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Mapping the metal uptake in plants from Jasper Ridge Biological Preserve using synchrotron micro-focused X-ray fluorescence spectroscopy

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Serpentine soil originates in the Earth's mantle and contains high concentrations of potentially toxic transition metals. Although serpentine soil limits plant growth, endemic and adapted plants at Jasper Ridge Biological Preserve, located behind SLAC National Accelerator Laboratory, can tolerate these conditions. Serpentine soil and seeds belonging to native California and invasive plants were collected at Jasper Ridge. The seeds were grown hydroponically and on serpentine and potting soil to examine the uptake and distribution of ions in the roots and shoots using synchrotron micro-focused X-ray fluorescence spectroscopy. The results were used to determine differences between serpentine-tolerant plants. Rye grown on potting soil was enriched in Ni, Fe, Mn, and Cr compared to purple needlegrass grown on serpentine soil. Serpentine vegetation equally suppressed the uptake of Mn, Ni, and Fe in the roots and shoots. The uptake of Ca and Mg affected the uptake of other elements such as K, S, and P.

I. INTRODUCTION

Serpentine soil is derived from ultramafic rocks abundant in Fe and Mg that originated in the mantle. Characteristics of serpentine soil include high concentrations of heavy metals such as Ni and Cr, low concentrations of essential nutrients such as N, P, and K, and a low ratio of Ca to Mg. The lack of Ca and abundance of Mg, along with the presence of potentially harmful heavy metals, are both cited as reasons for limited plant productivity on serpentine soil.[1] However, plants such as rye, an invasive species in California, have developed a tolerance for growing on serpentine soil.

Jasper Ridge Biological Preserve (JRBP), located in the foothills of the Santa Cruz Mountains, intersects the San Andreas Fault. The preserve contains regions of serpentine soil due to geologic activity along the fault. Serpentine-tolerant plants at the JRBP include California native purple needlegrass and squirreltail, while the common wild oat does not grow on serpentine soil. The uptake and distribution of ions in different plant tissues can be studied to determine how elemental uptake differs between serpentine and non-serpentine tolerant plants at JRBP.

Serpentine soil from JRBP has been found to contain three times more Mg than Ca[1]. In addition, serpentine vegetation contains higher concentrations of Fe, Cr, Ni, Mn, and Co in the roots than in the stems and leaves. This demonstrates that serpentine vegetation limits the uptake of metals in the plants' roots, a mechanism which confers serpentine tolerance[1]. The elemental uptake in plants from JRBP was studied using micro-X-ray fluorescence (μ-XRF) spectroscopy at

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the Stanford Synchrotron Radiation Lightsource (SSRL). In this study, the uptake and distribution of ions in the roots and shoots of plants grown on serpentine soil will be compared to the uptake and distribution in plants grown hydroponically and on potting soil. While serpentine soil and certain serpentine-tolerant vegetation has been studied in detail, the plants of interest include native and invasive species at JRBP that have not been studied using µ-XRF spectroscopy.

It is hypothesized that varying the concentration of Ca and Mg in plants grown hydroponically will not affect the uptake of other elements. The plants' roots and shoots will both suppress the uptake of metals, with higher concentrations present in the roots. The goal is to use the results to hypothesize the biological controls used by the plants. Model plant systems with a known genome will then be studied to identify genes that confer or remove serpentine tolerance.

II. METHODS

A. Sample collection and preparation

Samples of serpentine soil, along with purple needlegrass, squirreltail, rye, and common wild oat seeds were collected at JRBP by Courtney Roach and Sam Webb in May 2015. I placed two to three of each seed in 50 mL centrifuge tubes filled with either serpentine soil or Orchard potting soil. The tubes were wrapped in aluminum foil or electrical tape to mimic the lack of light in typical soil conditions, and placed behind SSRL. The seeds were monitored each day and watered when necessary to saturate the soil. I prepared four variations of Hoagland solution,[2,3] a hydroponic nutrient solution, by varying both $Ca(NO_3)_2$ and $MgSO_4$ in the original Hoagland solution to achieve different concentrations of Ca to Mg: 0% Ca/100% Mg, 25% Ca/75% Mg, 75% Ca/25% Mg, and 100% Ca/0% Mg. I soaked germination papers in the four Hoagland variations, the original Hoagland solution, and deionized water. Two seeds of each type were placed on the papers, which were then sealed in plastic bags and left to germinate.

When the seeds sprouted, samples of the root and shoot were covered in Paraffin and sectioned with a Leica RM2255 microtome. Seeds that did not sprout were also sectioned for comparison.

B. Analysis of soil samples and plant samples

µ-XRF spectroscopy was performed on beamlines 2-3 and 14-3 at SSRL. The 2-3 elements of interest were Mn, Ni, Fe, Cr, S, K, Ca, and Zn, while the 14-3 elements of interest were Ca, Mg, K, S, and P. The resulting µ-XRF elemental maps were compared using Sam's Microprobe Analysis Kit (SMAK) by first normalizing element intensity to account for fluctuations in the incoming beam, and then standardizing the element intensity for each sample on the two beamlines. The 2-3 samples selected for further study were the roots and shoots of sprouted purple needlegrass and common wild oat on serpentine soil, sprouted rye on potting soil, and sprouted squirreltail on the Hoagland solution germination paper. Samples of serpentine soil and potting soil were also examined. The 14-3 samples selected for further study were sprouted rye seeds grown hydroponically on the 25% Ca/75% Mg, 75% Ca/25% Mg, and original Hoagland solution germination papers in order to determine how the Ca and Mg uptake varied over an equally spaced range of concentrations. The mean element distribution and standard deviation in biologically similar areas of each sample was identified and recorded using SMAK, and plotted in Excel.

III. RESULTS

A. Beamline 2-3

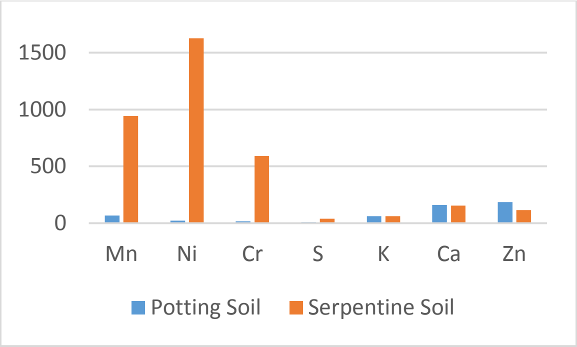

FIG. 1. Element distribution in serpentine and potting soil.

In Figure 1, the element of interest is plotted on the x-axis and counts, or intensity measured by the detector, is plotted on the y-axis. Accounting for error, the K and Ca levels are almost equivalent between the two soils at 61.92 counts and 159.44 counts for potting soil and 63.30 counts and 154.50 counts for serpentine soil, respectively. Zn is more common in potting soil, at 186.14 counts compared to 116.04 counts in serpentine soil.

In the remaining figures, the region of interest of the plant is plotted on the x-axis.

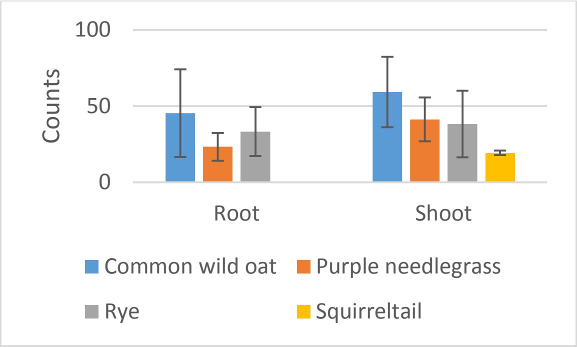

FIG. 2. Ni distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

According to Figure 2, Ni levels were greatest in the shoots of the serpentine sprouted plants at 59.19±23.07 counts and 41.21±14.36 counts, respectively, followed by the potting sprouted plant at 38.20±21.88 counts, and the hydroponically grown plant at 19.23±1.50 counts.

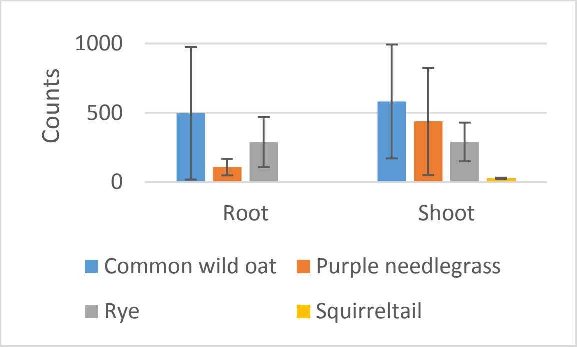

FIG. 3. Fe distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

Similar to Ni levels in Figure 2, Fe levels in Figure 3 were greatest in the shoots of the serpentine sprouted plants at 582.82±411.27 counts and 437.89±386.78 counts, respectively, followed by the potting sprouted plant at 290.21±139.58 counts, and the hydroponically grown plant at 27.53±4.17 counts.

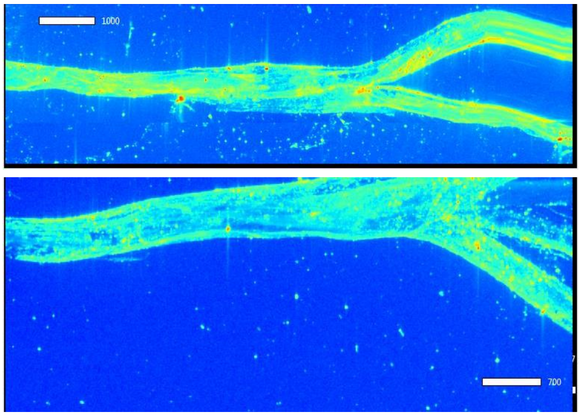

FIG. 4. Micro-X-ray fluorescence (μ-XRF) elemental maps for Fe in common wild oat sprouted on serpentine soil at 1000 μm (top) and purple needlegrass sprouted on serpentine soil at 700 μm (bottom). The regions of highest intensity are in red and the regions of lowest intensity are in blue.

Figure 4 depicts the μ-XRF mapping of Fe in the sprouted common wild oat and sprouted purple needlegrass, with regions of highest intensity in red and regions of lowest intensity in blue. The root is on the left and the shoot is on the right of each image.

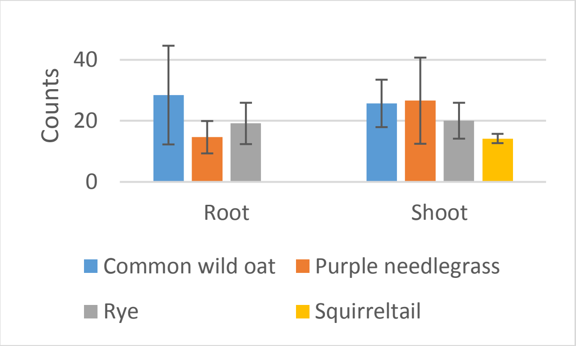

FIG. 5. Mn distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

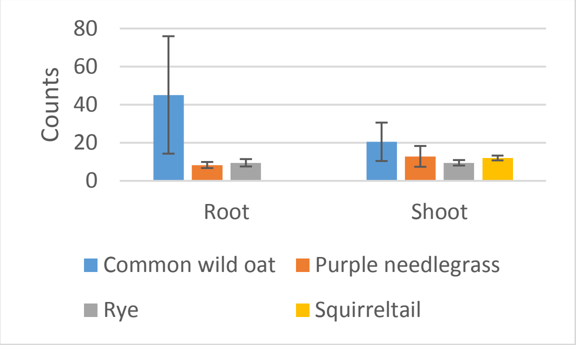

FIG. 6. Cr distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

Concentrations of Ni, Fe, Mn, and Cr in Figures 2, 3, 5, and 6 were greater in the sprouted rye root than in the sprouted purple needlegrass root. The rye counts were 33.22±15.99, 287.72±179.90, 19.16±6.77, and 9.43±1.95, while the purple needlegrass counts were 23.20±9.12, 108.25±59.15, 14.65±5.30, and 8.29±1.55. Accounting for error, the Mn, Ni, and Fe concentration differences between the shoots and roots of plants sprouted in serpentine soil was insignificant.

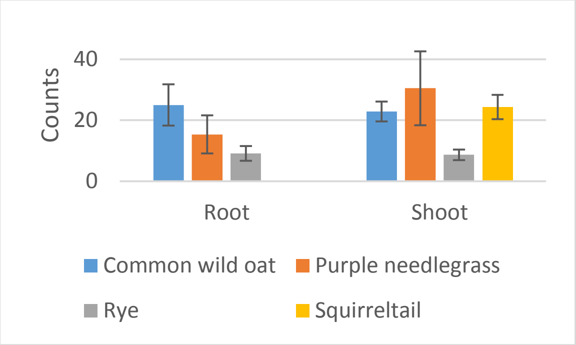

FIG. 7. S distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

In Figure 7, S was most abundant in the roots of the serpentine sprouted common wild oat and purple needlegrass at 25.00±6.78 counts and 15.35±6.22 counts, followed by the potting sprouted rye at 9.11±2.38 counts.

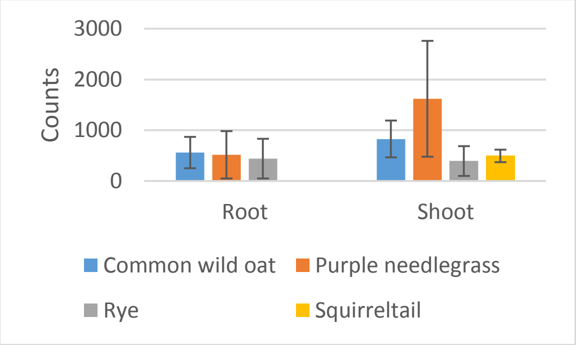

FIG. 8. K distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

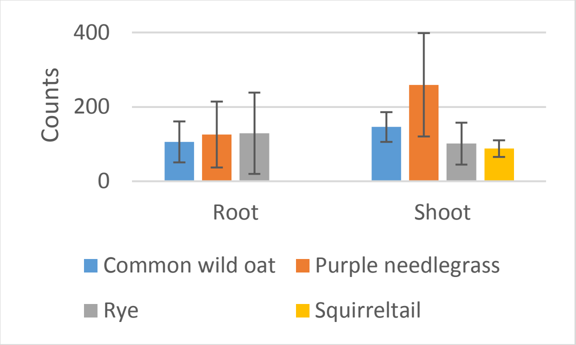

FIG. 9. Ca distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

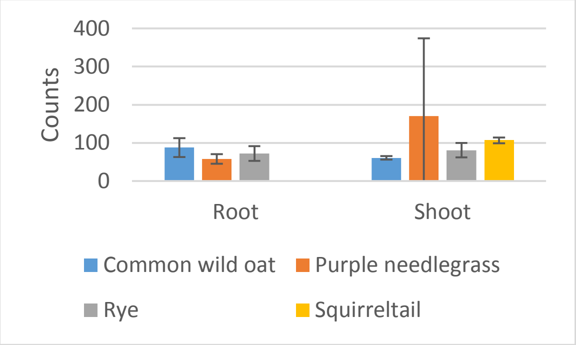

FIG. 10. Zn distribution in the roots and shoots of common wild oat sprouted on serpentine soil, purple needlegrass sprouted on serpentine soil, rye sprouted on potting soil, and squirreltail sprouted on the Hoagland solution paper.

Accounting for error in Figures 8-10, K, Ca, and Zn concentrations remained constant in the common wild oat, purple needlegrass, and rye roots. According to Figures 2, 3, and 6, the common wild oat shoot contained the highest concentration of transition metals (Ni, Fe, and Cr) while according to Figures 7-9, the purple needlegrass shoot contained the highest concentration of nutrients such as S, K, and Ca.

B. Beamline 14-3

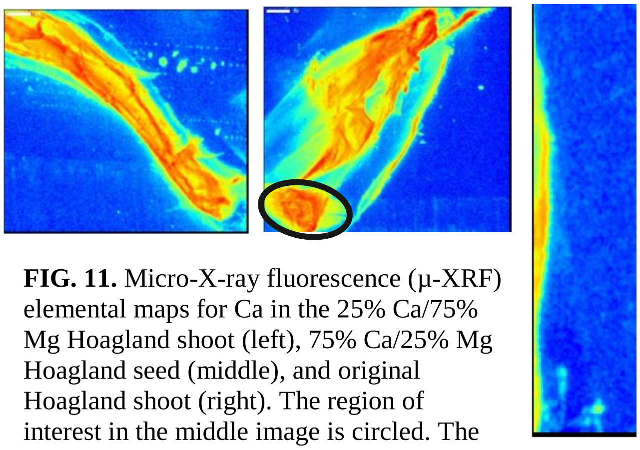

FIG. 11. Micro-X-ray fluorescence (µ-XRF) elemental maps for Ca in the 25% Ca/75% Mg Hoagland shoot (left), 75% Ca/25% Mg Hoagland seed (middle), and original Hoagland shoot (right). The region of interest in the middle image is circled. The regions of highest intensity are in red and the regions of lowest intensity are in blue.

Figure 11 depicts the µ-XRF mapping of Ca in the 25% Ca/75% Mg Hoagland shoot, 75% Ca/25% Mg Hoagland seed, and original Hoagland shoot. The shoot in the 75% Ca/25% Mg Hoagland seed is circled in black. The regions of highest intensity are in red and the regions of lowest intensity are in blue.

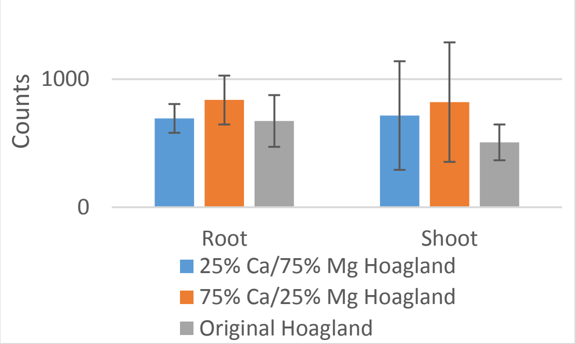

FIG. 12. Ca distribution in the roots and shoots of rye seeds grown hydroponically using the 25% Ca/75% Mg Hoagland solution, 75% Ca/25% Mg Hoagland solution, and original Hoagland solution.

According to Figure 12, the Ca concentration increased from 692.73±112.28 counts in the 25% Ca/75% Mg Hoagland solution root to 837.48±190.41 counts in the 75% Ca/25% Mg Hoagland solution root and from 716.55±423.22 counts to 821.21±465.63 counts in the respective shoots, but decreased to 673±201.37 counts in the original Hoagland solution root and to

507.03±139.48 counts in the original Hoagland solution shoot.

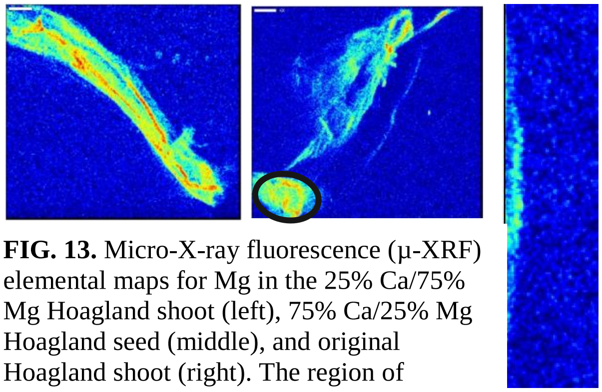

FIG. 13. Micro-X-ray fluorescence (µ-XRF) elemental maps for Mg in the 25% Ca/75% Mg Hoagland shoot (left), 75% Ca/25% Mg Hoagland seed (middle), and original Hoagland shoot (right). The region of interest in the middle image is circled. The regions of highest intensity are in red and the regions of lowest intensity are in blue.

Figure 13 depicts the µ-XRF mapping of Ca in the 25% Ca/75% Mg Hoagland shoot, 75% Ca/25% Mg Hoagland seed, and original Hoagland shoot. The shoot in the 75% Ca/25% Mg Hoagland seed is circled in black. The regions of highest intensity are in red and the regions of lowest intensity are in blue.

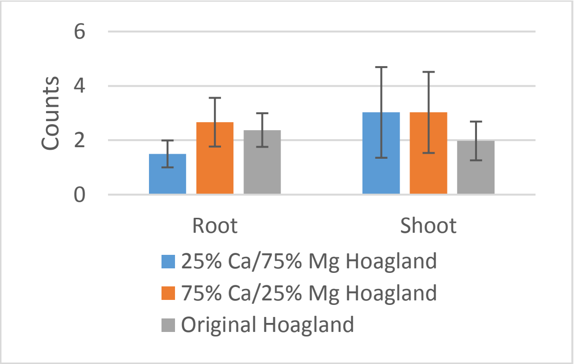

FIG. 14. Mg distribution in the roots and shoots of rye seeds grown hydroponically using the 25% Ca/75% Mg Hoagland solution, 75% Ca/25% Mg Hoagland solution, and original Hoagland solution.

Accounting for error in Figure 14, the Mg concentration in the roots was unaffected by the varying concentrations of Mg. The Mg concentration was constant in the 25% Ca/75% Mg Hoagland solution and 75% Ca/25% Mg Hoagland solution shoots at 3.03±1.67 and 3.03±1.49 counts, but decreased to 1.98±0.71 counts in the original Hoagland solution shoot.

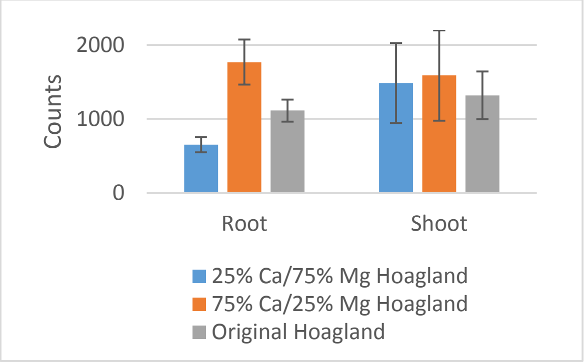

FIG. 15. K distribution in the roots and shoots of rye seeds grown hydroponically using the 25% Ca/75% Mg Hoagland solution, 75% Ca/25% Mg Hoagland solution, and original Hoagland solution.

Accounting for error in Figure 15, K levels remained constant in the shoots but varied in the roots.

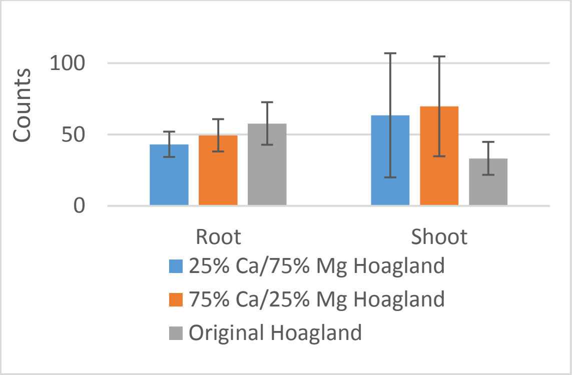

FIG. 16. S distribution in the roots and shoots of rye seeds grown hydroponically using the 25% Ca/75% Mg Hoagland solution, 75% Ca/25% Mg Hoagland solution, and original Hoagland solution.

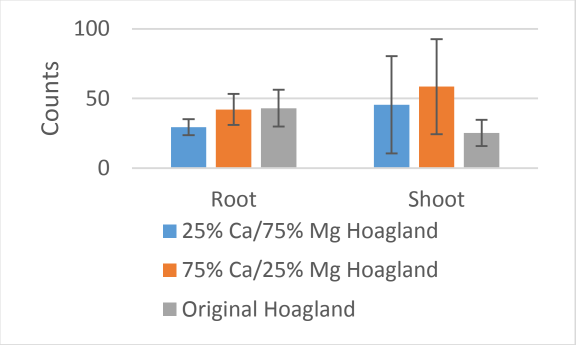

FIG. 17. P distribution in the roots and shoots of rye seeds grown hydroponically using the 25% Ca/75% Mg Hoagland solution, 75% Ca/25% Mg Hoagland solution, and original Hoagland solution.

According to Figures 16 and 17, S and P concentrations in the roots increased with increasing concentration of Ca. As the Ca concentration increased, the S concentration increased from 43.02±8.87 counts to 49.27±11.31 counts to 57.58±14.88 counts, while the P concentration increased from 29.39±5.72 counts to 42.10±11.14 counts to 43.06±13.23 counts. The S and P distribution in the shoots resembled the Ca distribution because S and P were more abundant in the 75% Ca/25% Mg Hoagland solution than in the original Hoagland solution. Between the 75% Ca/25% Mg Hoagland solution and the original Hoagland solution, S decreased from 69.66±34.95 counts to 33.12±11.52 counts, while P decreased from 58.52±34.03 counts to 25.29±9.48 counts.

IV. DISCUSSION

A. Beamline 2-3

According to Figure 1, K and Ca are two nutrients whose levels remain unchanged between serpentine and potting soil. This supports an earlier study, which found that although Mg is three times more common than Ca in serpentine soil at JRBP, serpentine soil is not low in Ca.[1] Mg was not examined on beamline 2-3. Serpentine soil is known to contain low concentrations of K, yet was also found to contain higher concentrations of K than non-serpentine soil at JRBP.[1] Zn is common in serpentine soil but is also a nutrient found in potting soil. The concentration of Zn has been shown to be higher in serpentine soil compared to non-serpentine soil at JRBP.[1]

Although common wild oat does not grow on serpentine soil, a sprout appeared in one of the tubes filled with serpentine soil. The sprout was classified as common wild oat but likely belonged to a serpentine-tolerant seed that ended up in the tube. The shoot trends observed in Figures 2 and 3 for Ni and Fe were expected because serpentine soil should contain the highest concentration of Ni and Fe, followed by the potting soil. The large Fe error bars for the serpentine sprouted plants arose because soil was present in the sample, which caused large deviations from the mean intensity. It was difficult to select a region of interest while avoiding soil particles, depicted in red in Figure 4.

According to Figures 2, 3, 5, and 6, the enriched concentration of Ni, Fe, Mn, and Cr in the sprouted rye root compared to the sprouted purple needlegrass root indicates the suppressed uptake of these elements primarily occurred in rye growing on potting soil instead of purple needlegrass growing on serpentine soil. The Mn, Ni, and Fe concentration differences between the shoots and roots of plants sprouted in serpentine soil were inconclusive regarding where the suppressed uptake of these metals primarily occurs.

Based on Figure 7, roots of common wild oat and purple needlegrass sprouted on serpentine soil are enriched in S compared to the rye grown on potting soil. According to Figures 8-10, common wild oat, purple needlegrass, and rye accumulated similar concentrations of K, Ca, and Zn in the roots. The different soil conditions did not affect the uptake of K, Ca, and Zn. According to Figures 2, 3, and 6, common wild oat preferentially sequestered transition metals into the shoot while according to Figures 7-9, purple needlegrass favored nutrients.

The results can be used to identify biological controls in common wild oat, purple needlegrass, and rye that control ion uptake and suppression.

B. Beamline 14-3

The concentration of Ca in the root and shoot of the Hoagland solution sprouted rye was lower than expected due to how the sample was sectioned. For example, each sample was sectioned to a depth which exposed key features of the plant. The depth also varied based on how the sample was oriented in the Paraffin. The correct plant biology of the Hoagland solution rye shoot was not accounted for because the sample was not centered, as shown in the right image of Figures 11 and 13. Compared to the uptake of Ca in Figure 12, the uptake of Mg was suppressed because only 2 to 3 counts were present in the roots and shoots, as demonstrated in Figure 14. The results in Figures 15-17 indicate that varying the concentrations of Ca and Mg did not affect the uptake of K in the shoots but did affect the uptake of S and P in the roots. The S and P distribution in the shoots was also affected by the sectioning of the Hoagland solution sprouted rye shoot.

Other plants from JRBP will be grown hydroponically to control the uptake of nutrients and metals such as Ca and Mg, and used to compare the element uptake to the uptake in rye.

C. Applications

The experiments performed on beamlines 2-3 and 14-3 provide further knowledge of the element uptake and distribution of serpentine-tolerant plants growing at Jasper Ridge, and can be applied toward similar naturally and artificially contaminated soil systems.

V. CONCLUSION

Serpentine soil contains high concentrations of metals such as Ni, Fe, Mn, and Cr that exceed levels found in other types of soil. In this study, plants grown hydroponically and on serpentine and potting soil were compared using µ-XRF spectroscopy to determine what differences exist between serpentine tolerant plants. Concentrations of Ni, Fe, Mn, and Cr were higher in the rye root compared to the purple needlegrass root. Accounting for error, the plants sprouted in serpentine soil contain similar concentrations of Mn, Ni, and Fe in the shoots and roots. The concentration of K, S, and P differed between the hydroponically grown rye sprouts.

The rye root was enriched in Ni, Fe, Mn, and Cr compared to the purple needlegrass root, meaning that rye on potting soil better suppressed the uptake of these metals compared to purple needlegrass on serpentine soil. The roots and shoots of serpentine vegetation equally suppressed the uptake of Mn, Ni, and Fe. Varying the concentration of Ca and Mg in plants affected the uptake of other elements such as K, S, and P.

In the future, different plants at JRBP will be grown hydroponically to control the distribution of metals and nutrients. These results will be used to further understand the uptake and distribution of ions in the roots and leaves and examine differences between various serpentine-tolerant plants. One can then hypothesize the plants' biological controls, and use known plant genomes to identify genes responsible for serpentine tolerance. The relationship between soil composition and elemental uptake in vegetation can be applied to similar metal-contaminated soil systems.

VI. ACKNOWLEDGEMENTS

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VII. REFERENCES

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[3] Emanuel Epstein and Arnold J. Bloom, *Mineral nutrition of plants: principles and perspectives*, 2nd ed. (Sinauer Associates, Sunderland, 2005).