# Preventing Aluminum Phytotoxicity through Phytoremediation

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5/11/06

**Abstract**

Aluminum toxicity is one of the leading factors resulting in decreased agricultural yields on acid soils, which comprise up to 50% of the world's potential arable land. While the final goal of much of the current research on aluminum phytotoxicity is to develop transgenic crops resistant to aluminum, there are current technologies that can potentially reduce aluminum phytotoxicity; phytoremediation, the cleanup of environmental contaminants with plants, is one such example. However, before an aluminum phytoremediation program can be implemented, an idea of the length of a program must be estimated. In this study, a crude model of aluminum phytoremediation was constructed by subjecting 17-day old common buckwheat (*Fagopyrum esculentum*) seedlings to increasing concentrations of aluminum in hydroponic culture, and then determining the rate at which aluminum was removed from the hydroponic solution. At the end of a six-day period, the rate of aluminum uptake was directly proportional to the initial concentration of aluminum in solution.

**Introduction**

Aluminum is the most abundant metal in the earth's crust, and is one of the most used metals in the world today (Aluminum Association, 2004). While aluminum is widely used by humans, it can be detrimental to many plants, including crop plants. Under acidic soil conditions (pH < 5.5 (von Uexküll and Mutert, 1995)) aluminum decreases the availability of essential nutrients and damages root systems, thereby decreasing agricultural yields (Kochian, 2004). While the threat and occurrence of aluminum toxicity to plants currently seems small on a global scale, much of this land is located in the tropics and subtropics where many countries are still developing, and where farmers may be less able to cope with the economic strain that would result from reduced crop yields. Areas of potential aluminum toxicity are also increasing due to the increased usage of aluminum, acidification of arable land, the conversion of forest land to cropland, and sludge disposal practices. In the future, plants will need to be made more resistant to aluminum, or be exposed to lower concentrations of aluminum, lest aluminum phytotoxicity become a more widespread problem.

Aluminum phytotoxicity can be prevented by genetically engineering plants to be more resistant to aluminum, or by removing aluminum from agricultural sites. Unfortunately, genetically engineering plants to be resistant to aluminum requires a level of knowledge of aluminum tolerance in plants that is still in its infancy (Kochian, 2000). Therefore a method of reducing aluminum concentrations in agricultural lands needs to be employed. Phytoremediation, the removal of contaminants from the environment using plants, is one such method (Chaney, 1997). Knowledge gained from phytoremediation research could also be used to benefit humans as well as plants, as aluminum has been considered a potential contributor to Parkinson's disease and Alzheimer's disease for many years (Environment Canada, 2000).

Before a phytoremediation project can be designed or implemented, a rough idea of the length of the program and the effectiveness of the phytoremediator should be ~~investigated~~determined. In this paper aluminum phytotoxicity and tolerance are reviewed, and a model is constructed that can be used to estimate the length of an aluminum phytoremediation program using common buckwheat.

**Literature Review**

Aluminum comprises an estimated 8% of the earth's crust, making it the most abundant metal in the crust (ATSDR, 1999). The largest natural sources of aluminum are located in Australia, Guinea, Jamaica, and Brazil (USGS, 2006). Aluminum is used extensively for packaging, for the transportation industry, and for construction, making aluminum one of the most widely used metals in the world (Aluminum Association, 2004). While aluminum is one of the most widely recycled materials in the world (Aluminum Association, 2004), a large amount still ends up in landfills. The amount of aluminum added to landfills is likely to increase as countries continue to develop economically; China is predicted to consume 8.8 million tons of aluminum per year by the year 2010, twice what China consumed in 2002 and more than the United States in 2002 (Hunt Jr., 2004). This is of concern because aluminum in landfills can reenter the environment by leaching into groundwater and surface water.

Aluminum can also be added directly to agricultural, and potentially agricultural, land. Throughout the world aluminum sulfate ($AlSO_4$) is added to municipal water supplies to reduce turbidity and to prevent algal blooms (Kennedy and Cooke, 1982). While the sludge that results is often disposed of in landfills, it has also been disposed of by spreading it over both forest and agricultural land (Environment Canada, 2000). The sludge can serve as a cheap and effective fertilizer (Van Rensburg, 2003) but such practices have led to a 200% increase in aluminum concentration in Canadian soils (Environment Canada, 2000).

The toxic concentration of aluminum varies by plant species and cultivar, but in laboratory experiments concentrations of aluminum as low as 0.13 parts per million (ppm) have severely decreased root elongation in an Al-sensitive cultivar of wheat (Delhaize and Ryan, 1995), while concentrations of 2.70 ppm have resulted in a 60% in root elongation in a resistant cultivar of buckwheat (Zheng et al., 2005). The low phototoxic concentrations of aluminum are of concern because concentrations of aluminum in crop soils can be quite high. For example, in Canada the concentration of available aluminum in a cropland soil was between 403 ppm and 543 ppm (Environment Canada, 2000).

Fortunately, aluminum is only toxic in acidic soils, which only accounted for 12% of agricultural land in a 1995 study (von Uexküll and Mutert, 1995). However, as much as 50% of the world's potential arable land is acidic, and the potential for agricultural lands to become acidic is high. Agricultural lands can be made more acidic by two main mechanisms – introduction of acidifying agents to soils, and converting land with already acidic soils to agricultural land. It is common practice in agriculture to add nitrogenous fertilizers to agricultural land, but these fertilizers can also decrease soil pH by dissociating into a nitrogenous compound and an acid (Kochian, 2000), thereby leading to acidification of soil. Acid rain can also lead to increased bioavailability of aluminum (Cronan and Schofield, 1979).

Conversely, naturally acidic land can be converted to cropland, thereby exposing crop plants to acid soils; a prime example of this is currently taking place in Brazil, where 40% of the forestland could be converted to agricultural use by 2050 if current deforestation trends continue (Soares-Filho et al., 2006). When considering that most of the soils in Brazil are considered acidic (von Uexküll and Mutert, 1995) and Brazil has a large amount of aluminum in the soil, the likelihood of aluminum phytotoxicity is high; phytotoxicity could even exacerbate the deforestation problems that Brazil is currently experiencing.

Under neutral pH, aluminum in the form of $Al(OH)_3$, otherwise known as gibbsite, is not soluble and is considered non-phytotoxic. However, as pH decreases from about 6.5 to 4.0 aluminum is dissolved and is made soluble. The most toxic forms of aluminum are considered to be $Al(H_2O)_6^{3+}$, commonly referred to as $Al^{3+}$, and $[AlO_4Al_{12}(OH)_{24}(H2O)_{12}^{7+}]$, commonly referred to as $Al_{13}$, the latter of which forms when the ratio of aluminum to hydronium ($H_30$$H_3O^+$) ions rises above $10^{8.8}$. However, it is currently unknown whether the latter form of aluminum occurs in nature. $Al(OH)_2^+$ and $Al(OH)^{2+}$ are also toxic, but are not considered as toxic as the above forms (See Kochian 1995 for a more detailed discussion).

**Mechanisms of Aluminum Toxicity**

When looking at aluminum toxicity at the level of the entire plant, aluminum slows cellular function (cell signaling, enzyme function, cell division) and induces phosphorous deficiency, causing stunted root growth and stunted plant growth. The stunted root growth results primarily from the decreased rate of mitosis because fewer cells are being produced in a given period of time. The cells that are produced may be deficient in phosphorous, resulting in slower cellular expansion from a decreased metabolic rate. In turn, the plant's root system is not able to take up enough nutrients to support the growth of the rest of the plant, leading to stunted growth.

Aluminum has been long associated with damaging plant root systems (Eisenmenger, 1935), but the actual mechanisms by which aluminum acts are just now being understood. It has been observed that aluminum primarily acts on younger cells in the roots that are actively dividing (Foy, 1978), leading to wider reaching implications for the entire plant. These cells are typically located in the first 5-10mm (Kochian, 1995). Aluminum may have direct effects on the leaves and shoots of plants as well, but the majority of research on this subject has been focused on how aluminum affects the roots, as they are the portion of the plant that is directly exposed to aluminum. As such, the effects that aluminum directly has on non-root of the plant portions (i.e. not caused by the root damage) are largely unknown. However, within the roots themselves aluminum interferes with the normal functioning of the cell in some very serious ways.

Aluminum can profoundly affect the metabolism, and slow the functioning, of plant cells (Kochian et al., 2004). One possible mechanism by which this occurs is through a decrease in mitochondrial function, as indicated by reduced production of adenosine triphosphate (ATP) in the cell (Yamamoto et al., 2002). ATP is vital for many cellular functions. It activates and deactivates proteins through phosphorylation, and it provides the energy that drives unfavorable reactions forward. It is also responsible for organelle movement through the cell via microtubules, among other things (Baskin, 2000). ATP is the driving force behind cell metabolism, and without it the cells could not function properly, if at all.

Despite only comprising 0.2% of the minerals in plants, phosphorous is essential for plants to function. Phosphorous is a primary component of the nucleotides that form DNA strands, the aforementioned ATP molecule, and the phospholipids that form the membranes that surround organelles. Phosphorous also controls enzymatic reactions through common cell-signaling molecules, such as ATP and GTP (guanosine triphosphate) (Kochian, 2000). Without phosphorous, there would be no cell function. The importance of phosphorous to plants is most strongly represented in white lupine's (*Lupinus albus*) response to phosphorous deficiency. White lupine will secrete up to 25% of its total carbon in the form of organic acids in order to take up phosphorous from the soil (Kochian, 2000). Aluminum can lead to phosphorous deficiency in the cell, both by interfering with aluminum uptake, and by interacting with phosphorous in the cell itself. When aluminum reacts with phosphorous outside of the cell, phosphorous is precipitated out of solution, making it unavailable to plants. So while the soil in which the plant is growing may contain a high concentration of phosphorous, it will not be usable by the plants if aluminum concentrations are high. These same types of complexes can occur within plant cells, reducing the availability of phosphorous that is already in the cell (Kochian, 2000).

Aluminum also promotes tubulin assembly into microtubules more strongly than magnesium (the ion that normally aids in microtubule production), and interferes with microtubule depolymerization (Macdonald et al., 1987). The implications that aluminum-produced microtubules has in the cell can be quite wide reaching, as components of the cell will not be transported to their proper place if microtubules are not functioning properly. This has the greatest implications during mitosis, where precise microtubule control is required. During anaphase, improperly placed chromosomes could result in a longer amount of time being required for mitosis and cytokinesis, as the cell plate cannot form until anaphase is complete (Doerner, 2000). Malfunctioning microtubules could even cause the two daughter cells formed during cytokinesis to have a different number of chromosomes, resulting in two improperly functioning cells, or even cell death.

**Aluminum Resistance Mechanisms and their potential uses:**

Mechanisms that confer resistance to aluminum in plants have been the focus of much research for a long time, yet they are just now being understood. Aluminum resistance mechanisms can be divided into two broad categories; mechanisms that work by preventing aluminum from entering the plant, and mechanisms of internal aluminum detoxification. Understanding both of these mechanisms can help scientists enable plants to better survive in aluminum-infused acid soils; specifically through genetic engineering and phytoremediation.

**Exclusion Mechanisms:**

There are a number of defense systems that plants employ to keep aluminum out of their roots. The mostly widely observed of these mechanisms is the release of organic acids (OA), specifically citric, malic, and oxalic acids, from the roots. As OA is exuded from the plant roots it reacts with aluminum in the soil forming a complex that does not easily pass across cell membranes (Kochian et al., 2004). The exudation is usually confined to the first 1-2cm of the root, as this is the area in which aluminum toxicity begins. But the exact location of exudation varies widely by plant species and cultivar (see Kochian 2004 for more details). OA release has been observed in cultivars of wheat (*Triticum aestivum*), maize (*Zea mays*), buckwheat (*Fagopyrum esculentum*), sorghum (*Sorghum bicolor*), and soybean (*Glycine max*), among others (Kochian 2004), and has been shown to increase with increasing concentrations of aluminum (Piñeros et al., 2005).

There are two other exclusion mechanisms that have been observed, although they are not nearly as prevalent as OA release. Piñeros and Kochian (2002) observed that some cultivars of wheat raise apical zone pH by varying uptake and efflux patterns of hydronium. Phosphorous exudation has also been observed in the Atlas 66 cultivar of wheat in response to aluminum stress (Pellet et al., 1996). Phosphorous exudation may seem counter-intuitive, but aluminum resistance is commonly associated with increased phosphorous-use efficiency (Zhu et al., 2002 as cited by Zheng 2005), so some plants can "afford" to exude some of their phosphorous.

**Internal Detoxification Methods:**

Some plants have the opposite response to aluminum, and will take aluminum up in large quantities. Since an effective exclusionary resistance mechanism would prevent aluminum accumulation by a plant, accumulator resistance must be conferred by an internal resistance mechanism (Kochian, 2004). The main mechanism of aluminum resistance in aluminum accumulators is compartmentalization, the transport of aluminum into a location in the cell where it cannot damage the cell. Using confocal microscopy Zheng et al. (2005) showed that buckwheat may detoxify aluminum by storing aluminum-phosphorous complexes in its cell walls. The other prime location for compartmentalization is in the cell's vacuole. Common buckwheat has been shown to detoxify aluminum in this location through an aluminum-oxalic acid complex (Ma et al., 1997).

**Genetic Engineering:**

Genetic engineering has promise for developing crop plants that are resistant to aluminum. Genetic engineering is achieved by inserting a section of DNA containing a desired trait into the DNA of an organism. If the new trait is exhibited, and is not accompanied by any other negative changes, the genetic engineering attempt is considered a success. But before genetic engineering can take place, genes conferring aluminum tolerance must first be identified, isolated, and successfully inserted into plant cells. The first gene isolated and cloned for conferring aluminum resistance through an aluminum-activated malate ion channel, known as *ALMT1*, was recently inserted into *Xenopus* oocytes, rice and cultured tobacco cells (Sasaki et al., 2004). While this finding is definitely intriguing, and a first of its kind for aluminum, the effectiveness of the ion channel in other organisms has yet to be determined, and it is unknown if any undesired side effects will accompany the genetic change. Therefore, other methods of preventing aluminum phytotoxicity need to be considered.

**Phytoremediation**:

Phytoremediation is the practice of using plants to clean up environmental contaminants. In phytoremediation plants can be planted in a medium that has been contaminated by a specific pollutant, at which point the plants remove the contaminant from the medium, or break the contaminant down into a form that is no longer toxic. Plant tissues are then removed from the site of contamination and can be converted to a form that is much easier to dispose of than massive amounts of soil. Phytoremediation is of particular use because it is a more environmentally friendly alternative to other methods of removing contaminants(Chaney, 1997); such methods include removing the contaminated material from the site of contamination, and either putting the contaminated material in a landfill, or decontaminating it through incineration (EPA, 2004). If using plants to remediate soil, plants can be harvested using traditional farming equipment and techniques, thereby reducing the cost and complication of cleaning soil contamination.

The main type of phytoremediation that is applicable to the aluminum threat to agriculture is phytoextraction, the removal of contaminants from soils, which operates under the principle of plants being harvested to complete the removal of contaminants from the soil (Chaney, 1997). Currently, alpine pennycress (*Thlaspi caerulescens*) is showing promise as a phytoextractor of zinc and cadmium, and brakefern (*Pteris vitatta*) is showing promise a phytoextractor of arsenic (Lassat, 2002).

There are a number of ideal characteristics that phytoremediators must have. Plants must be resistant to the contaminant that they are going to be taking up, lest they die before the program can be completed. The plants must also take up a large amount of the target contaminant relative to other plants, and store it in their leaves (Chaney, 1997). If the contaminant is not stored in the leaves or the shoots, the contaminant will have to be removed again the next growing season. It is also important that a plant be able to generate a large amount of biomass because a plant with larger biomass will theoretically be able to take up more of the target contaminant (Becker, 2000).

**Buckwheat as an aluminum phytoremediator:**

Common buckwheat is a prime candidate for an aluminum phytoremediation program. In laboratory research, common buckwheat has exhibited aluminum resistance, defined as exhibiting greater root growth when exposed to aluminum when compared to other plants (Kochian 1995), and has been shown to accumulate the majority of aluminum in its leaves (Ma et al., 1998). Buckwheat also grows very fast, meaning it can generate a large amount of biomass in a short period of time.

The mechanisms of aluminum tolerance in buckwheat are quite complex, as they exhibit both exclusionary resistance mechanisms and internal detoxification mechanisms. Zheng et al. (1998) showed that the exudation of oxalic acid by buckwheat was in response to aluminum stress and not stress from other heavy metals, nor was it in response to phosphorous deficiency. In that same year, Ma et al. (1998) showed that buckwheat also detoxifies aluminum internally through aluminum-oxalic acid complexes in the leaves. In fact, when a cultivar of corn was subjected to treatment with the aluminum-oxalic acid complex purified from buckwheat leaves there was no inhibition of root growth, while the root elongation of plants exposed to only aluminum was half that of the other groups. Further research has shown that aluminum tolerance can also be attributed to superior phosphorous-use efficiency (Zhu et al., 2002 as cited by Zheng 2005).

To test the phytoremediatory abilities of buckwheat, an unnamed cultivar will be grown in solutions containing increasingly greater concentrations of aluminum. Based on the rate at which aluminum is removed from these solutions, a model will be constructed to predict the length of time an aluminum phytoremediation project will take with a given concentration of aluminum present in soil or water.

## Material and Methods:

### Initial Growth:

Common buckwheat (*Fagopyrum esculentum*) seeds were purchased from Johnny's Selected Seeds (Winslow, Maine). Seeds were germinated in the dark at 25$^{o}$C in Petri dishes sealed with Parafilm that contained paper towel saturated with deionized water. On day 3 the tops of the Petri dishes were removed and the seedlings were exposed to light for the first time. On day 10 plants were transferred to 4-in. plastic, thin-walled azalea pots with the bottoms replaced with 2 mm plastic mesh (Figure 1b). The pots contained approximately ¼ in. of perlite to provide support for the plants. The plants' roots were threaded through the 2 mm plastic mesh. There were five plants per pot and sixteen pots total.

The pots were placed in a hydroponic system constructed from a 31.8 L Rubbermaid container with wooden dowel rods extended across it to support the pots (Figure 1a). The plants were grown in aerated 1/5th strength Hoagland's solution with full strength micronutrients (Hoagland and Arnon, 1938). When plants were 17 days old, they were subjected to the following aluminum treatments.

**Aluminum Treatments:**

Pots were randomly divided into four groups of four pots and were treated with 0ppm (control), 100ppm, 200ppm, or 400ppm of aluminum from $Al(NO)_3$. The aluminum treatment was added to 1/5th strength Hoagland's solution that did not contain phosphorous with full strength micronutrients. The solution was then diluted to a volume of 1.00 L. Phosphorous was not included due to potential aluminum-phosphorous interactions. Aluminum treatments were administered in 1.2L Rubbermaid TakeAlong containers covered with Parafilm with a hole for the pot cut out of it (Figure 1b). The purpose of this covering was to reduce water loss through evaporation. Each day the aluminum solutions were adjusted with 0.1M HCl to a pH of 4.0 ± 0.05 (Hanna Instruments pH electrode, attached to a Corning pH/ion analyzer 350). With the exception of the first two days the plants were in a Percival Scientific 101 series growth chamber (Percival Scientific, Perry, IA) with a 16h / 25°C – 8h / 20°C day-night cycle with an average light intensity of 300-350 microeinsteins, as measured by a quantum meter (Apogee Instruments, Logan, UT). Relative humidity was not controlled. On day 24, the plants were harvested and stored as detailed below.

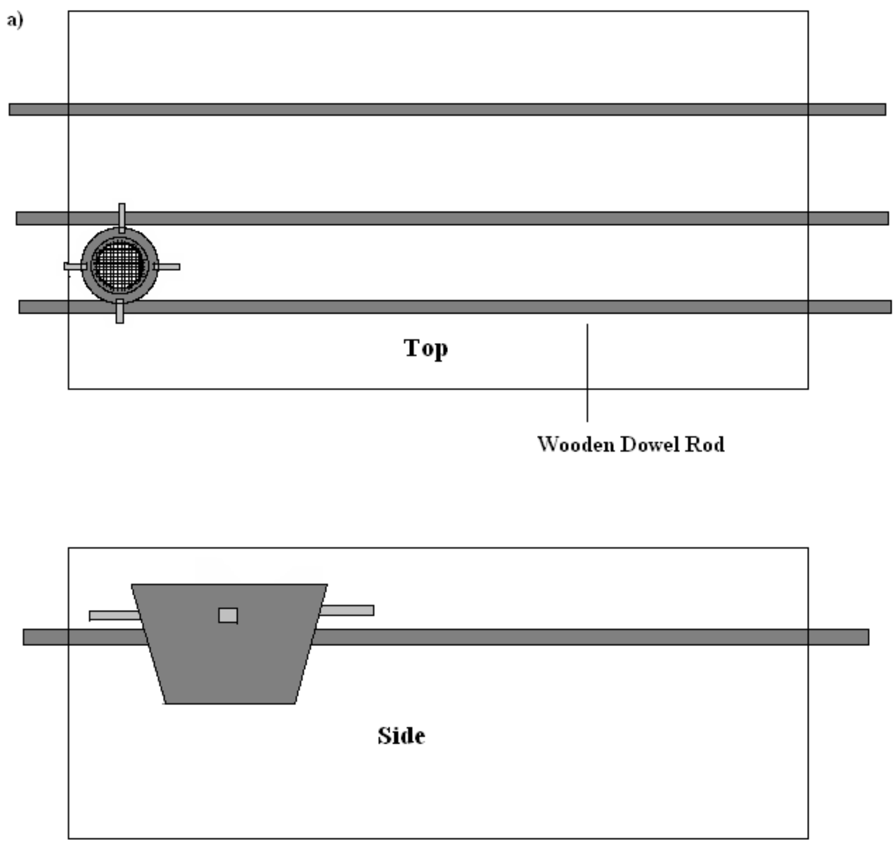
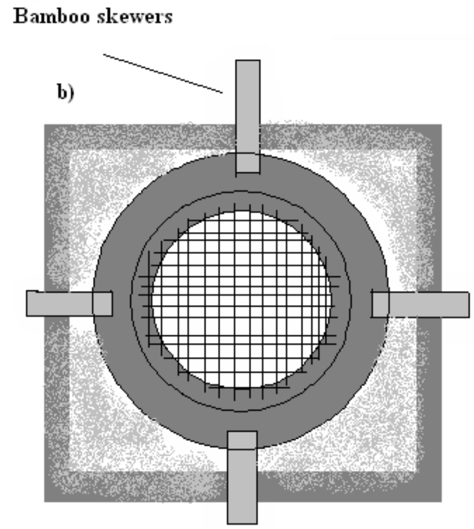



**Figure 1.**

a) Diagram of large Rubbermaid container where initial plant growth took place.

b) Diagram of aluminum treatment container. The gray, speckled area is Parafilm.

Note: Diagrams not to scale




**Sample collection, storage, and analysis**:

Every day of the aluminum treatment, 10.00mL samples of each growth solution were obtained via a glass 10.00mL volumetric pipet. The samples were stored in HCl-rinsed plastic tubes. All samples were preserved with nitric acid and refrigerated until the time of analysis (approximately 3-4 weeks from collection). Harvested plant samples were dried in Pyrex beakers at 95$^{o}$C for 18 hours, after which plant material was transferred to brown envelopes and refrigerated for storage. For logistical reasons, only samples from days 1, 3, 5, and 7 and from groups 1-3 were analyzed. Samples were analyzed spectrophotometrically (Spectronic 20, Bausch and Lomb, Rochester, NY) using the eriochrome cyanine R method (method 3500-Al, Standard Methods 20$^{th}$ Ed), and in duplicates.

**Data and Statistical Analysis**

After samples were analyzed the rate of aluminum uptake by buckwheat was determined by solving for k in the equation $\frac{C_f}{C_i} = \exp(kt)$. $\frac{C_f}{C_i}$ is the fraction of aluminum remaining in solution, k is the decay constant, and t is time. This data was then used to develop a model that can be used to estimate the length of a phytoremediation program using this particular cultivar of buckwheat. Single-factor ANOVA ($\alpha$=0.05) was used to determine if significant differences between data sets were present.

**Results:**

**Plant Observations**

While plant root system size was not quantified, qualitative observations were made (pictured in Figure 2). There was a noticeable difference in the size of the root systems of the plants than were exposed to aluminum, with all of the experimental groups exhibiting smaller root systems than the control group. The shoot portions of the control group plants were also larger than the groups exposed to aluminum.

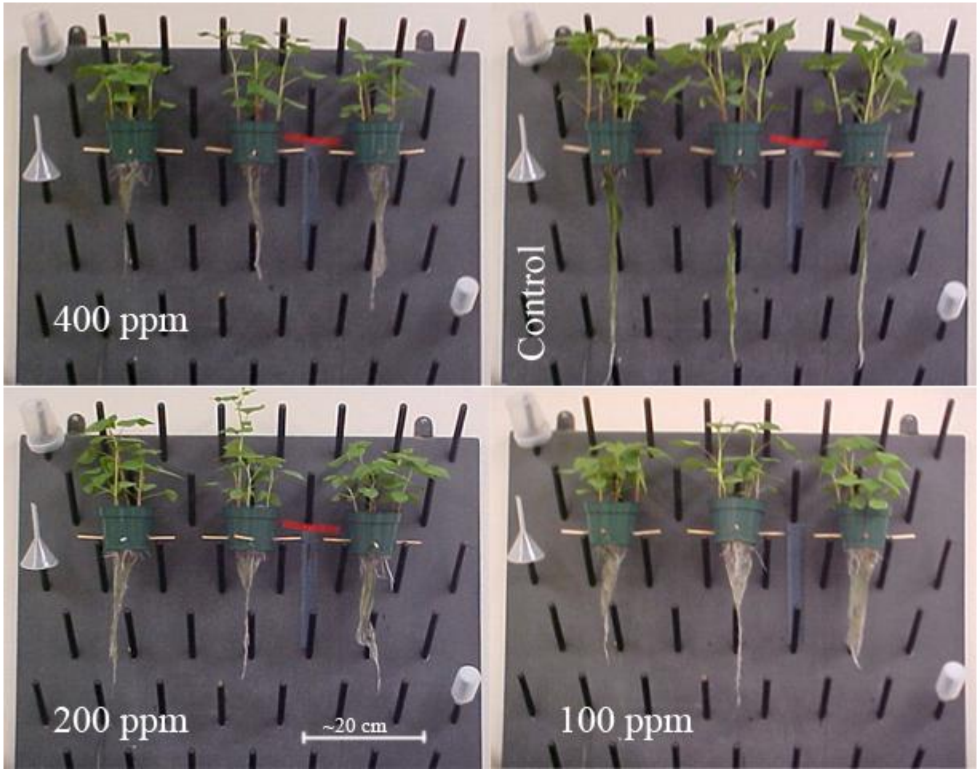



**Figure 2:** 24-day old buckwheat seedlings after 6 of exposure to varying concentrations of aluminum.

**Aluminum uptake trends**

Through the third sample period, the trend of aluminum uptake (as indicated by the percent of aluminum remaining in solution, Figure 3) for the 100ppm and 400ppm groups was very similar, in that both groups experienced a decrease in their initial uptake rates before the rate of uptake increased again (after the fifth sample period), after which the rate of uptake varied dramatically. After the third sampling period, the 100ppm and 200ppm groups had nearly the same rate of uptake. The two groups were nearly offset by the difference in percent aluminum remaining that was observed after sample period 3. Single-factor ANOVA analyses indicated no significant difference between the three groups that comprised each experimental group (data not shown), and significant differences in aluminum removal began developing after the third sample period (Table 1).

**Table 1**: Single-factor ANOVA of the % aluminum remaining in solution after a given sample period. Bold values indicate significant difference (α = 0.05).

| | df | F-Ratio | P-value |
|---|---|---|---|
| SP3-100vs200 | 1 | 2.270 | 0.163 |
| SP3-100vs400 | 1 | 0.224 | 0.646 |
| SP3-200vs400 | 1 | 3.091 | 0.109 |
| **SP5-100vs200** | **1** | **8.083** | **0.017** |
| **SP5-100vs400** | **1** | **8.060** | **0.018** |
| **SP5-200vs400** | **1** | **25.612** | **<0.001** |
| SP7-100vs200 | 1 | 6.836 | 0.026 |
| **SP7-100vs400** | **1** | **63.358** | **<0.001** |
| **SP7-200vs400** | **1** | **11.894** | **0.006** |
| SP3-All | 2 | 1.643 | 0.226 |
| **SP5-All** | **2** | **14.805** | **<0.001** |
| **SP7-All** | **2** | **21.744** | **<0.001** |

**Phytoremediation Model:**

While there was large variability between the three experimental concentrations, the average k-values were proportional to the –ln(initial concentration) ($R^2$ = 0.9991) of aluminum. This relationship allowed the following model to be constructed:

$$t = \frac{\ln\left(\frac{C_f}{C_i}\right)}{\left((-0.058 * -\ln(C_i)) - 0.4014\right)} * \frac{t_{ff}}{12} * [Al(aq)]$$

In this equation t is the time in days, $C_f$ is the final concentration, $C_i$ is the initial concentration, $t_{ff}$ is the average time in months between the final and first frosts, and $[Al_{(aq)}]$ is the soluble fraction of aluminum in the soil. $[Al_{(aq)}]$ can be determined by interpolation on a graph such as that in Figure 4, or through a chemical speciation program such as GEOCHEM-PC.

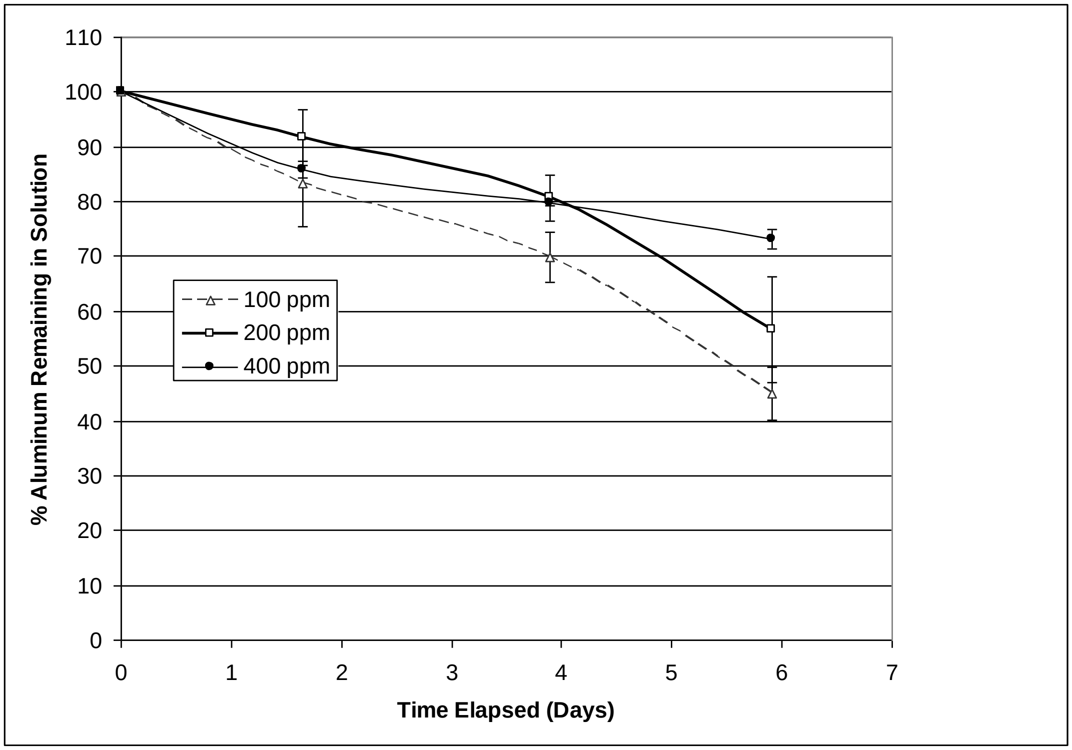



**Figure 3**: % of the original aluminum remaining in treatment solutions at a given time. Points on the graph represent odd-numbered sampling periods. Error bars represent SD (n=3)

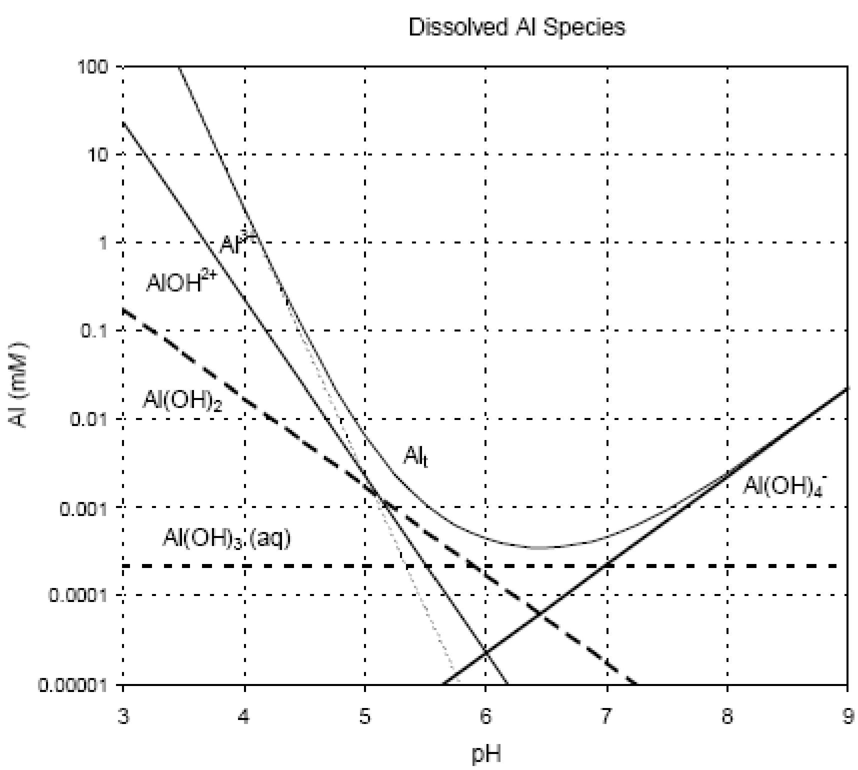



**Figure 4 (Left).** Solubility of aluminum species (and total aluminum, Alt) in relation to pH in a system in equilibrium with microcrystalline gibbsite (0.001 mM =0.027 mg/L). (Source: Environment Canada, 2000).

## Discussion:

### Plant Observations:

At the end of the aluminum treatments, there was a noticeable difference between the root systems of the control group and the groups exposed to aluminum (Figure 3). The root systems of the control plants were considerably longer than those of the other plants, indicating that aluminum toxicity did occur. However, there was no clear indication as to how the three experimental concentrations affected the plants relative to one another. It appears as though the 400 ppm group was affected the most, but given the variability between plants within and across the experimental groups, the differences may be attributed to variations in root systems prior to the aluminum treatments. Without root measurements prior to aluminum treatments, it was impossible to determine if any of the differences in root systems was due to increasing aluminum concentrations.

The shoot portions of the control plants also appear to have more biomass, indicating that reduced root systems will lead to decreased shoot systems. The decreased biomass with root system damage supports the assertions of Kochian, Foy, and others (1995 and 1978, respectively) that aluminum leads to lower agricultural yields.

### Aluminum uptake trends explained

In early research on aluminum phytotoxicity Stoklasa (as cited by Eisenmenger, 1935) observed that high concentrations of aluminum resulted in lower aluminum uptake than more dilute concentrations due to cellular damage. The observations of Stoklasa correlate strongly with what was experienced during this experiment. When comparing the highest and lowest aluminum concentrations, the response was initially very similar, but as the aluminum treatment persisted, the rate of uptake from the 400ppm group was much lower than that of the lowest concentration. Also, the high amount of variability within the 200ppm groups could indicate that 200ppm is the concentration threshold at which aluminum toxicity affects aluminum uptake.

Also, the lower rate of uptake for the 200ppm group (when compared to the 100ppm group) may not be due to root system damage. After the third sampling period, the rate of uptake for both groups was nearly identical, but with the % aluminum remaining being offset by the difference between them at the third sample period. Had aluminum been damaging the plant root system the rate of aluminum uptake would have slowed, as it had with the 400ppm group. Uptake may have been slowing at the end of the sampling period, the difference in % aluminum remaining was getting larger as time progressed, but no conclusions can be drawn without allowing the experiment to go on longer.

**Use of the Model**

The model generated in this experiment is the standard $\frac{C_f}{C_i} = \exp(kt)$ equation with the equation of the k-value vs. –ln(initial concentration) curve (not shown) in place of the k-value. In order to use the model, one would only need to know the initial concentration of aluminum in the soil, and have a goal that needed to be reached. However, there are two other pieces of the model that may be important depending on the target application. They are modifiers that take into account the length of the growing season in which buckwheat can survive, and the fraction of soluble aluminum in the soil.

The growing season piece of the model is very important in determining the length of a phytoremediation program. Since buckwheat is frost sensitive and can only be grown in warm months, the amount of time out of the year that phytoremediation can take place is going to greatly affect the length of a phytoremediation program, and the method in which the program is implemented. The importance of the solubility factor varies depending on what the application is going to be. For agricultural use, the solubility factor is irrelevant. The amount of aluminum that is dissolved in the soil is a good enough indication of how long a phytoremediation program is going to take, provided there are no large pH fluctuations. However, if buckwheat's ability to remove aluminum from soil is going to be used for some purpose outside of phytoremediation, the solubility factor would be very important because it determines whether or not its use is viable.

**Implementation of a phytoremediation program**

Phytoremediation is an eco-friendly method of reducing contaminant concentrations in a medium, be it soil or water, through the use of plants. However, phytoremediation can take a long time depending on the amount of a contaminant in the soil. Therefore proper planning is essential when designing and implementing a phytoremediation program. The importance of proper planning is amplified in poorer regions of the world where farmers cannot afford reduced crop yields, as they receive a small amount of money per unit of crop produced, and may be reluctant to give up any portion of the land they work for a purpose that has no immediate benefit. Following are two possible methods of implementing an aluminum phytoremediation program on agricultural land that would be efficient in removing aluminum from the soil, but the principles are still valid for the phytoremediation of other contaminants on agricultural lands.

The first method is to grow buckwheat in the crop planting "off-season," the time during which a tract of land is not used to grow main crops. Since no major crop would be growing on a tract of land during this point, there would be little loss in revenue for the farmer stemming from a reduction in the area that a crop is grown on. It would be an ideal time to plant a fast-growing and quick aluminum accumulating plant such as buckwheat. However, such an implementation would require extra labor in the form of additional seed sowing and crop harvest. It could also lead to less nutrients being available in the soil, due to the existing plants not being tilled under before the next crop is to be planted, thereby resulting in increased fertilizer use by the farmer. Also, buckwheat is highly sensitive to frost. In warm portions of the world this is not an issue, but in the temperate regions of the world cold temperatures will limit the time at which buckwheat could be planted; perhaps to the point of delaying crop plant growth if a phytoremediation program is to be implemented.

A potentially more versatile alternative is to implement a phytoremediation program in the form of crop rotation. Crop rotation is often employed as an agricultural method that will vary the nutrient demands placed on the soil, resulting in agricultural land that may be able to replenish nutrients between growing seasons. A crop rotation phytoremediation method is useful in that it will reduce aluminum concentrations in the soil while allowing farmers to continue growing the crops that they normally do. Carrying out a phytoremediation program via crop rotation would only require a marginal increase in the labor required to carry out the program, as the farmers that would be implementing such a program would already be familiar with the process of sowing and harvesting different crops.

**Prospects for Aluminum Phytoremediation using Buckwheat**

While phytoremediation is a very promising biotechnology and has proven its worth in the past, a serious evaluation of using phytoremediation to remove aluminum from crop soils is required. The majority of phytotoxicity associated with aluminum is going to be as a result of persistent aluminum sources. So even if aluminum is removed from agricultural land, there is still going to be aluminum outside of the rhizosphere that can leach back into the rhizosphere. Given the low concentrations of aluminum required to induce toxicity, it is possible that aluminum concentrations could reach phytotoxic levels between the time buckwheat is harvested and the time the next crop begins to grow. This constant return of aluminum to the rhizosphere would make a phytoremediation program very difficult to justify when low initial concentrations of aluminum are in the soil.

However, there are going to be instances where a tract of land is going to contain a high concentration of aluminum. In a case such as this, phytoremediation would be a very viable method of reducing aluminum concentrations in the soil, because even with aluminum leaching back into the rhizosphere, there would usually be some reduction in aluminum concentrations after a given period of time. At the end of the program, there would be the problem of trying to remediate soils with low concentrations of aluminum, but the concentrations of aluminum would be easier for plants to cope with.

As an extension of implementing a phytoremediation program on soils containing high concentrations of aluminum is the practice of phytomining. Phytomining is an extension of phytoremediation, in that plants are used to take up a specific metal from the soil, and are harvested after a certain amount of time. But in phytomining, the plant material is ashed with the express purpose of refining it to obtain the target metal. Phytomining nickel and cobalt is currently being research (Becker, 2000), but it could just as easily be applied to aluminum. Refining aluminum metal from plants could be easier than refining aluminum metal, which requires a huge amount of energy, but as of now there has been no effort to investigate this. Therefore, the actual practicality of aluminum phytomining is only speculative at this time.

**Sources of Variance**

*The Eriochrome Cyanine R method:*

The eriochrome cyanine R method used to analyze samples in this experiment is not the most precise method available. There was an average percent relative standard deviation (% RSD) of over 5.5% between replicate measures of the same sample during the course of this study. Large % RSDs are commonplace in this method; when a sample of aluminum containing only aluminum and distilled water was analyzed by 27 laboratories, there was a relative standard deviation of 30% (Standard Methods) and a standard error of 1.7%. The high standard deviation and low standard error indicate that while this method is not very precise it is accurate, so long as no interferences are present. Interferences from the formation of orthophosphates, a possibility in this experiment, have been associated with an 8.9% standard error (Standard Methods). In this experiment, interferences were not accounted for during sample analysis.

*Assumptions during Analysis*

There were a number of assumptions made during data analysis that could have affected the determined aluminum values. It was assumed that pH adjustments did not dilute the nutrient solution that the plants were growing in, pH remained consistent throughout the experiment, and nutrient solution was lost only to sampling.

The dilution during pH adjustment assumption is relatively minor. After the initial pH adjustment no more than 2mL of acid was required to reach the targeted pH range. At the end of the experiment, the error from this assumption would be positive and on the order of 1-2% for the lowest concentrations, and lesser still for the highest concentrations.

The pH stability assumption has potentially larger implications. The amount of dissolved aluminum in solution is pH dependant, with solubility increasing as pH decreases. Therefore, any large change in the pH of an experimental solution could lead to aluminum precipitating out of solution making less aluminum available for the plants to take up, and less aluminum in solution during sampling. Both of these cases could lead to an inflated rate of aluminum uptake that would lead to an underestimation of the length of an aluminum phytoremediation program. However, after the initial pH adjustments, a pH greater than 4.5 was never observed.

The assumption that no growth solution was lost to evapotranspiration is the most likely source of error from all of these assumptions. Plants will use water and water will evaporate. There is no possible way to prevent evapotranspiration that would not result in the death of the plants. But attempts, in the form of covering containers that were used for the aluminum treatments with Parafilm and trying to keep the relative humidity in the growth chamber high, were made to limit water loss. There was no attempt to quantify the effectiveness of the evapotranspiration measures. The main problem with this assumption is that it underestimates the effectiveness of the plants in removing aluminum from a solution because there is an unquantified dilution factor inherent in any calculation that occurs

### Future Directions

Due to the prevalence of aluminum in the environment, aluminum phytoremediation should still be investigated, but in a lower concentration range than what was used in this study, and with a persistent aluminum source. The most effective phytoremediator of aluminum also needs to be determined. Also, as of now, phytoremediation has largely been studied on a case-study basis, but a more concerted effort needs to be made to model phytoremediation. Successful modeling of phytoremediation will give governments and private entities a greater level of confidence in phytoremediation projects by removing some of the guesswork involved with its implementation. Finally, aluminum phytomining, and the refinement of aluminum from plants, should also be researched to determine if phytomining is a viable method of obtaining aluminum metal for industrial processes.

## Conclusion:

Aluminum phytoremediation was able to be modeled, but the accuracy of the model has yet to be tested in field experiments. Given the persistent nature of aluminum in the environment, it is unknown if aluminum phytoremediation will keep aluminum levels in the soil below phytotoxic levels. Regardless, phytoremediation is a promising method of preventing aluminum phytotoxicity and it needs to be investigated further.

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