Report of the Small-Scale Economies Project Seminar “Plant-based remediation of arsenic-contaminated soil: Successes and Challenges” on July 1st, 2016

Soil contamination is one of the serious environmental issues that our planet is currently dealing with. Plant-based remediation has been drawing attention as its new countermeasure. The sub-project of Long-term Sustainability through Place-based, Small-scale Economies: Approaches from Historical Ecology” (hereinafter, the Small-scale Economies Project) titled “Towards sustainable remediation of arsenic-contaminated soils,” which is led by Prof. Celine Pallud at University of California, Berkeley, focuses on the study of plant-based remediation of arsenic-contaminated soils using the brake fern (*Pteris vittata L.*). Mr. Sarick Matzen, the project member, visited RIHN on July 1st and gave a talk on the progress and challenges of the research.

Plant-based remediation of arsenic-contaminated soil: Successes and Challenges by Mr. Sarick Matzen

The goal of the sub-project is to develop sustainable and “green” soil remediation techniques to mitigate the threat of soil contamination, particularly with arsenic, while avoiding the conventional method of soil excavation. Arsenic soil contamination occurs globally with various sources including agriculture, mining, contaminated groundwater, the use of arsenical pesticides and arsenic-treated wood. Arsenic is a contributor to the health hazards, causing various kinds of cancers, skin lesions, cardiovascular diseases, paralysis, blindness and so forth. Our team works with a fern called *Pteris vittata L.*, which is a known arsenic hyperaccumulator. The fern absorbs arsenic from soil through phosphate intake pathways in roots, translocates it from roots to fronds and stores it in fronds at very high concentrations, up to 1,000mg/kg. So, harvesting the fern fronds and disposing them as hazardous waste means removing arsenic from the site. This plant-based remediation technique is called phytoremediation.

While it is potentially a very attractive green and sustainable technique, there are a number of challenges that need to be addressed before arsenic phytoremediation is used under real-life conditions. Firstly, the efficiency is expected to be lower under complex field conditions where arsenic uptake is affected by climate, soil factors and the presence of other contaminants. Current remediation rates are relatively slow, on the order of decades. Secondly, arsenic uptake with *Pteris vittata L.* is limited to the root zone, therefore approximately only the top 30cm of the soil. Thirdly, there is the possibility of inadvertently leaching the containment out of the soil into the groundwater, through fertilizer application to the fern.

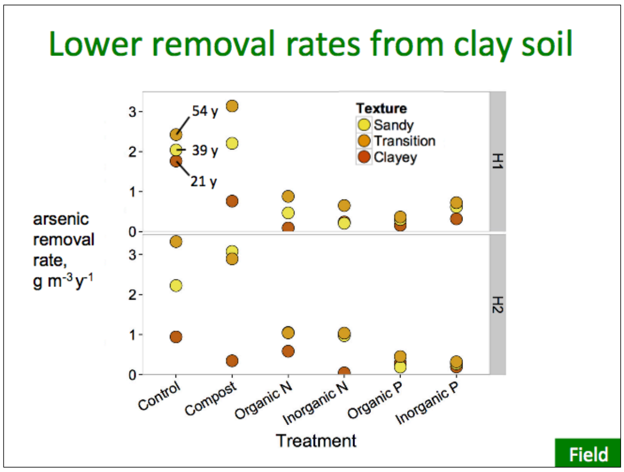
Arising out of those challenges, the research objective was framed: To test the fern’s performance under real-life conditions, and specifically to determine the effects of soil texture and fertilization on arsenic extraction by the fern in order to optimize remediation efficiency.

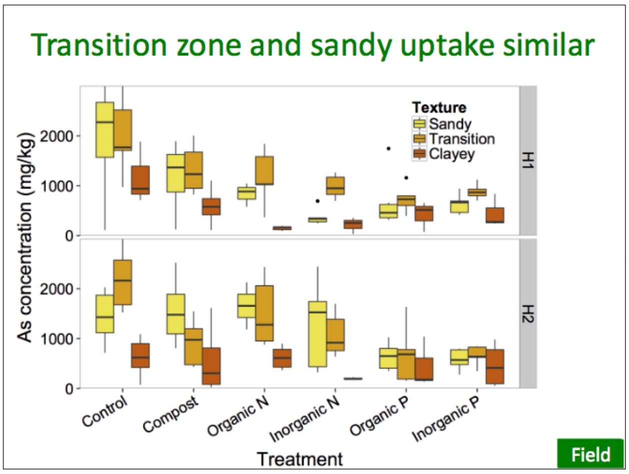
Our field site is a former railroad grade (compacted soil with fill) called the Santa Fe Right of Way in

Berkeley, California. The soil is impacted with arsenic ranging from 10 to 200 mg/kg (source unknown). After tilling the soil in an effort to homogenize it, a total of 1,600 ferns were planted. The plot was then divided into six sub-sections and following treatments were applied at standard agricultural rates accordingly: 1) control (no treatment) on 400 ferns, 2) compost (mixture of nitrogen and phosphorous – 151kg N/ha and 34kg P/ha) on 400 ferns, 3) organic nitrogen amendment (50kg/ha) on 200 ferns, 4) inorganic nitrogen amendment (50kg/ha) on 200 ferns, 5) organic phosphorous amendment (85kg/ha) on 200 ferns and 6) inorganic phosphorous amendment (85kg/ha) on 200 ferns. It should be noted that despite our efforts to homogenize the soil, three different zones of soils were found in the experimental plot soil: 1) clayey zone with up to 47 ppm arsenic, 2) sandy zone with up to 83 ppm, and 3) transition zone, a gradient from clayey to sandy, with up to 119 ppm. The results of our first two frond harvests (8 months after planting (H1) and 21 months after transplanting (H2)) will be shown later on. 10% of the harvested ferns were analyzed, and then the plant biomass and plant arsenic concentrations so as to calculate the remediation rates were measured.

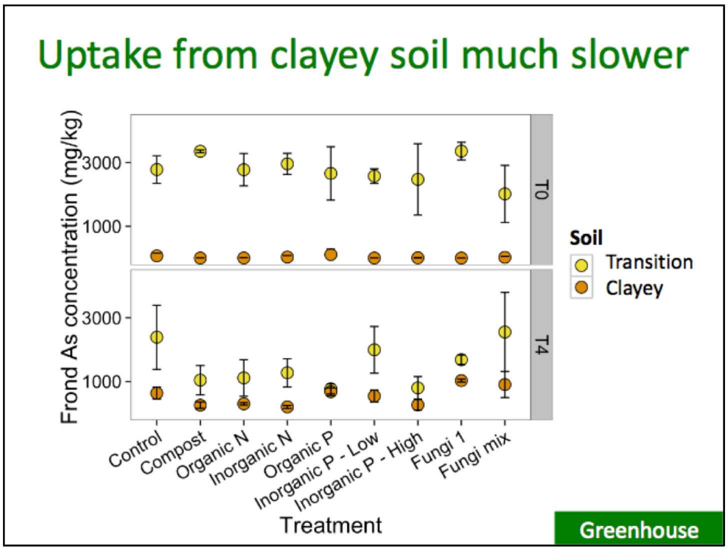
Then, a parallel greenhouse pot study was conducted to understand the system under controlled or more ideal conditions, using the soil excavated from the field site: the transition zone in the middle with 22% clay and 160 ppm arsenic, and the clay zone with 27% clay and 91 ppm arsenic. In this parallel study, a total of eight treatments were applied with three replicates each: the same five fertilizers used in the field and additional three kinds of soil treatment including two types of mycorrhizal fungi and another form of phosphorus. The fern fronds were harvested monthly for four months after treatment and arsenic availability in the post-study soil was examined.

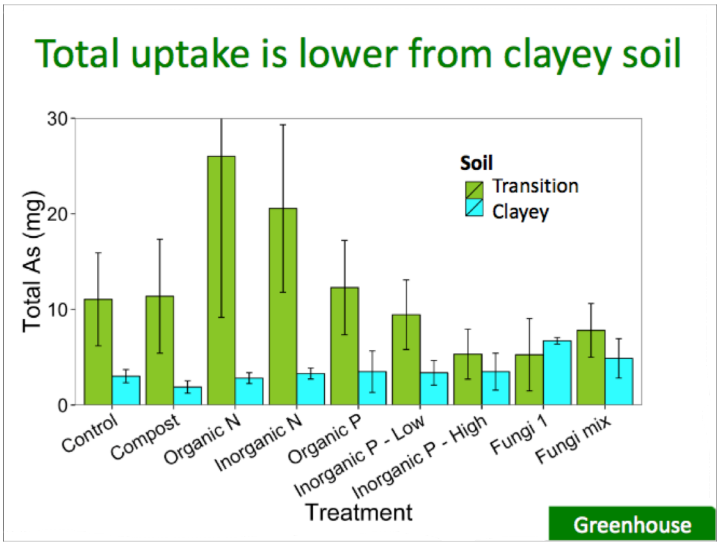
The results of the field site samples show that arsenic removal rates ($g\ m^{-3}\ y^{-1}$) are the fastest in the control and compost plots of the sandy and transition soils. In the plants that received the nitrogen and phosphate amendments (both organic and inorganic), much lower rates were observed. This seems to indicate that the fertilizers are interfering with arsenic uptake in the fern. In fact, it specifically appears that the phosphate fertilizers do really bring about a negative impact. It is possible that the fern is preferentially taking up phosphate compared to arsenic, so that is decreasing the arsenic uptake. In terms of soil texture, the clayey soil has the lower removal rate in comparison to the sandy and transition soils. Fertilizers do not appear to impact arsenic uptake in the clayey soil significantly, and in particular no effect was observed in the clayey soils in H2. Similar arsenic uptake is observed in the sandy and transition zones even though the latter contains 50% more arsenic. Hence, the soil texture seems to function independently of arsenic concentration.





The results from the greenhouse study exhibit similar patterns with those from the field study – specifically the phosphate arsenic competition. Comparing arsenic concentration in fronds (mg/kg) harvested from the transition soil at time zero (T0, after 8 weeks of pre-treatment growth) and four months after fertilizer application (T4), it can be pointed out that the fertilizer application decreased the frond arsenic concentrations in the four-month timeframe. However, no decrease was found in the transition soils with two mycorrhizal fungi added. On the other hand, in terms of the soil texture, a very different behavior was exhibited in the fern fronds growing in the clayey soil – arsenic uptake is almost none and very low even after four months. However, one outstanding result is a positive effect of fungi treatments, which is statistically significant. It can be presumed that the fungal hyphae might be growing into the pores in the clayey soil that are too small for the fern roots to grow into and transport arsenic from those small pores to the fern roots. Looking at total arsenic uptake (mg), the most total uptake was found in the transition soil that received the nitrogen treatments, which should be due to a biomass effect. As explained above, the frond arsenic concentrations in the ferns receiving the nitrogen treatments are low. That is because nitrogen increases the fern biomass that leads to a dilution of arsenic in the fern. Thus, the most total arsenic can be removed due to the large size of the ferns in this case. This behavior is contrary to the results of the field samples, which might be that a compost is more effective in delivering nitrogen under real-field conditions whereas these pure nitrogen amendments are more effective under greenhouse conditions. In terms of the total arsenic uptake in the clayey soil, it is much lower compared to the transition soil. Recalling that the clayey soil has only about 60% of the arsenic that is in the transition soil, yet control ferns in the

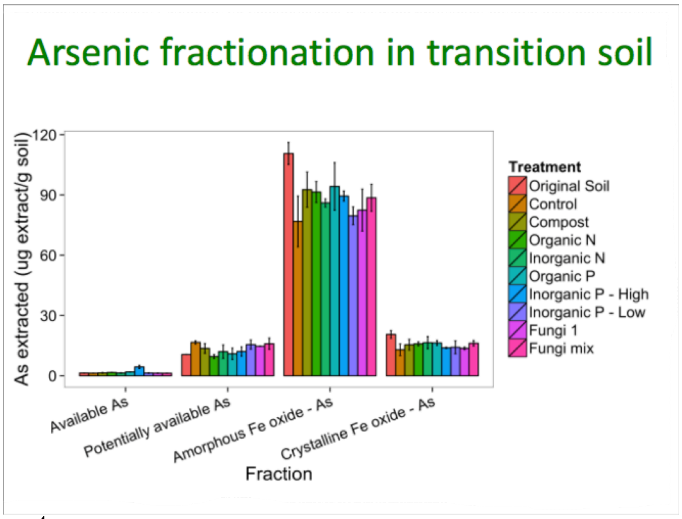




clayey soil absorbed only 30% of arsenic as those in the transition soil, it can be deemed that arsenic uptake is not directly proportional to the soil arsenic concentration, but that there is a distinct role of soil texture. Again, a positive effect by the fungi treatments is another remarkable finding.

The research results so far seem to circumstantiate that both soil texture and arsenic concentration affect arsenic uptake. The uptake rates are lower in the clayey soil, which had lower arsenic. Fertilizers interact differently with the sandy and clayey soils, and therefore the soil texture needs to be taken into consideration when applying fertilizer. And, fertilization decreases uptake rates. Based on the results, the best remediation time was calculated to be 21 years to remediate a clayey soil with approximately 50 ppm arsenic. The soils are an active partner in plant-based remediation, but not all soils are alike. It is of great importance to build a body of research that considers the role of soil characteristics in plant uptake of contaminants. This has applications to remediation, including phytoextraction, and also to the uptake of contaminants in food crops.

The other crucial question to be addressed here is: How do soil characteristics affect arsenic availability after fern growth? To address the issue of the arsenic availability in our soils after fern growth, we are using a method known as sequential extractions, where we use chemicals of increasing strengths (starting with a simple salt solution, ammonium sulfate) to extract arsenic based on the form or the availability of arsenic in the soil. The five fractions are known as: (1) readily exchangeable arsenic, which is the most available, (2) potentially mobilizable arsenic, (3) arsenic associated with amorphous and poorly-crystalline hydrous oxides of Fe and Al, (4) arsenic associated with well-crystallized hydrous oxides of Fe and Al and (5) residual arsenic, which is the least available.



Based on this procedure, it is illuminated that relatively little arsenic in our soils is available, which is probably the case in the Japanese soils with a contaminant threshold of 100 ppm arsenic, which is much higher than thresholds in the US. It is one of exciting findings that most of the arsenic in our soils is not going to pose an environmental risk. However, we also found an interesting relationship between the phosphate and the arsenic availability, with phosphate application increasing arsenic availability. We have previously saw that arsenic uptake was not high in the phosphate treated ferns, which contradicts this finding. It seems that the phosphate is playing two roles: 1) it is exchanging with the arsenic adsorbed to the soil and making the arsenic more available, and 2) the phosphate is also being taken up through the phosphate pathways, thus blocking the arsenic. While a high phosphorous application can increase the amount of available arsenic, there is no corresponding increase in arsenic uptake in the ferns. This could possibly lead to arsenic leaching into ground water.

At this point, our study results suggest that phytoextraction is too slow for rapidly turning over land for use, but could be useful in longer term applications. Whereas we see little environmental risk from the arsenic in these soils, it is crucial to tread with caution when growing food crop in arsenic contaminated soil because phosphate fertilization and other factors in the rhizosphere could mobilize arsenic. Regarding the future direction of our research, we will be directly addressing the issue of arsenic uptake into food crops by conducting a vegetable uptake study in our soils. At the same time, we are starting up a Meso-scale study by planting the fern in a column so that we can determine the arsenic-phosphate biogeochemical cycling in a mechanistic manner. Mineralogy and the form of arsenic in the soil are also being analyzed using X-ray absorption spectroscopy. Finally, we are setting up a second field study in soil with multiple contaminants such as arsenic, lead, zinc and copper, which allow us to further look into how the ferns take up arsenic in real-life conditions.

Q&A and Discussion

Q: Does the existence of arsenic in soil have some impact on the fern's growth rate?
A: It seems that the fern grows better in the presence of arsenic, however, with an (unidentified) upper limit.

Q: When you say the soil texture, is it physical properties or chemical?
A: Texture specifically refers to particle size, but then there are certain particles of soil that have certain chemical characteristics.

Q: How did you compare the sandy soil with the clayey soil? Did you consider the existence of minerals since the clayey soil should contain much more minerals?
A: The preliminary results of X-ray diffraction work, though not fully analyzed, suggest that there is quite a bit of quartz and feldspar and also a signature of a copper arsenic phase. The work that has been done to date is to characterize the mineralogy in our soil to try to understand the source of an arsenic pollution. Next, synchrotron-based work using X-ray spectroscopy will be carried out so as to identify the form of arsenic in the soil to understand what minerals the arsenic is associated with.

Q: This fern is generally known to grow in the area where the soil is contaminated with heavy minerals, so they are heavy metal tolerant. When studying the uptake of the arsenic, did you also consider the uptake of other heavy metal elements and the impact to the arsenic uptake from the intake of other metals.
A: That will be addressed in the second field site that is being set up now in Richmond. The soil there is contaminated with pyrite ore, thus containing arsenic, lead, zinc and copper. As explained thus far, the fern can tolerate arsenic by hyperaccumulating and storing it in its vacuoles. However, there have been conflicting reports about its ability to tolerate lead, zinc and copper contamination possibly through a different mechanism.

Q: Brake fern has evolved so that they are able to grow in the area where it is difficult for other plants to grow. What will happen if it is planted in the soil that has more phosphate and arsenic?
A: It will take up the arsenic and phosphate through the same transporter system, but the fern does not hyperaccumulate phosphate, only arsenic.

Q: How do you procure the seed or seedlings from the fern? Do you plant the seed directly in the contaminated soil or do you firstly grow the seedlings?
A: The process or the act of using this plant for arsenic remediation has been patented and the patent is held by the professor who first discovered arsenic hyperaccumulation and the fern, and also by the remediation corporation. We purchased the young ferns from the corporation. It is not legally possible to reproduce the fern from the spores from those specific plants.

Q: In the course of your research that extended for more than several years, you must have encountered some generation changes of the fern. Did they change the tolerance to the arsenic or the accumulation rate for the arsenic uptake?
A: With regards to the change in arsenic uptake in the fern over time, two things were observed. Firstly, the arsenic uptake goes down over time in the control group on the field site. Different chemical forms of arsenic are present in the soil to make up the total concentration of arsenic, and the chemical form affects how easily the plant can take it up. We would expect that the fern would take up the most available arsenic first, and then the arsenic would become less available over time, resulting the decrease in the uptake. At the same time, the root biomass is the other factor that affect arsenic uptake. This could be evinced by an increase in arsenic uptake in the nitrogen treated ferns between H1 and H2.

Q: What is the final remediation process under real-life situations? What is going to happen to the contaminated ferns?
A: That is a crucial issue. If we do not effectively deal with contaminated waste, it means that we are creating waste that could potentially contaminate another side. In our case just within the constraints of our study, we have to send our harvested ferns to a hazardous waste incinerator. However, more ideal conditions would be that the biomass would be digested in a way that you would at least further reduce the volume of waste, and

then potentially you could extract arsenic because arsenic is still being used in a whole host of industrial processes. It is crucial to balance how hazardous the arsenic is in the soil with the amount of waste that would be produced if you excavated the soil, with the amount of waste produced by extracting the arsenic and disposing of the contaminated biomass.

Q: Is there a possibility of genetically engineering the ferns to have more phosphate pathways or providing them with a better ability to concentrate more arsenic?
A: It is certainly a good question that frequently comes up in remediation research. In this case, the phosphate intake pathway is what is known as a high affinity pathway, which functions to take up phosphate at low concentrations because phosphate in general is not very available in the soil. Arsenic hyperaccumulation depends on up-regulating the transporters responsible for arsenic movement across membranes, which could include the phosphate transporter system. It certainly could be possible to genetically modify the fern. However, considering the complicated system within the fern and the number of genes involved, experts think it is better to look at existing plants than to spend time trying to modify this whole suite of genes. Of course, the other major issue when dealing with genetically engineered plants is whether or not they will remain under human control or spread over international environments. That is a major concern in phytoextraction or any sort of plant-based remediation because we really do not want to create invasive plant problem when we are using this plant-based technology whether or not it is genetically engineered.

Q: Will there be a change in physical characteristics after absorbing arsenic over generations?
A: As far as I know, there are not any changes either in terms of the physical characteristic or the ability of the plant to take up arsenic at the concentrations we are working with. However, in some studies that examined concentrations as high as over 5,000 ppm arsenic in the soil, those populations seem to have developed an ability to basically employ two tolerance mechanisms: one to tolerate the arsenic by taking up into the fronds and sequestering there, and the other by actually taking up less arsenic. Consequently, those populations are less useful for remediation in sites with lower amounts of arsenic.

Q: To what extent is the behavior of hyperaccumulation applicable to other kinds of ferns?
A: There is a handful of species: a few in the Pteris family and one in the Pityrogramma family and so forth, but it is not a characteristic that is bound for over most ferns.

Q: One of the soil samples that you are dealing with has 119 ppm of arsenic, is it supposed to be a quite high level of concentration compared to the general condition? In Japan, there are quite a few areas where the arsenic concentration in the soil is around 100 ppm, does the food crop cultivated in this type of soil have any health impact to the human body?
A: 119 ppm is only a moderate level. I believe that 100 ppm is the regulatory limit in Japan, which is certainly the regulatory limit in Australia and much higher than that in the US. However, the fact is that often very little

of the total concentration of arsenic is actually available to have negative health effects on the human body or to be taken up by plants, due to a high iron oxide content in the soil.

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