Uptake of Thallium from Artificially Contaminated Soil into Brassica oleracea acephala L.

Morgan Skalsky
Carroll College, Helena, MT

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Uptake of Thallium from Artificially Contaminated Soil into
Brassica oleracea acephala L.

Morgan Skalsky
Carroll College
2007 Honors Thesis
This thesis for honors recognition has been approved for the Department of Natural Sciences by:

Dr. Kyle Strode, Thesis Director

Dr. Marilyn Schendel, Reader

Dr. Dawn Bregel, Reader

Date
Acknowledgments:

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Abstract:

A method to monitor inductively coupled plasma atomic emission (ICP-AE) was used for analysis of available thallium in the soil and hyperaccumulated thallium in three kale cultivars (Winterbor, Redbor, and Reflex). The soils were artificially contaminated in increments ranging from 0-300 ppm. Half of the 150 pots (with 1-2 plants per pot) were also spiked with potassium to investigate the relationship between thallium and potassium. There was no significant competitive inhibition between potassium and thallium for the two cultivars, Winterbor and Reflex. At high levels of available thallium, there appeared to be competitive inhibition for Redbor. I also found that Redbor, a cultivar not previously studied, is a moderate hyperaccumulator of thallium and that thallium seems to be toxic when the plant reaches concentrations between 40 and 120 ppm.
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Introduction:

Thallium is not an element typically found in large quantities in most soils. When it is found in substantial amounts the cause is usually anthropogenic. One common way that this happens is through residual mining contamination. There are numerous no-responsible-party abandoned mine sites on public lands in western Montana.¹ Water with elevated levels of thallium was used for center post irrigation on private land near the Kendall Mine outside of Lewistown, Montana.² The affected soil is thought to have correspondingly high levels of thallium, and portions of the downstream water used by ranchers in the Lewistown area have also shown elevated levels of thallium.

Thallium is known to be toxic to humans and other living organisms.³ Some of its first uses were as a treatment for syphilis, gonorrhea, tuberculosis and ringworm.⁴ Another domestic use was as a poison for rats, prairie dogs and other unwanted pests.⁴ The use of thallium for these purposes was discontinued in the mid 1960’s following reports of unintentional poisonings and death of humans was reported.⁴

The fact that thallium is so toxic makes it important to develop efficient methods for remediating the contaminated ground water and soil. Candytuft, Iberis intermedia (a mustard family-plant) was one of the first plants to be shown to be a thallium hyperaccumulator.⁵ Candytuft has also proven effective by the U.S. EPA for the purposes of phytoremediation and phytomining.⁶ Moreover, Kurz et al.⁷ showed that there is a lack of information about the differences in thallium uptake between cultivars of the same species, specifically kale. The Kurz et al.⁷ study also showed vast differences in hyperaccumulation of cultivars.
The actual amount of thallium in the soil that is available to the plant (soil-available) has not been tested. Thallium tends to bind to the organic matter of soil and therefore the amount of thallium available to the plant is less than the total thallium concentrations. “The solubility of thallous compounds is relatively high so that monovalent thallium is readily transported through aqueous routes into the environment. Tl can be transferred from soils to crops readily and accrues in food crops.”

Thallium is known to have about the same hydrated radius as potassium. Therefore, I tested the hypothesis that potassium inhibits the uptake of thallium through competitive inhibition and studied the relationship between soil-available thallium and plant uptake of thallium in three different cultivars of kale. I conducted a pot experiment with artificially contaminated soil in order to study my hypotheses. We had hoped to obtain soil from the Kendall Mine site, but were unable to gain access to the land. Two of the kale cultivars that Kurz et al. studied, Winterbor and Reflex, were used as controls and a kale cultivar that had never been studied, Redbor, was used as my test subject.

**Materials and Methods:**

**Soil Collection:**

Soil known to have low potassium concentrations was collected in June, 2005 on N. Montana Ave. in Helena. Eight, five gallon buckets of soil were taken from the top two inches of the surface.
Treatments:

Treatments included five different Tl concentrations in both low and high (artificially treated) potassium soil. Thallium increments were 0, 10, 30, 100, and 300 ppm. The original soil was analyzed by ICP-AE and had about 280 ppm of potassium. High potassium treatments had a concentration 10 times that of low potassium, therefore high potassium treatments had approximately 2,800 ppm potassium.

Soil Preparation:

The collected soil was dried for three days and sieved using #10 sieves and electric shakers. A ceramic aerating agent (Shultz Clay Soil Conditioner) was then added at 500 mL for every 10 gallons. A non-potassium fertilizer (Scotts Natural Bone Meal 6-12-0) was also added at 100 mL for every 10 gallons. Each 10 gallon portion was then mixed in a cement mixer for 1 minute, to ensure homogeneity.

For ease of mixing, soil was prepared in 3 kg portions. Each treatment had a total of 9 kg of soil. Each 3 kg portion was mixed with either no potassium or 19 g of a potassium salt and the respective amount of a thallium salt diluted in deionized water to 500 mL. Each portion was added to the two other 3 kg portions to make enough soil for the treatment. The portions were mixed by hand to ensure homogeneity. These treated soils were allowed to dry for 24 hrs.

Seed Germination:

Seeds were started in early June, 2005. Three types of kale were chosen: Winterbor, Reflex, and Redbor. Medium sized Petri dishes were used along with ashless filter paper to keep the dishes moist. Each dish contained 10-15 seeds with filter paper both above and below them. The paper was moistened with deionized water and kept
moist for the trial period in which the seeds germinated. The Reflex seeds germinated in about four days while the other two types took about seven days.

Planting:

The germinated seeds were planted one week later. A total of 150 pots were used and, three to four plants were planted in each pot. Plants were watered every 2-3 days depending on how dry the top soil appeared. Some pots had white crystals forming on the tops of the soils, which were later determined by ICP-AE analysis to be potassium crystals of a potassium salt. The plants with higher T1 levels did not grow as well as the plants with no or low T1 and therefore some had to be replanted. One seed was placed in each hole and covered with soil. Each pot was replanted so that there were at least three plants growing.

Soil Analysis:

Soil analysis was done using a modified 1M ammonium acetate extraction. This was made by diluting 77.1 g of ammonium acetate in 900 mL of deionized water. The pH was adjusted to pH 7 by addition of glacial acetic acid. The samples were allowed to dry in a 95°C drying oven for two hours. One gram portions of soil were measured out into 25 mL Erlenmeyer flasks. Five milliliters of ammonium acetate extracting solution were added to each flask. The samples were then filtered and analyzed using ICP-AE (Perkin Elmer Optical Emission Spectrometer Optima 2000DV).

Plant Analysis:

All of the plants were harvested during the first week of August, 2005. They were then allowed to dry in a 175°C drying oven for 48 hours and were broken up or ground by hand in order to weigh them directly into the acid washed 10 mL volumetric
flasks. Samples were weighed accurately using analytical balances. The samples were, at most, 0.250 g. All samples were used regardless of weight. Plant analyses were tested using different plant amounts, concentrations of nitric acid, and temperatures. The best digestion used 0.250 g of dried plant matter and 5.0 mL of 16 M nitric acid at about 125°C. The 5 mL of nitric acid was added 1 mL at a time. Samples were swirled and left to digest over night. One mL of hydrogen peroxide was added to each sample a drop at a time to complete the digestion. No plant matter remained. Samples were then diluted to 10 mL with deionized water and inverted 20 times to mix. The digested portions were stored in plastic bottles until they were analyzed by ICP-AE.

Standard Preparation:

The aforementioned plant analysis was done to prepare the standards used in the ICP-AE analysis, but quantities were multiplied by 100. Twenty-five grams of dried, ground kale from the Real Food Store in Helena, MT was used. This was digested using 500 mL of concentrated trace metal grade nitric acid. The solution was left to digest overnight. The next day 15 mL of 50% by weight hydrogen peroxide was added very slowly to complete the digestion. The digest was then heated for another 24 hours. The next day another 15 mL of the hydrogen peroxide were added and the digest was heated and stirred overnight. A typical aliquot of digested plant material before dilution was about 5.25 g, so that amount was weighed into five different 10 mL flasks. Then the required amount of Tl was added to each flask to make 0, 10, 30, 100, and 300 ppm standards for ICP-AE analysis of the experimental kale plants.
Results:

The first objective of this research was to study the relative hyperaccumulation ability of Redbor, a previously unstudied cultivar of kale. Redbor, when compared to Winterbor and Reflex, appears to be an intermediate hyperaccumulator at low thallium treatments and a high hyperaccumulator at high thallium treatments in soil that is high in potassium (Fig. 1). However, the elevated thallium hyperaccumulation at high thallium treatments is not statistically significant. It also appears to be an intermediate hyperaccumulator at all of the thallium levels studied in low potassium soil (Fig. 2).

Figure 1. Comparison of Reflex, Winterbor, and Redbor plants grown in low potassium soils.
The second objective of this study was to examine the relationship between the uptake of potassium vs. the uptake of thallium to determine if competitive inhibition exists between these two similar elements. Neither Reflex nor Winterbor show significant inhibition (Figs. 3 and 5) while Redbor appears to exhibit competitive inhibition at high levels of available thallium (Fig. 4), however this is not statistically significant.
Figure 3. Comparison of thallium levels in Reflex plants grown in both high and low potassium soils.

Figure 4. Comparison of thallium levels for Redbor plants grown in both high and low potassium soils.
The third objective of this study was to examine the relationship between hyperaccumulation and soil available thallium. Table 1 illustrates the experimental design that was used and the relationship between the amount of thallium added to the treatments and the amount that was actually available for plant uptake. Only one plant at the highest thallium treatment survived, implying a toxicity level somewhere between the two highest treatments. Table 2 shows hyperaccumulation values that document the ratio of thallium taken up by the plants and the thallium that was available in the soil. For example, Winterbor in a 10 ppm thallium treatment and at high potassium levels accumulates ~140 times the amount of thallium that is found in the soil.
Table 1. Experimental Design and Plant Survival

<table>
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<th>Treatment (ppm)</th>
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<th>10</th>
<th>30</th>
<th>100</th>
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<tr>
<td>Soil-available Tl (ppm)</td>
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<td>7.1</td>
<td>16.6</td>
<td>41.4</td>
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<td>Redbor</td>
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<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Low K</td>
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<td>4</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>High K</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Winterbor</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
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<td>2</td>
<td>0</td>
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<tr>
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Table 2. Hyperaccumulation Factors

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<th>100</th>
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<tr>
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<td>65.96</td>
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Discussion:

Both kale \(^7\) and candytuft \(^3\) have been identified as thallium hyperaccumulators and exhibit the ability to uptake large amounts of thallium in both nutrient solution and pot experiments. \(^7\) Data accumulated in the present study support this observation and extend our understanding by testing a previously unstudied kale cultivar, Redbor. Along with Kurz et al. \(^7\) I found Winterbor to be a high hyperaccumulator and Reflex to be a low hyperaccumulator. Hyperaccumulation experiments with Kale have also been done using a three compartment rhizobox \(^9,10\), yielding similar qualitative results with respect to Winterbor.

Hyperaccumulation factors using soil-available thallium were also obtained. This is important because it gives us a clearer picture of just how much thallium can be taken
up by kale. This could also be helpful in reclamation situations where a soil could be pre-treated with different fertilizers in order to decrease the amount of thallium that is organically bound, thus increasing the total thallium that can be removed from the soil.

Kale is a potentially useful plant in both phytoremediation and phytomining,\(^9,10\) therefore Redbor was tested in the present study in hopes of finding an even more aggressive thallium hyperaccumulator. Phytoremediation studies have historically been conducted using *Iberis intermedia* (candytuft).\(^5\) The use of kale is relatively new to hyperaccumulation studies, but has shown promising results. Phytoremediation and phytomining are quickly gaining popularity. Phytomining is seen as a cost-effective, environmentally-friendly, alternative technology\(^11\) for reclamation of contaminated lands, both public and private. The development of effective phytoremediation techniques could play a major role in the clean up of the over 8,000 no-responsible-party, abandoned mine sites located in western Montana.\(^1\) The cost of phytoremediation could be off set by selling the extracted thallium, with the metal recently having a world market price of about $300 USD/kg.\(^3\)

Kale is known to be naturally rich in potassium.\(^12\) The similarities between thallium and potassium led me to believe that competitive inhibition may be present. This would have led to the testing of more potassium rich plants in order to find the best thallium hyperaccumulator; however the results do not appear to show any competitive inhibition between the two elements. This implies that they are taken up by two completely separate mechanisms. The next step in finding the best thallium hyperaccumulator may be to conduct mechanistic studies on several different species in order to gain a better understanding of exactly how thallium is being hyperaccumulated.
REFERENCES


