Phytoremediation of Arsenic and Lead Using *Brassica rapa*

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BRIEF. An analysis of the ability of *Brassica rapa* to remove arsenic and lead from contaminated soil.

ABSTRACT. Polluted soil poses serious health risks to wildlife and humans particularly due to dust inhalation. A method called phytoremediation can be used to absorb contamination, such as heavy metals, from the soil. This consolidates the contamination for disposal and establishes growth on soils to prevent erosion and more widespread contamination. *Brassica rapa* is a common field mustard, which has a rapid lifecycle and can be grown under a variety of conditions. This plant could be a practical, wide-ranging tool for soil cleanup. The purpose of this study was to determine *Brassica rapa*'s ability to absorb arsenic and lead from soil. Plants were grown in soil containing three levels of both arsenic and lead with target pH values of 6, 7, and 8. After contamination, the highest levels of arsenic prevented the plants from growing. No other contamination had any effect on the plant height. After determining the specific amount of lead and arsenic in the plant material, it was found that all of the plants contaminated with arsenic had absorbed some of it. However, only two out of nine samples had absorbed any lead. Therefore; under these specific conditions, Brassica rapa is able to absorb arsenic but not lead.

INTRODUCTION.

Soil contamination is a major environmental issue facing the world today. Standard methods of removing heavy metal contamination include bulldozing the soil and moving it to a landfill. This is neither cost nor time effective and only relocates the problem instead of consolidating or eliminating it. A more efficient method of remediating soil is a process called phytoremediation, which is the use of plants to remediate soil. A specific type of phytoremediation known as phytoextraction is the process of using natural hyperaccumulators or high biomass plants to retain toxins to be disposed at a later time [1]. This consolidates the contamination, makes disposal more simple and straightforward, and establishes plant growth on possibly barren soils to prevent further dispersion.

As and Pb are hazardous heavy metals, and soil contaminated with these metals is a major health concern. As and Pb poisoning can lead to cancers, birth defects, and other extreme health issues. Industrial sites are the most common locations for this heavy metal contamination, although many fertilizers originally contained As and Pb. Brownfield sites contaminated with these toxins are often barren, which increases the risk of large scale contamination because soil can be easily transported by wind or runoff. Therefore, a plant which can be quickly established in its environment, withstand, and absorb contamination is necessary. Phytoremediating plants have been found for As, but often the plants themselves only grow in certain conditions. For example, ferns have been shown to be hyperaccumulators of As [2,3,4], but grow in shady areas and are hard to grow on a large scale. It would be more effective to have a versatile plant to use for short-term phytoremediation and for quickly establishing vegetation on an otherwise unpopulated site. A wide variety of plants have also been shown to uptake Pb in varying amounts (Table 1) but there is still a need for a plant which is an effective remediator on a more short-term scale.

Plant	Metal
Tobacco (*Nicotiana tabacum* L.) [5]	Cadmium (Cd) Lead (Pb)
Chinese Brake Fern (*Pteris vittata*) [2,3,6]	Arsenic (As)
Indian Mustard (*Brassica juncea*) [2]	Lead (Pb)
Fern (*Pteris cretica*) [7]	Arsenic (As)
Fern (*Pteris longifolia*) [7]	Arsenic (As)
Fern (*Pteris umbrosa*)[7]	Arsenic (As)
Indian Lotus (*Nelumbo nucifera*) [8]	Cadmium (Cd) Copper (Cu)

Table 1. Plants shown to be effective at phytoremediation.

Plants in the mustard family, *Brassica*, are often effective at phytoremediation [2]. *Brassica rapa*, also known as field mustard, is very versatile and can grow in many different soil textures, pH levels, and amounts of shade [9] The species used for this study, known as Wisconsin Fast Plants, has a rapid growth rate [9] because it has been bred to withstand constant light. The natural variety has very similar characteristics and would be easy to grow and would quickly establish growth at contaminated sites. Further field studies should be done to confirm the similarity between the two varieties for phytoremediation. *Brassica rapa* has the potential to be a practical, wide-ranging tool for soil cleanup.

The purpose of this study was to determine the ability of *Brassica rapa* to absorb soluble As and Pb from soil and to analyze the effects of soil pH on this process. Based on standard sorption trends, it is expected that the lower the soil pH, the more Pb will be absorbed and the higher the soil pH, the more As will be absorbed.

MATERIALS AND METHODS.

Plant Preparation.

Seven flats with 24 cells each were set up for planting. Every other cell was used for plants. Each flat had a different contaminant concentration and within each flat there were three different soil pH levels, resulting in 4 plants for each of 21 different sets of conditions, including 12 control plants with the 3 soil pH valued but no contamination. Trays underneath the flats were used for constant water supply and strips of cotton were used as wicks. Each tray was divided into three water tight sections to prevent any hydrogen ion transfer which could alter the soil pH.

Flats were placed on racks with fluorescent lights 18 cm above the top of the containers. Since there was a window beside the plants, the flats were rotated every 4 days to counteract possible uneven light presence. Approximately 30 grams of dry soil mixture were used for each cell. The soil used was a 1:1:1 ratio of compost, vermiculite, and peat. The soil was limed to target pH levels of 6, 7, and 8 using 1% CaCO3, 1.25% CaOH, and 2% CaOH respectively. Two to three Brassica rapa seeds were planted in each cell.

After the seeds were planted, 500 mL of de-ionized water was added to each section. Miracle Grow containing 24% total N, 16% soluble potash, 8% available PO_4, and <1% of B, Cu, Fe, Mn, Mo, and Zn, was dissolved in the water, approximately 0.24 grams per 500 mL. On day 4, 300 mL of water were added to replenish the water supply. By this time, all cells had at least one sprouted seedling. On day 5, the plants were thinned to one plant per cell. Another 300

mL of water was added on day 6. During growth, plant height was measured and true leaves were counted.

As and Pb contamination was introduced as soluble salt in the water for the plants to focus on the plants ability for absorption rather than the contaminants availability. The contamination solutions were created using $PbNO_3$ and $Na_2HAsO_4 \cdot 7H_2O$. The Pb concentration levels were 100, 800, and 2,000 ppm and the As levels were 10, 100, and 1,000 ppm. These levels were chosen based on EPA regulatory limits and the limits chosen for other studies [1, 2, 4, 5, 8]. On day 12, 300 mL of this contamination was added and again at day 16 when they had absorbed all the water. Plants continued to grow until they were collected on day 19, at which point at least 50% were blooming.

Height of the plants was measured once a day to determine the growth rate with and without contamination. The average height of the control plants was compared to the average heights of the low, medium and highly contaminated plants before and after contamination.

Sample Preparation and Analysis.

After 19 days of growth, 42 plants were pulled up, including roots. Two plants of each set of four were randomly selected by tossing a coin. The soil on the roots was rinsed off with de-ionized water. All samples were then dried at 110°C for 75 minutes and then re-dried for 5 minutes prior to grinding.

The plant samples were ground into a powder form using an agate mortar and pestle. This powder was put into Parr Microwave Acid Digestion Vessels and digestions were conducted using the vessels' operating instructions [10]. Approximately 2.5 mL of 76% HNO_3 were added to the digestions vessels, along with less than 0.1 gram of plant material. Samples were heated in the microwave for 2 minutes 30 seconds then left in fume hood to evaporate for 2 days. When the solution had completely evaporated, 5mL 2% Nitric Acid was added to re-dissolve.

The Pb and As solutions were tested using ICP-OES after nineteen days of growth to determine As and Pb concentrations. The dilutions of the 1,000 ppm As solution, 800 ppm Pb solution, and 2,000 ppm Pb solution used for analysis were 500 ppm, 400 ppm, and 500 ppm respectively based on the allowed concentrations for the ICP-OES.

RESULTS.

After approximately 13 days of growth more than half of the plants began to droop. This was not from wilting but most likely from unstable soil. The drooping plants were staked using coffee stirrers and thread. Overall, physical characteristics of the plants seemed appropriately random.

The plants highly contaminated with As began withering and turning brown after day 13 (Figure 1), the day that contamination was added.

Arsenic Results.

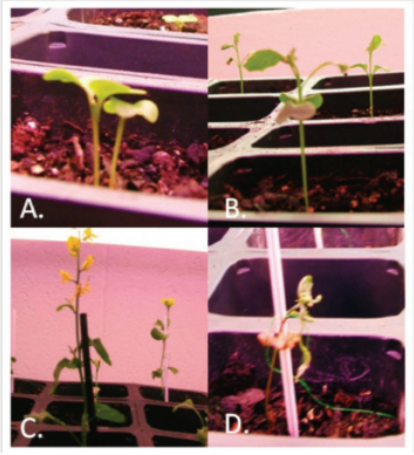

Figure 1. A. Control plants on day 4. **B.** Control plants on day 11. **C.** Control plants on day 19. **D.** Highly contaminated AS plants on day 19.

Before contamination there was no significant difference between the heights of the control plants and the contaminated plants. After contamination there was a significant difference (p=0.001) between the height of the control plants and the highly contaminated plants.

However, based on p-value tests, there was no significant difference (P=0.894, P=0.697) between the control and the low and medium contaminated plants (Figure 2).

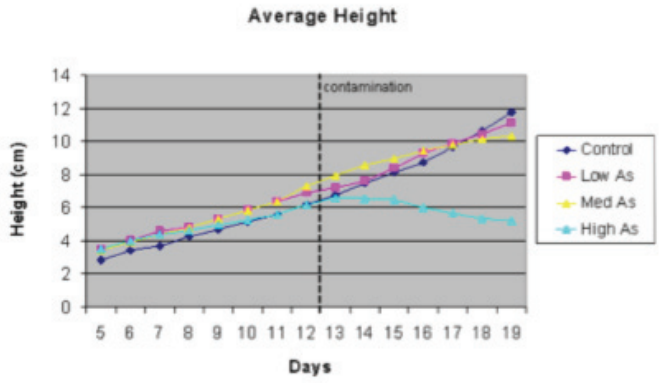

Figure 2. Comparison of As contaminated plant heights over nineteen day growth period. Control vs. Low before P=0.244 after P=0.894, Control vs. Medium before P=0.269 after P=0.697, Control vs. High before P=0.503 after P=0.001.

In all nine samples of plant matter As was detected. The plants with low levels of contamination (10.142 ppm) absorbed 3.420, 0.067, and 0.204 ppm, for an average absorption rate of 12.10% contamination. The plants with medium contamination (108.17 ppm) absorbed 0.291, 0.200, and 0.841 ppm. This is an average of 0.40%. The highly contaminated plants (526.547 ppm) absorbed 26.982, 2.438, and 6.287 ppm. This is an average absorption of 2.30% of the contamination present.

Lead Results.

There was no significant difference (P>0.05) in plant height before or after Pb contamination (Figure 3).

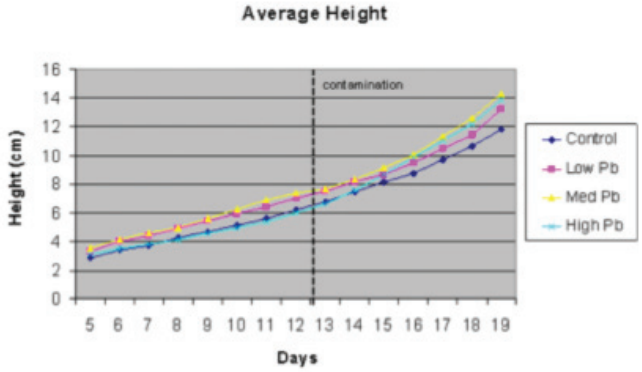

Figure 3. Comparison of lead contaminated plant heights over nineteen day growth period. Control vs. Low, Medium, and High p > 0.05.

When the plant material was analyzed, only two of nine samples contained Pb. Medium Pb with high pH contained 192.171 ppm out of 379.142 ppm. This is absorption of 50.70% of the present contamination. High Pb with medium pH contained 63.690 ppm out of 484.531 ppm. This is 13.10% absorption. All other samples had no detectable Pb.

DISCUSSION.

Under these specific conditions, Brassica rapa was able to consistently absorb

As but not Pb. Further analysis of soil is being conducted to determine the amount of contamination that may have undergone stabilization or degradation as this would make the contamination unavailable for plants to uptake.

The obvious impact on plant height suggests that As was absorbed and accumulated within the plant material. This was confirmed by plant material analysis. However, the amount absorbed was a very small percentage of the total amount of contamination. Other studies have found that in soil containing 338 mg Pb/kg soil, Brassica juncea contained 423 mg Pb/kg dried shoot material without chelators, and 1,302 mg Pb/kg dried shoot material with the addition of EDTA to the soil [2]. That is approximately a 125% and 385% absorption rate respectively. The soil in this study absorbed 12.10%, 0.40%, and 2.30% depending on the contamination level. This is a drastic difference and may be attributed to the soil pH levels used in each experiment.

The plants contaminated with Pb had no significant visual health differences from plants without contamination. Plant material analysis showed that Pb was only absorbed in two of nine treatments. The pH levels were most likely not low enough to facilitate phytoremediation by Brassica rapa. Pb compounds need highly acidic soil with pH levels lower than six to mobilize [11]. Lowering the pH would possibly facilitate greater absorption; however, Brassica rapa may have trouble surviving in more drastic conditions. It has been determined that when using mustard plants for Pb extraction, a chelator such as EDTA, which is an organic compound that sequesters metal ions, is most likely essential [2]. This is supported by the findings of this study because no chelator was used and Pb was not effectively absorbed.

A continuation of this study including further condition varieties such as different soil types, light exposure levels, and planting methods would help to better understand overall methods of aiding this process. Changing the soil pH at a more drastic level may mobilize the metals more effectively and therefore promote further absorption. Beginning contamination at different times in the plants lifecycle may change the rate of absorption based on the growth rate of the plants. Also, this study only covered 19 days and ended when the plants began blooming. A study over the complete lifecycle of the plants could show a continuous rate of absorption, although since the plants are not continuing to accumulate biomass while they are being pollinated it is more likely that the rate would decrease. Field studies must be conducted using the natural strain of Brassica rapa to determine whether this phytoremediation method could be feasible outside of the lab.

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