

## **Abstract**

Soil pollution is becoming a greater threat to the environment, especially as populations and industrial economies expand. There are studies to suggest that several plant species may be useful in reducing the migration of such pollution further down the soil column or perhaps even into the ground water. Given its widespread natural habitat, dandelions (*Taraxacum officinale*) are an appealing prospect for such soil remediation. The bentgrass family (genus *Agrostis*) is also an attractive candidate for study, as some species of this group have also been studied for metal uptake. Uptake of metals by a plant is when the plant takes the metals out of the soil and somehow stabilizes it away from the rest of the soil matrix.

This experiment will examine the ability of dandelion and a native-Alaskan strain of bentgrass to uptake metal contaminants from its growth medium, which is most often soil. For purposes of the control of extraneous variables, the soil will be replaced by a hydroponic nutrient solution and glass wool for stabilization.

Research of this nature is necessary for the Alaskan landscape: since this state has a colder climate than the other areas in which phytoremediation has been studied, cold-tolerant plants have rarely been studied for this ability. In addition, invasive species need to be excluded from this type of study in Alaska, as the preservation of wild flora is important to our state ecosystem. If a native species of plant with phytoremediation abilities can be found, this plant can be planted along the roadsides of Alaska to lessen the detrimental impact of pollution from vehicular traffic. Depending on the approximate location of the isolated metals, it may also be possible to harvest these plants and process them to extract the metals.

## **Purpose/Aim**

- 1)** To see if some Alaskan plants can phytoremediate metal-polluted soils as simulated by metals added to a hydroponic growth medium.
- 2)** To see if such plants can be more effective in metals uptake with the application of the chelator ethylenediaminetetraacetic acid (EDTA).

## Introduction

With an ever-increasing population and expanding industrial economies, there is a corresponding increase in environmental pollution. Contaminants in roadside soil can easily be found in many areas of the world, and these contaminants, some of which are metals, can be identified and quantified.

Studies have been performed on roadside soils and dust to analyze anthropogenic sources of metal contamination (Jaradat & Momani, 1999; Nouri & Naghipour, 2002; Ayodele & Oluyomi, 2011; Zhu, Bian & Li, 2008; Duong & Lee, 2011; Cervantes, 2005; Amusan, Bada & Salami, 2003). Other sources of other metals have been attributed to vehicles and industry, such as copper, iron, and manganese from vehicle break pad use and general engine wear.

Some metals, such as lead and manganese, are not biologically useful. Even if metal has biological functions, they can exist in too high of concentrations so as to be toxic, such as the case with iron, zinc and copper. This toxicity leaves plants struggling to live in such polluted soils. The contamination can also leach through the soil strata and eventually into the local water supply, a resource essential to every land-dwelling organism.

To decrease the detrimental impact of this pollution, some changes to the environment can be made. Phytoremediation, or the rehabilitation of soils by use of plants, can be easily utilized: by planting certain species of plants, contaminants in the soil can be isolated by various means in different parts of the plant. Phytoremediation can be more specifically categorized any of the following:

- phytoextraction, the concentration of contaminants in the plant and subsequent removal of the plant;
- phytodegradation, using the plants and associated organisms to degrade the contaminants into nontoxic substances;
- rhizofiltration, the root system absorbing and isolating the contaminants;
- phytostabilization, the use of plants to limit the bioavailability of contaminants; and
- phytovolatilization, where the plant removes contaminants from the soil and volatilizes them into the air (Salt, Smith & Raskin, 1998).

Many plants have been identified as candidates for each of the aforementioned remediation methods.

Chelators like ethylenediaminetetraacetic acid (EDTA) have proven to be helpful in making metals more bioavailable, allowing more of the contaminants to be isolated from the soil (Cooper, Sims, Cunningham, Huang, & Berti, 1999; Epelde, Hernandez-Allica, Becerril, Blanco, & Garbisu, 2008). EDTA can have negative consequences though, as its application to plants can also lead to sickly or underdeveloped plants (Alkorta, Hernandez-Allica, Becerril, Amezcaga, Onaindia & Garbisu, 2004; Thayalakumaran, Robinson, Vogeler, Scotter, Clothier & Percival, 2003).

Several plants have been studied for this ability, including several grasses like bentgrass (genus *Agrostis*) (Humphreys & Nicholls, 1984; Wu & Antonovics, 1975). One particular species of bentgrass that is native to the state of Alaska, rough bentgrass (*Agrostis scabra*) is an option for phytoremediation in an area with a grazing wildlife population. If grazing animals eat plants that are used for phytoremediation, they too will be accumulating the toxic metals. Rough bentgrass has low palatability and low nutritional value, so it is less likely to be eaten by grazing animals (Matthews, 1992).

The ubiquity of the common dandelion makes it another candidate for phytoremediation. Dandelions are tolerant of many adverse growing conditions, including frosts, overcrowding, low nutrient concentration, and relatively dry soil. This tolerance is ideal for roadside planting, which is an area often overlooked for maintenance. One dandelion study conducted by Keane, et al, gathered dandelion leaves from rural, urban, suburban, and industrial areas of Ohio, Indiana, Colorado, Illinois, and Kentucky (2001). Results from this and other studies have been inconclusive, but there is some question as to what part of the experiment is producing the most uncertainty.

This experiment will examine the ability of dandelion and a native-Alaskan strain of bentgrass to uptake metal contaminants from its growth medium, which is most often soil. For purposes of the control of extraneous variables, the soil will be replaced by a hydroponic nutrient solution and glass wool for stabilization. Similar phytoremediation studies have looked into the abilities of plants in the field, where many other factors can confound results. This experiment will control for these possible interferences, as there will be no soil and highly controlled inputs into the experimental system.

Since the cold climate and relatively wild landscape makes Alaska unique, research must be done to study the effects of pollution, as it may have different consequences for the distinctive natural landscape. Traffic pollution is a growing problem anywhere there is an expanding population, so natural and locally-orientated ways of controlling this pollution is essential for the preservation of the Alaskan environment. Phytoremediation can be another tool for preserving the environment.

## **Experimental Design**

### *Contaminant Evaluation*

A sample of roadside soil will be taken from a major Alaskan highway and analyzed by inductively-coupled plasma – mass spectrometry (ICP-MS) for metal content (as per EPA method 200.8). The content and concentration of these metals will be the basis of the induced pollution in the hydroponic growth medium.

### *Samples*

Seeds will be purchased from online sources to guarantee their genetic identity. These seeds, both dandelion and rough bentgrass, will be dried and surface sterilized, then set up in a sterilized wet environment to promote germination.

When adequately sprouted, similarly-sized seedlings will be placed in a glass wool matrix for stabilization in a glass growing tube containing nutrient-enhanced water. Six replicates of each test will be performed. To maintain sterility of the environment, everything going into the tube will be autoclaved, the seedlings will be planted while in a sterile-environment hood, and the tubes capped with a gas-permeable membrane to keep any other organisms out of the experimental samples.

Growth conditions will consist of consistent ambient laboratory temperature, fluorescent lights on a timer programmed to turn on for 16 hours of the day, and a buffered nutrient solution of macronutrients, micronutrients, and vitamins with a pH appropriate for the chosen plant's ideal growing conditions.

One third of the samples will be allowed to grow in pure water with nutrients and the described growing conditions. Another third of the samples will receive a metals mix that simulates the observed metal pollution of the soil, all with concentrations based on the roadside soil analysis. The final third of the samples will have the metal mix as well as EDTA, a chelator that has been found to increase the bioavailability of metals in a soil matrix. According to one article, 0.265g EDTA per liter of hydroponic solution is an approximation of a 0.1g/kg application to soil (January, Cutright, Van Keulen & Wei, 2008).

An additional set of tubes will be set aside, prepared in the same ways as the aforementioned samples, only without the plant. This will serve as a baseline understanding of what the metals will do if left in the growth matrix without any additional intervention.

When the sample plants have sufficiently grown, they will be harvested. The charts below show how each set of plant samples will be divided amongst the tests:

To grow:

	Nothing	Metals	Metals + EDTA
3x	Water only	Water only	Water only
12x	Plant	Plant	Plant

Table 1 - Sample distribution during plant growth

To harvest:

	Nothing	Metals	Metals + EDTA
3x	Water	Water	Water
6x	whole plant water	whole plant water	whole plant water
6x	Leaves/stem Roots Water	Leaves/stem Roots Water	Leaves/stem Roots Water

Table 2 - Sample distribution of digestions and analysis

### *Chemical Analysis*

Half of the plant samples will be individually homogenized and digested, then this digest and the water each of those plants grew in will be analyzed for metal content. The remaining half of the plants will be partitioned, separating the roots from the upper portion of the plants to determine the approximate location of the isolated metals.

To prepare the samples for analysis, biological samples will be digested in nitric acid and hydrogen peroxide as per EPA method 3050B and diluted. A seven-level calibration set of standards will be prepared and run on the ICP-MS along with the digested & diluted samples. The ICP-MS will be operated according to the EPA method 200.8.

Analysis will be conducted on the concentrations of metals. The metals will be selected based on the observations from the roadside soil sample. The sample distribution of digestions and analysis is displayed in Table 2. The first set of samples will consist of three replicates of only the growth water with no additional inputs; three samples of the growth water with the metals mixture; and three samples of the growth water with the metals and EDTA. The second set will be six samples of the whole plant from each type of water. The final set of samples will be similar to the second set, though the plants will be additionally separated into two samples each, the upper portion of the plant and its roots.

### *Statistical Analysis*

Each sample of this experiment will be prepared and analyzed in six replications. A seven-level linear calibration curve will be constructed from analysis of known standards and experimental data will be correlated with this calibration curve. Basic descriptive statistics and variance analysis will be utilized to compile the results of this experiment.

## **Anticipated Results**

When presented with a growth medium spiked with heavy and nonessential metals, many plants are able to remove these contaminants from the growth matrix and isolate them in nontoxic ways. Dandelion and bentgrass are each expected to follow this trend: there is the anticipation that the metals uptake will only be partial but statistically significant compared to the plants grown without metals. Additionally, EDTA is expected to increase the proportional amount of metals removed from the growth matrix with relation to the plant mass, though the application of EDTA is expected to stunt the growth of the plants. Recommendations for phytoremediation may be presented to the State of Alaska depending on the results of this experiment.

**Budget**

Lights (\$13 each) & bulbs (\$7 per pair), 12 set-ups:	\$240
Racks: 3x 72" wire racks, \$70 each	\$210
Power regulation for lights:	
Timers, 3x \$15 each	\$45
Power strip, 3x \$10 each	\$30
Plant nutrients, \$1/L, 5 liters; shipping	\$10
Seeds (dandelion & rough bentgrass); shipping	\$10
Deactivated glass wool	\$250
Glass deactivation:	
Dimethyldichlorosilane	\$50
Chloroform (2L)	\$100
2L volumetric flask	\$360
Metal salts	\$800
Digestion:	
Nitric Acid (1.5L)	\$150
Hydrogen Peroxide 30% (1L)	\$225
PE vials:	
50mL	\$250
15mL	\$200
10mL disposable syringes	(200 qty) \$220
0.45 micron syringe filters	(200 qty) \$300
ICP-MS:	
Argon, \$125/ tank, 4 tanks	\$500
Maintenance	\$400
Miscellaneous lab tools	\$650
TOTAL: \$5000	

**Budget Rationale**

Lights, racks, timers: This experiment involves growing plants, so the most secure and consistent growing conditions can be provided by growing in a laboratory. Since the lab has no constant source of natural light, fluorescent lights will be used to simulate the sunshine. Plants also need regular day and night cycles, so the lights need to be on timers to simulate these day/night cycles at regular time intervals. Three setups are needed for growing both types of plants.

Nutrients, glass, glass deactivator: Without soil, growing plants need some other source of nutrients and vitamins, which can be provided by nutrient additives to their water source. Glass wool will stabilize the seedling in the water, and it needs to be deactivated so that the metals will not adhere to the glass. Dimethyldichlorosilane in chloroform (mixed to a specific ratio in a 2L volumetric flask) will be used to deactivate the surface of the glass culture tubes.

Metals: This experiment focuses on metal analysis, so therefore a definite source of metals is needed for spiking the water & nutrient source. These metals need to be of the highest purity possible to remove the chances of contamination from extraneous metals.

Digestion: Following EPA method 3050B, nitric acid and hydrogen peroxide will be used to digest the samples individually in PE vials. These samples will then need to be filtered through 0.45 micron filters. Each sample will need one 50mL vial and one 15mL vial.

ICP-MS: Running 216 samples plus calibration curve & periodic calibration checks, there will be a total of 245 samples to run. With about 65 samples per tank of argon, four tanks of argon will be needed for this experiment. Maintenance fees are necessary for the upkeep and proper functioning of the ICP-MS machine.

Miscellaneous lab tools include nitrile gloves and personal protective equipment; forceps for use with the glass-deactivating agents; non-metallic tools for sample preparation, such as plant separation and homogenization; and a water filtration cartridge for the deionized water filter.



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## **Timeline**

October 2011: Gather supplies, germinate seeds

November 2011 – January 2012: Plant seeds, monitor growth

February 2012: Prep samples for ICP-MS, run samples on ICP-MS

March 2012: Analyze data

April 2012: Present results at Undergraduate Research and Discovery Symposium

May 2012: Submit final expenditure report; submit final written report