Uptake and translocation of AsIII and AsV by *Zea Mays* (Corn): Effect of Boron

ENEV079T

Purpose

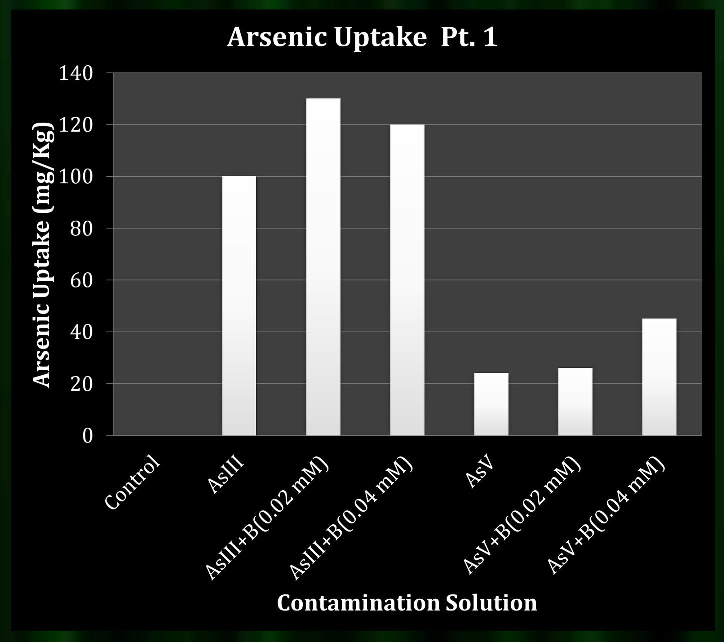
To better understand arsenite (AsIII) and arsenate (AsV) uptake in environmentally realistic concentrations via *Zea Mays* in correlation to the presence of Boric acid (an Arsenic analogue). If Boron significantly reduces As uptake, it can be manipulated into an Arsenic contamination prevention method in growing corn.

Boron

- Boron (B) is found in either an anion or neutral state in natural conditions, Boron is a neutral molecule which is fairly permeable across biological membranes (Graham et al.).
- When B levels are low, as in most external conditions, then B transport is facilitated by the plant.
- The expression of *AtNIP5;1* from *A. thaliana* increased the sensitivity of yeast to arsenite and antimonite, as well as As accumulation in the yeast cells. Interestingly, AtNIP5;1 has been identified as a boric acid transporter essential for boron uptake by *A. thaliana* roots (Takano et al.).
- B deficiency prevents root elongation, reduces leaf expansion and causes the loss of fertility, depending on the plant species and developmental stage (Gupta).
- Plants need B to avoid deficiency and toxicity problems. Originally, Boron transport was believed to be a passive process, but B transporters were later identified, suggesting that plants sense and respond to the B conditions and regulate transporters to regulate B at optimum levels (Miwa et al.)

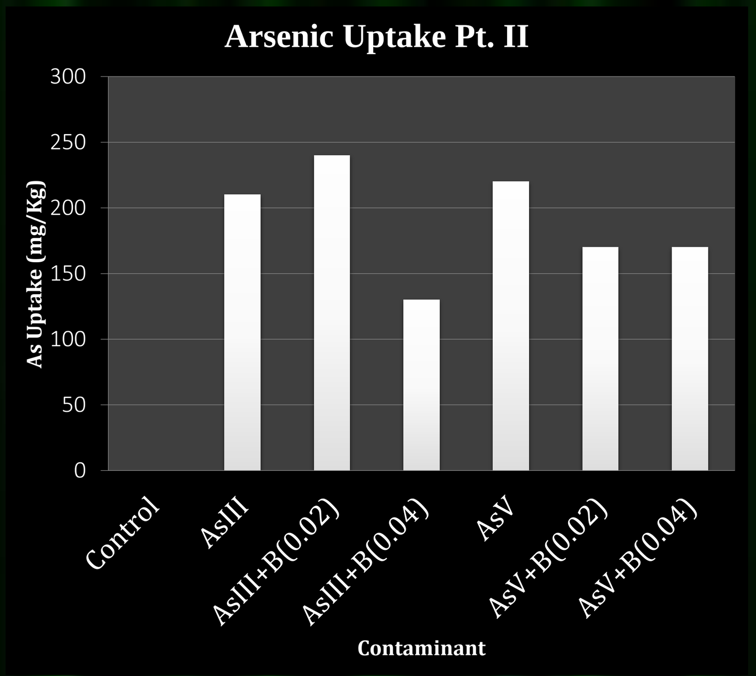
Significance

- Human exposure to arsenic has been linked to increased instances of several cancers, including pancreatic cancer. (Akter)
- Environmental levels of arsenic exceeding safe standards set by the WHO and EPA are found at many locations worldwide such as India (Patel), Bangladesh (Smith et al.), China (Xia and Liu), Vietnam (Berg et al.), Australia (Smith et al.) and the U.S. (Durant et al.)
- In South-East Asia, particularly in Bangladesh, millions of people are exposed to elevated levels of arsenic via drinking As- contaminated groundwater. This means one in five deaths in Bangladesh can be attributed to arsenic poisoning. (Hirji)
- Contaminated food sources of rice and vegetables are also a large source of arsenic toxins. *Corn is one of these food sources.*



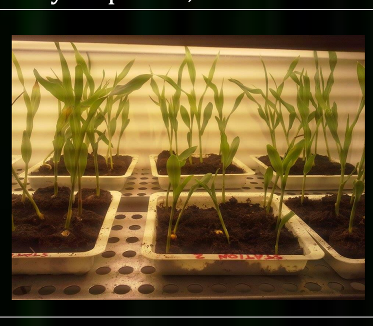
Contamination	As Uptake(mg/Kg)
Control	0
AsIII	100
AsIII+B(0.02 mM)	130
AsIII+B(0.04 mM)	120
AsV	24
AsV+B(0.02 mM)	26
AsV+B(0.04 mM)	45

Improved experiment with controlled pH



Contamination	As Uptake(mg/Kg)
Control	0
AsIII	210
AsIII+B(0.02 mM)	240
AsIII+B(0.04 mM)	130
AsV	220
AsV+B(0.02 mM)	170
AsV+B(0.04 mM)	170


At first, experiment planned to involve hydroponics, which was unsuccessful.

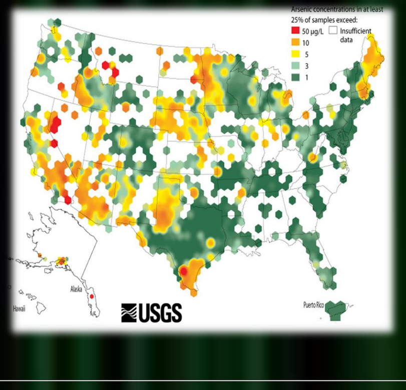



While the first experiment did not use pH buffers.
The second experiment was conducted where the pH was carefully monitored around 6.5.

Arsenic

- Arsenic in groundwater is largely the result of minerals dissolving from weathered rocks and soils. Heavy metals such as Arsenic are also released from human activities such as mining and industry.
- Arsenic is carcinogenic and toxic to both plants and animals, as well as humans. Arsenic contamination is a concern because of biomagnification through the food chain.
- Arsenate (AsV) and Arsenite (AsIII) are the two most toxic and common arsenic species in the environment. (Zhao et al.)
- For example, in Bangladesh, AsIII accounts for 67-99% of total arsenic in most tube wells. (Ahmed) Plants growing in aerobic environment may encounter a mixed pool of AsV and AsIII.

Map detailing arsenic concentrations throughout the U.S. Rocks and soil dissolve and pollute groundwater with Arsenic. Additionally, in 2001 the US Environmental Protection Agency set the limit of Arsenic in groundwater to 10 ug/L. (Riker)



Rationale

- In a previous experiment (Wang et al.), the effect of boric acid on arsenic uptake and speciation by *Pteris vittata* was investigated after exposure to AsIII or AsV. Data from the experiment showed that boric acid had no significant effect ($P > 0.05$) on AsIII or AsV uptake. It was notable that more arsenic was depleted from the AsIII treatment than from the AsV treatment ($P<0.05$).
- However, other research investigating Silicon, showed that AsIII shares silicon (Si) transporter 'Lsi' for entry into rice roots, signifying that Silicon is an effective competitor of AsIII for rice uptake. Therefore, Si suppressed arsenic accumulation in rice. Whether a similar mechanism occurs with Boron in *Zea Mays* is unknown- and the focus of this research.

Hypothesis

AsIII contamination

- Boric acid will inhibit AsIII accumulation in Zea Mays because of a shared aquaporin channel. Similar to both glycerol and Silicon, Boron is taken up by transport proteins akin to ones utilized in AsIII uptake. According to studies of known plants like *Arabidopsis thaliana,* AtPht5;1 is responsible for both Boron and Arsenic uptake.

AsV contamination

- Boric acid will compete with AsV for uptake via the P transporters. In rice plants, boric acid successfully inhibited Arsenate uptake. This method of inhibition will have similar results when *Zea Mays* is exposed to boric acid.

Methods

Golden Bantam, heirloom, organic, non-GMO, sweet corn seeds (*Zea mays* convar. *saccharata* var. *rugosa*) of identical size, obtained from M&B Sales Seed Company (Tampa FL, USA) were used. 105 seeds were grown in Jiffy 36 mm Peat Soil Pellets Seeds Starting Plugs for ten days under the same temperature (High; 32.2 degrees C, Low: 16 degrees C).

Two sections based on arsenic analogue

Control where As is absent

45 *Zea mays* seeds in soil contaminated with solution of 1 uM AsIII

- Addition of 0.02 mM Boron
- Addition of 0.04 mM Boron
- Addition of no Boron

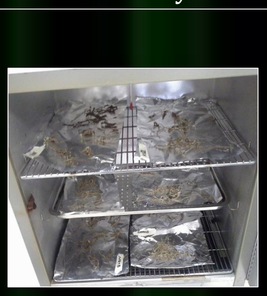
45 *Zea mays* seeds in soil contaminated with solution of 1 uM AsV

- Addition of 0.02 mM Boron
- Addition of 0.04 mM Boron
- Addition of no Boron

15 *Zea mays* seeds in soil with no AsIII or AsV contamination (Control)

Note:
pH was controlled by adding acetic acid and KOH
Boron was in the form of H_3BO_3
AsIII was in the form of $NaAsO_2$
AsV was in the form of $Na_2HAsO_4 \times 7H_2O$

Dimensional Analysis for concentration creation is in logbook.



Left: Corn plants (roots only), placed in oven to be dried.
Above (right): Grounded plant roots ready for arsenic analysis
Above Roots crushed in mortar

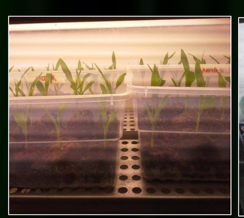






Each of the seven groups of plants growing after contamination (with the exception of the control)

Method 6010B for spectrometry was performed.

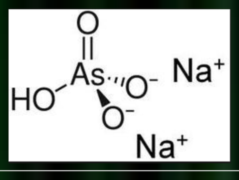


All photos taken by student unless otherwise stated.

Arsenate (AsV)

As a phosphate analog, AsV is taken up via the P transporters in higher plants and AsV competes with P for plant uptake. (Wang et al.)

Sodium Arsenate Structure ("ChEBI")

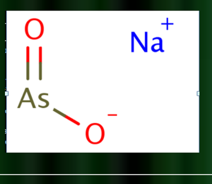


After it is inside the cell, Arsenate is reduced to Arsenite which is thiol reactive, meaning it reacts with compounds that have the group --SH.

Arsenite (AsIII)

On the other hand, AsIII is taken up by plants via glycerol channels, known as aquaporins.
Arsenite may be forced out of the cells or sequestered in the vacuoles by the ABC transporters (part of the ATP binding cassette family), which allow the Arsenite to travel across membranes.
Arsenite is a Silicon analog as well, meaning its uptake mechanism can also be through the Lsi2 transporter which uptakes Silicon.

Sodium Arsenite Structure ("Sodium Organic Chemicals")



Conclusion

- As Boron increased, Arsenic accumulation increased for both AsIII and AsV in plant roots. However, when pH was controlled, AsIII and AsV uptake was increased by the presence of Boron.
- AsIII showed a 30% increase when 0.02 mM Boron was added, and 20% when 0.04 mM Boron was added. On the other hand, AsV showed a 8.3% increase when 0.02 mM Boron was added, an a 87.5% increase when 0.04 mM Boron was in the contaminant solution of the soil. However, in the second experiment, AsIII uptake decreased by 45.9% as Boron concentration increased from 0.02 mM to 0.04 mM, while AsV uptake decreased by 32.8% in the presence of both levels of Boron. Results conclude that Boron competes with Arsenic for uptake in *Zea Mays.*
- The Arsenic was in the soil for 24 hours, under a controlled temperature of 23.8 degrees Celsius and a 12 hour light period, whether these results would have changed significantly under a longer time period is still to be uncovered.
- The results are the opposite of the assumed conclusion under the hypothesis under the original experiment. With controlled pH, Boron is found to have a similar effect on Arsenic uptake in Corn as Silicon has on *Pteris Vittata*.
- At first, results are inconclusive due to the effect of the confounding variable of pH. pH can change the rate of As accumulation. However, in the second experiment, pH did prove to effect the uptake levels as the Arsenic levels decreased with the increase in Boron.
- In all trials, both in this experiment and prior research, it was evident that AsIII was remediated more efficiently than AsV, although this trend was not verified by the second experiment.

Discussion

- Because of the addition of Boron and Arsenic, pH fluctuates, making pH buffers highly significant for accuracy.
- In terms of *Pteris vittata,* at pH levels less than or equal to 5.21, the greatest amount of As was uptaken (s. Tu et al. 2003). Similarly, the pH of the soil becomes more acidic or basic as Boron and Arsenic are added, thus affecting As uptake.
- The uptake mechanism of AsIII and AsV in *Zea mays* was determined to be through a similar channel as Boron uptake.
- Research can be expanded to either increase or decrease As uptake in corn to either remove As from soil so it can be used for other commodity crops or to decrease and minimize As uptake in corn so as not to expose consumers to unsafe amounts of As in food.
- To further verify whether AsIII uptake by Zea Mays was via aquaporin, a well-known aquaporin inhibitor HgCl2 should be added.

Future Research

- Since the AsIII transporter genes have now been identified as within the Lsi family, studies should be done after removing this gene from the plant.
- Similarly, for AsV, remove the genes coding for the P transport system, which are the AtPht 1 family. By removing the gene, AsV may or may not be be able to be accumulated.
- Test varying levels of Arsenic contamination from 1 uM to 30 uM to find the threshold of when the level of Arsenic is too toxic to be accumulated or prevented by boron.
- Modeling after the experiment conducted on *P. vittata* (S. Tu et al. 20013). In this case, there would be three factors in a five-level composite design, where we would test the effects of each-pH, Silicon, and Boron-on AsIII and AsV uptake.
- Test both roots and shoots separately in *Zea Mays* or another plant.
- In this case, Silicon should be tested as well because of the history of competitive inhibition between Silicon and AsIII in prior research on rice.

Bibliography (abridged)

- Ahmed, K.M., Bhattacharya, P., Hasan, M.A., Akhter, S.H., Mahbub Alam, S.M., Hossain Bhuyian, M.A., Imam, M.B., Khan, A.A., Sracek, O., 2004. Arsenic enrichment in groundwater of the alluvial aquifers in Bangladesh: an overview. Appl. Geochem. 19, 181–200.
- Akter KF, Owens G, Davey DE, Naidu R. Arsenic speciation and toxicity in biological systems. Rev Environ Contam Toxicol. 2005;184:97–149.
- Berg M, Tran HC, Nguyen TC, Pham HV, Schertenleib R, Giger W. Arsenic contamination of groundwater and drinking water in Vietnam: a human health threat. Environ Sci Technol. 2001;35:2621–2626.
- Bienert GP, Thorsen M, Schüssler MD, Nilsson HR, Wagner A, Tamás MJ, Jahn TP. 2008b. A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes. *BMC Biology* **6**: 26.