass, *Deschampsia beringensis*, was released in 1981 by the University of Alaska Agricultural Experiment Station as a forage and revegetation grass in northern areas. Norcoast is recommended for revegetation use in coastal

- regions of western Alaska to southwestern Alaska and possibly in the northern maritime regions (Mitchell, 1985).
- i. 'Nortran' Tufted Hairgrass was also released by the University of Alaska Agricultural Experiment Station. Intended use is similar to Norcoast; however, this cultivar is better adapted to northern regions of Alaska (Mitchell, 1985).
 - j. 'Tundra' Glaucous Bluegrass, *Poa glauca*, was originally collected in Arctic Alaska. The cultivar was released by the University of Alaska Agricultural Experiment Station for revegetation in extreme northern areas with severe environmental conditions (Mitchell, 1979).
 - k. 'Caiggluk' Tilesy Sagebrush, *Artemisia tilesii*, was developed and released by the Alaska Plant Materials Center in 1989 as a reclamation species. This forb has a wide range of adaptations throughout Alaska (Wright, 1992).
 - l. 'Gruening' Alpine Bluegrass, *Poa alpina*, was released by the Alaska Plant Materials Center in 1986. The species is widely adapted throughout Alaska. As the name implies, the species is adapted to high elevation areas. It also performs well on sites drier than those tolerated by Kentucky bluegrass. Seed availability is limited. Before this cultivar is included in a planting plan, you should research the availability of the seed.
 - m. 'Nugget' Kentucky Bluegrass, *Poa pratensis*, was released and developed by the University of Alaska Experiment Station in 1966. The source of this cultivar was a single plant collection made in 1957 at Hope, Alaska. Nugget has outstanding winter survival (USDA 1972), and is used extensively in Alaska for turf and lawns.
 - n. 'Park' Kentucky Bluegrass, *Poa pratensis*, was developed by the Minnesota Agricultural Experiment Station in 1957 (USDA 1972). Hardiness of this cultivar is not as good as Nugget in extreme northern areas of Alaska. However, it is still used in volume in Alaska. Like Nugget, its use tends to be limited to landscape and lawns.
 - 'Merion' Kentucky Bluegrass, *Poa pratensis*, was released in 1947 by the USDA Plant Service Research Division, ARS and the U.S. Golf Association Green Section. The cultivar is more adapted to close mowing than any other Kentucky bluegrass (USDA 1972). Merion is often used in lawn mixes in Alaska.
 - p. 'Reeve' Beach Wildrye, *Elymus arenarius* (*Leymus arenarius*), is a 1991 release of the Alaska Plant Materials Center. The cultivar has high potential in coastal restoration, especially in the fore dune and other sandy sites throughout coastal Alaska (Wright 1991a).
 - q. 'Benson' Beach Wildrye, *Elymus mollis* (*Leymus mollis*), is a cultivar of native species released by the Alaska Plant Materials Center in 1991 (Wright 1991b). Unlike Reeve, Benson is available only from vegetative cuttings (sprigs). Seed will not be available. Benson was selected for use in sandy areas of high erosion potential. Revegetation with sprigs is a preferred method of revegetating at highly erosive areas.
 - r. Annual & Perennial Ryegrass. There are no cultivars called for in these species since long-term survival is not critical and may not be desirable. These species provide a quick, temporary cover and should be limited to 10% or less of a seed

mix. These species use nutrients that are intended for the perennial species included in the mixes and can produce a heavy plant cover, which can slow the growth of the perennial species. Annual and perennial rye grasses are also very attractive to herbivores, which may cause vehicle/animal conflicts.

Native Species

The goal of tailing and mine waste revegetation in northern Canada should be to encourage the return of the original flora through the use of native plants, especially pioneer species (Johnson and VanCleve, 1976,1986). Invasion by native species will be hindered if commercial species have been introduced. They can out compete the native species when high rates of fertilizer are applied (Younkin, 1973,1974; Grime, 1973a,b; Dabbs, 1974, Withers, S., 1999.). Rapid growing commercial species are not adapted to extremes in temperatures, short growing seasons or the long winters of northern regions.

Native plants are adapted to poor nutrient conditions (Antonovics et al., 1967 , Densmore et al 1990, Roundy et al. 1997) and are hardy against cold temperatures and less susceptible to frost damage than fast-growing commercial species (McCowen, 1972, 1973; Wielgolaski, 1975, Munshower 1994, Brown 1997). There is no danger of creating a new 'weed' as might happen with introduced species (Johnson and VanCleve, 1976, 1986). Seeds from native species have also shown genetic variability (Antonovics et al., 1967; Johnson and VanCleve, 1976, Bradshaw, 1997, Stanley 2000).

The lack of an available seed source for large-scale use is a disadvantage of using native species (Sutton, 1975). While many of the commercial graminoids can easily be purchased ([Arctic Alpine Reclamation Group](http://www.aaseed.com/comp/profile.html) <http://www.aaseed.com/comp/profile.html>) Northern native perennial species are poor seed producers, especially in northern climates where species have adapted to asexual underground reproduction (Grime, 1974; Wielgolaski, 1975). Native perennial species grow slowly. However, in toxic tailings this slow growth could be an advantage enabling them to survive on tailings because of slower uptake and metabolism of toxic elements compared with the commercial species with higher growth rates.

Much of the information about the potential of native species for tailing revegetation was derived from studies of natural succession in disturbed sites in arctic, arctic/subarctic, and boreal regions (Van Cleve et al. 1986). Pioneer species such as *Calamagrostis canadensis*, *Arctophila fulva*, *Poa arctica* and *Luzula confusa*, are all successful invaders of mesic disturbed sites whereas, *Eriophorum scheuchzeri*, *Carex aquatilis* and *Senecio congestus* are invaders of wetter sites (Hernandez, 1972, 1973).

The potential of various grasses (e.g. *Arctagrostis latifolia*, *Calamagrostis canadensis*, *Poa lanata*, *Hierochloe alpina* and *H. ordorata*) for invading disturbed sites has been studied by Klebesadel (1965, 1969); Younkin (1973, 1974); Mitchell (1974, 1976); Bliss et al., (1972), Von Frenckell-Insam and Hutchinson 1993. On tailings in the N.W.T., Taylor and Gill (1974) found that some pioneer species (e.g. *Hordeum jubatum*, *Epilobium angustifolium*, *Equisetum arvense*, *Eriophorum angustifolium*, *Carex aquatalis*) had invaded where the tailings were shallow enough to allow plant roots to penetrate into the original soil surface.

Some native legumes (i.e. *Lupinus*, *Astragalus*, *Oxytropis*, *Vicia*, *Lathyrus* and *Hedysarum*) occur on acidic soils and their potential usefulness has been investigated (Kiebesadel, 1971, 1973). Other non-leguminous nitrogen fixers are worth considering (e.g. *Alnus crispa*). Presently, *Alnus glutinosa* has been utilized on tailings in the U.K. (Sheldron and Bradshaw, Taylor 1976; and Fedkenheur and Heacock 1979. Field trials with native tree species on tailings as well as coal mine spoils have been described: Van Cleve 1972,1973; Taylor and Gill 1976, Aldon 1975; Mitchell 1972; Darmer Brown and Johnson 1976 Densmore 1996. Poplars and willows can be easily propagated from viable rootstocks (Zuffa, 1971; Laidlaw, 1974, Densmore 1987). Woody plants could provide nuclei for invasion of herbaceous species that might germinate in the litter accumulated under the trees and shrubs (Yarranton et al., 1974; Miller, 1978).

Johnson and VanCleve 1976, also, Roundy et al. 1997 discuss the advantages of native species in revegetation in regions in the far north. However, reference is made mainly to disturbed areas, which are non-toxic. With specific reference to toxic sites the reader is referred to the work of Mitchell 1972, Leroy 1973, Neuman et al. 1993, Roundy et. al. 1997, and Stanley et al. 2000.

Field Trials in the North

Few reports exist of native plant field trials on contaminated sites, with only a handful dealing with species that live in the Arctic (Kuja 1980). Often these represent one or two species tested on a site (eg. Laidlaw 1974- Aspen, McNaughton 1974 – *Typha latifolia* Younkin, 1974 - *Arctagrostis latifolia*, Wu and Antonovics 1975 - *Agrostis stolonifera*, Densmore 1977- *Rosa acicularis*, Densmore 1987- Willow, Von Frenckell-Insam, and Hutchinson, 1993- *Deschampsia caespitosa* Schnoor 2000 - Poplar trees (see reference section). The most extensive record found is the “Native Plant Revegetation Manual for Denali National Park and Preserve, USGS (2000). Essentially, they documented the success or failure of various plantings of truly native species. The following table is taken from that work.

Table 9. Species tested and/or used for native plant revegetation in DNPP from 1976 to 1994.

Scientific name*	Propagation method	Location*	Year planted	Comments and status in 1999
Trees				
<i>Picea glauca</i>	seedlings	16	1992	Planted in regraded placer mine tailings; experienced high mortality and poor growth; best survival and growth where planted under alder
	transplant	2	1976	Vigorous, 4-5 m tall
		3c	1990	Healthy
		3d	1991	Healthy but slow growing; most plants DBC
		3e	1989	Trees root-pruned a year before transplanting died or grew poorly, but unpruned trees dug up individually or with sod squares and immediately replanted are healthy
		6b	1985	Transplanted trees effectively blocked abandoned road, permitting natural revegetation
		8	1988	Healthy; trees effectively blocked off-site camping and allowed natural revegetation
<i>P. mariana</i>	transplant	3e	1989	Healthy
<i>Populus balsamifera</i>	cuttings	12	1976	Few surviving; 2 m tall; surrounded by natural revegetation
		16a	1989	Low survival, but survivors with slow-release fertilizer healthy; 1-2 m
<i>P. tremuloides</i>	transplant	3e	1989	Trees are alive but not vigorous
		3f	1992	Dead or barely alive
Shrubs				
<i>Alnus crispa</i> (= <i>Alnus viridis</i> ssp. <i>crispa</i>)	seedlings	3f	1991	Vigorous
		16a,b	1989	Vigorous
<i>Betula nana</i>	seedlings	16a	1992	Healthy but growing very slowly
	transplant	3e	1989	Healthy; some plants which initially died back resprouted
		11a,b	1989	Healthy
		14a	1989	Most vigorous, but some plants which lost soil from roots during transplanting died back and did not fully recover
		15	1988	Vigorous
<i>Cornus suecica</i>	transplant	14a	1989	Healthy
<i>Empetrum nigrum</i>	transplant	3e	1989	Healthy
		11a	1989	Healthy
		14a	1989	Healthy
<i>Ledum palustre</i>	transplant	3e	1989	Died back; cover decreased after transplanting
		11a	1989	Healthy
		14a	1989	Healthy, but some plants which lost soil during transplanting died back
<i>Linnaea borealis</i>	transplant	14a	1989	Healthy
<i>Potentilla fruticosa</i> (= <i>Pentaphylloides floribunda</i>)	seedlings	5	1986	Vigorous
		10	1986	Vigorous
	rooted cuttings	3b,c,h	1991	Vigorous
		3d	1991	Vigorous until DBC
		6a	1987	Vigorous until DBC
		10	1987	Healthy
		11a	1989	Vigorous
		13	1987	Vigorous
<i>Rosa acicularis</i>	transplant	3e	1989	Healthy
<i>Salix alaxensis</i>	seedlings	10	1986	Died
	cuttings	2	1976	Browsed by moose to snow level for approximately 15 years; now 4-5 m tall
		3f	1991	Healthy
		7	1994	Sprouted vigorously from brush bar and stabilized slope
		10	1987	Barely alive, 20 cm tall; slope too dry for cuttings
		12	1986	Many healthy plants 2-3 m tall but obscured by natural revegetation which grew rapidly once visitor traffic was blocked by plantings
		16a	1989	Healthy on well-drained nutrient-poor placer mine tailings with slow-release fertilizer; died without fertilizer
		16b	1991-92	Vigorous; growth much faster with slow-release fertilizer

Scientific name	Propagation method	Location	Year planted	Comments and status in 1999
Shrubs (continued)				
<i>S. depressa</i> (= <i>S. bebbiana</i>)	transplant	3e	1989	Healthy
<i>S. glauca</i>	transplant	11a	1989	Healthy
<i>S. pulchra</i>	transplant	3e	1989	Healthy
(= <i>S. planifolia</i> ssp. <i>pulchra</i>)		11a,b	1989	Healthy
		14a	1989	Healthy
		15	1988	Healthy
<i>Shepherdia canadensis</i>	seed	15a,b	1993	Good establishment, even under coconut fiber mats; slow growth to 10-20 cm diameter
	seedlings	10	1987	Vigorous; plants 75-120 cm diameter
		15a,b	1993	Slow initial growth; plants 30 cm diameter
<i>Vaccinium uliginosum</i>	transplant	3e	1989	Died back; cover decreased after transplanting
		14a	1989	Healthy, except for some plants which lost soil from roots during transplanting
		15	1988	Healthy
<i>V. vitis-idaea</i>	transplant	3e	1989	Died back; cover decreased after transplanting
		14a	1989	Healthy, except for some plants which lost soil from roots during transplanting
Forbs				
<i>Arnica alpina</i>	seedlings	1	1988	Healthy; plants have produced hundreds of new plants from seed
<i>A. trigloa</i>	seed	4	1985	Plants grew slowly even though fertilized with slow-release fertilizer; cover per plant only 10 cm ² after two years, but healthy until DBC
	seedlings	3a,d	1991	Vigorous with many flowers but many plants DBC
		3c	1991	Vigorous with many flowers, up to 30 cm diameter, but plants by and under entrance sign badly trampled by visitors photographing sign
		3h	1991	Healthy
		6a	1987	Vigorous with many flowers, up to 30 cm diameter, until DBC
		10	1986	Healthy but slow growing
		13	1987	Alive but overgrown by taller vegetation; must be planted in open areas
<i>Artemisia tilesii</i>	seed	13	1992	Seeded with autumn seed blitz method; many healthy plants
	seedlings	1	1988	Few alive; not vigorous
		3a,b	1991	Vigorous
		5	1986	Healthy; reproducing from seed
		10	1986	Variable with healthy and sickly plants
		11a	1989	Vigorous
<i>Aster sibiricus</i>	seed	4	1985	Plants were healthy but grew slowly even though fertilized with slow-release fertilizer; cover per plant only 20 cm ² after 2 years; DBC
	seedlings	3a,c,h	1991	Vigorous, spreading vegetatively into mats; many plants DBC
		5	1986	Healthy, spreading vegetatively
		6a	1987	Vigorous until DBC
<i>Epilobium angustifolium</i>	seeds	3a	1991	Failed on gravel fill, even with slow-release fertilizer
		3f	1992	Healthy and flowering; seeded into "seed trap" depressions fertilized with slow-release fertilizer and composted dog manure
		14a	1989	Many plants, but poor growth on subsoil
		14b	1989	Many plants but not vigorous growth
	transplant	3e	1989	Very successful; scattered plants in sod spread rapidly and are vigorous
		11a	1989	Vigorous
<i>Mertensia paniculata</i>	rhizomes	3f	1992	Failed
	seed	4	1985	Plants grew very slowly
	seedlings	3c,d	1991	Healthy
		6a	1987	Vigorous until DBC
	transplant	11a	1989	Vigorous
<i>Myosotis alpestris</i>	seedlings	3a,c	1991	Dead; some dug up by visitors
(= <i>Myosotis asiatica</i>)		6a	1987	Vigorous until DBC

Scientific name	Propagation method	Location	Year planted	Comments and status in 1999
Forbs (continued)				
<i>Saxifraga tricuspidata</i>	seedlings	10	1987	Vigorous unless planted with fertilized grass seedlings; must be planted in open areas
		11a	1989	Vigorous
<i>Senecio lugens</i>	seed	4	1985	Plants grew very slowly, but healthy until DBC
	seedlings	3a,c,h	1991	Vigorous, but many plants DBC
		6a	1987	Vigorous until DBC
<i>Silene acaulis</i>	seedlings	11a	1989	Dead, probably trampled
		13	1987	One vigorous plant in open area, 30 cm diameter; most of plants overgrown by taller plants; must be planted in open areas
<i>Solidago multiradiata</i>	seed	1	1987	Plants vigorous, spreading from seed
		4	1985	Plants grew very slowly, but healthy until DBC
	seedlings	1	1988	Vigorous
		3a,c,h	1991	Vigorous, some DBC
		6a	1987	Vigorous until DBC
Leguminous forbs				
<i>Astragalus euosmus</i>	seeds	4	1985	Unhealthy plants
	seedlings	5	1986	Poor growth; died
		6a	1987	Poor growth until DBC
<i>Hedysarum alpinum</i>	seeds	1	1987	Vigorous, spreading from seed
		3a	1991	Vigorous, spreading from seed
		3g	1994	Plants vigorous but sparse relative to seeding rate
		4	1985	Plants grew rapidly, cover per plant 300 cm ² after 2 years; flowered third year; plants vigorous until DBC
	seedlings	3a	1991	Most seedlings died when overwintered outside in containers; remainder vigorous and spreading from seed
		5	1986	Healthy
<i>Lupinus arcticus</i>	seeds	6a	1987	Vigorous until DBC
		3a-e	1991	Failed to germinate even when scarified; probably needed more scarification
		4	1985	Good germination; coat of each seed nicked with a file before planting. Plants grew rapidly; cover per plant 340 cm ² after 2 years; flowered third year; plants vigorous until DBC
	seedlings	6a	1987	Failed to germinate
		6a	1987	Seedlings grew poorly in greenhouse even though inoculated with <i>Lupinus arcticus</i> nodules; died after planting
<i>Oxytropis campestris</i>	seeds	3a	1991	Vigorous, self-seeding
		3f	1993	Chaff from 1992 seed cleaning contained a surprising number of seeds which established many plants
		3h	1994	Plants vigorous but sparse relative to seeding rate
		4	1985	Plants grew rapidly, cover per plant 300 cm ² after 2 years; flowered third year; vigorous until DBC
	seedlings	7	1994	Vigorous
		1	1988	Vigorous; spreading from seed
		5	1987	Original plants dying back but spreading from seed
		6a	1987	Vigorous until DBC
<i>O. deflexa</i>	seedlings	5	1987	Grew poorly, died
Grasses				
<i>Agropyron macrourum</i> and <i>A. violaceum</i> (= <i>Elymus macrourus</i> and <i>E. alaskanus</i>)	seeds	1	1987-88	Plants vigorous and spreading from seed
		3a	1991	Vigorous stands
		3f	1993	Chaff from 1992 seed cleaning contained a surprising number of seeds which established many plants
		3g	1994	Stand sparse, but plants healthy
		4	1985	20 plants/m ² produced 10% cover after 2 years; plants vigorous until DBC
		7	1994	Vigorous stand
		11b	1989	Healthy; sown with mix of species collected on site

Scientific name	Propagation method	Location	Year planted	Comments and status in 1999
Grasses (continued)				
		14a	1989	Seeded on trampled areas with a mix of species collected on site and covered with coconut matting which was reduced after 10 years to plastic netting and nylon string; scattered plants, not vigorous
<i>Arctagrostis latifolia</i>	seeds	3c	1991	Failed
		11b	1989	Healthy; sown with mix of species collected on site
		13	1992	Healthy plants dominant in mixed species stand produced by autumn seed blitz technique
		14a	1989	Sown on trampled areas with a mix of species collected on site; few plants established; not vigorous
<i>Calamagrostis canadensis</i>	seeds	3a	1991	Plants did not establish on gravel fill
		14a	1989	Seeded on trampled areas with a mix of species collected on site; some areas covered with coconut matting; not vigorous
		14b	1989	Initial growth good but declined when yearly fertilization stopped
	transplant	11a,b	1989	Vigorous; increased growth when sod transplanted
		14a	1989	Vigorous; increased growth when sod transplanted
<i>C. purpurascens</i>	seedlings	3d	1991	Vigorous, but most DBC
<i>Elymus innovatus</i> (= <i>Leymus innovatus</i>)	seedlings	3d	1991	Vigorous and spreading, but many plants DBC
		10	1987	Vigorous; spreading vegetatively
		13	1987	Healthy and spreading; patches up to 2 m across
<i>Festuca altaica</i>	seeds	4	1985	Slow growing; 130 plants/m ² produced 8% cover after 2 years; healthy until DBC
		13	1992	Seeded with autumn seed blitz technique; scattered healthy plants
<i>F. rubra</i>	seedlings	10	1986	Died
	seed	10	1986	Did not establish on unstable subsoil
		11b	1989	Vigorous stands; seed collected from site, fertilized with slow release fertilizer, and visitor trampling restricted
		13	1992	Seeded with autumn seed blitz technique; many healthy plants
		14b	1989	Seeded with mix of species collected on site; many plants not vigorous but still stabilizing soil
	seedlings	10	1987	Alive, but cover decreased when fertilizer was used up; roots and litter stabilizing slope even with decreased cover
		11a	1989	Vigorous, forming sod which has stabilized slope
		13	1987	Vigorous and spreading
<i>Phleum commutatum</i> (= <i>P. alpinum</i>)	seed	13	1992	Seeded with autumn seed blitz technique; scattered healthy plants
<i>Poa alpina</i>	seed	1	1987-88	Few plants, not vigorous
		3c	1991	Healthy even though trampled by visitors
		4	1985	Slow growing; after 2 years, 50 seedlings/m ² provided 2% cover, but plants healthy until DBC
	transplant	6a	1987	Sod was grown from seed in nursery bed and was transplanted to create waterbars; effectively controlled erosion until DBC
<i>Trisetum spicatum</i>	seed	11b	1989	Healthy; seeded from mix of species collected on site

*Nomenclature follows Hultén (1968); updated nomenclature from U.S. Department of Agriculture Integrated Taxonomic Information System (<http://www.itis.usda.gov>) is listed in parentheses.

¹See Fig. 24 for location of vegetation sites. (1) Parks Highway, mile 231; test plots by DNPP south entrance sign. (2) Parks Highway, mile 232.6, old road. (3) Park Road, mile 0-1.6, (a) roadsides, mile 0-1.2, (b) cut slopes, mile 0-1.2, (c) area around DNPP main entrance sign, mile 0.1, (d) abandoned road to Riley Creek Campground and along road to dump station, (e) Visitor Access Center, (f) abandoned parking lot on both sides of airport access road, (g) cut slopes around airplane parking area on airstrip, and (h) access trail between hotel and train station. (4) DNPP Headquarters. (5) Test plots on gravel fill, mile 5. (6) (a) Savage Cabin gravel pad and trail and (b) Savage Campground, abandoned road. (7) Road cut and fill slopes, mile 20. (8) Sanctuary Campground. (9) Teklanika Campground. (10) Road cut slopes in Sable Pass, mile 42.5. (11) Polychrome Comfort Station, (a) cut slopes behind buildings and (b) trampled areas above buildings. (12) Abandoned Toklat Campground. (13) Eielson Visitor Center. (14) Wonder Lake Campground, (a) abandoned campsites and (b) sod roots on buildings. (15) Wonder Lake Ranger Station, leach field. (16) Glen Creek, placer-mined watershed, (a) regraded placer mine tailings above the active floodplain and (b) placer mine tailings regraded to construct new floodplains.

Unfortunately, this is an extremely limited sample. There are a few items on contaminated sites but one could hardly argue this is scientific sample. There are a couple manuals with descriptions of native plant autecology (see Withers 1999), but again there are no specific trials of species. The fact that there is so little research on these sites and species is not surprising. Typically, engineers did mine site restoration, if they were re-vegetated at all. One only has to travel to the nearest mine to see the success we have had. The newer closure plan legislation has made this much more imperative. Because of the large size of the areas restoration methods borrowed from agriculture are frequently used. Many of these methods apparently (by their success) are not suitable for the harsh habitats in the north.

Mosses

Mosses clearly represent the most exciting opportunity for re-vegetating severely damaged sites. As discussed throughout this paper, mine waste differs from soil due to the absence of organic matter. Mosses are the natural pioneers of rock based sites. Although there is extensive research on vascular plants (see above) for re-vegetating high metal sites, only a few cases have been conducted on mosses. Traditionally, botanists have ignored this huge group of plants probably because they cannot be eaten.

Among plants, mosses have been extensively studied as sorbent material for use as environmental indicators particularly for atmospheric pollution. Mosses have been used as a bio-indicator for metals (Gerdol and Cenci, 1999; Loppi and Bonini, 2000; Genoni et al., 2000; Gerdol et al., 2000; Guidotti et al., 2000) and polycyclic aromatic hydrocarbons (Tsakovski et al., 1999). Standardized procedures for the use of mosses in trace metal deposition, biomonitoring have been recently proposed by Castello et al. (1999) and by Cenci (1999). Other researchers proposed the use of moss in the removal of heavy metals from wastewater and contaminated sites (Al Asheh and Duvnjak, 1999; Aldrich and Feng, 2000)

Why Use Mosses For Overburden Revegetation?



Restoration Using Common Mosses on Grasberg Mine area, Irian Jaya, Indonesia (pH <2.8). Two years after establishment (Stanley et. al. 2000ab).

Besides Stanley et al. (2000ab) there appears to be no record of mosses being used in revegetation, although as discussed above there is ample evidence to suggest that they would be successful mosses are:

- 1) Successful natural pioneers. Mosses are amongst the first natural colonizers of moraines, disturbed soils, and overburden. Indeed they frequently invade many of the former Arctic sites.



Pioneer mosses at Giant Mine NWT.

- 2) Initiators of the soil formation process. Moss mats trap silt, spores and seeds, and decaying moss tissue builds up humus, which all contribute to the initial stages of soil formation.
- 3) Able to improve vascular plants establishment. In some situations, mosses have improved germination, emergence and survival rate of juvenile vascular plants.
- 4) Relatively successful in harsh environments. Mosses can tolerate repeated cycles of drying and re-wetting, and grow at altitudes above the limits of vascular plants.

- 5) . Tolerant of heavy metals. Many moss species are common colonizers of metal-contaminated habitats (e.g. mine tailings) and survive in some of the most toxic of micro-sites.
- 6) . Tolerant of low pH. Many mosses have been shown to have the ability to grow and develop in very acid conditions.
- 7) Easily propagated using vegetative fragments. Effective methods were already developed for fast establishment of mosses from vegetative fragments. Since these fragments are collected locally the gene pool and natural bio diversity are preserved.

Stanley et al. (2000ab) provide good methods and directions for re-establishing bryophyte communities on severally degraded low pH rock communities. Furthermore, there is a substantial volume of Canadian research in the re-establishment of *Sphagnum* mosses (see Rochefort et al., 1995; Campeau and Rochefort, 1996; Quinty and Rochefort, 1997; Rochefort, 2000). *Sphagnum* typically grows on acidic conditions, and many believe because of its high cation exchange capacity that it may be a very suitable plant for some of the northern wetland treatment methods and tailing re-vegetation. Briefly, the techniques used are based on 1) active re-introduction of peatland plant diaspores (collected *Sphagnum* plants), 2) the use of mulches to ameliorate the otherwise harsh substrate conditions in which diaspores are developing and 3) blockage of the natural drainage to induce flooding. In some instances, low bunds or shallow basins are also used in order to improve water retention at the site, thereby improving the summer hydrological conditions of the peat surfaces.

While Stanley et al. 2000 represents the only large-scale trial of mosses, they commonly invade mine sites. The photos above clearly illustrate the pioneer moss *Polytricum juniperinum* invading the giant mine site. By physically establishing a moss layer one creates the following:

1) Active nutrient cycling and soil genesis process; 2) maintains erosion control; 3) locks up heavy metals as few species eat mosses; 4) provides habitat for mycorrhizas considered essential for metal tolerant plant survival; 5) provides habitat for invasive native plants Densmore 1994, and 5) does not preclude any other planting or cutting technique; 6) uses site specific seed sources (from the actual site, avoiding genetic and cultivar problems; and, 7) Stanley et al 2000 as well as Rochefort 2000, both suggest these are extremely economical methods.

Since one of the fundamental ecological principals in arctic and boreal forest ecology is the nutrient cycling through the moss layer (Vierek 1966, Van Cleve et al 1986) it only seems logical that the re-establishment of this soil forming layer is critical for successful revegetation.

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