Phosphorus and metal detection in freshwater mussels and water willow and biologic mitigation efforts in an urban stream restoration.

M. Behrendt, S. Carani, H. Easterday, M. Gerbuyos, T. Huber, A. Luna,
D. Vine, and K. Beardsley

Glenbard East

Argonne National Laboratory

ABSTRACT

The introduction of American water willow (*Justicia americana*) (Figure 1) and freshwater mussels, giant floater (*Pyganodon grandis)* and white heelsplitter *(Lasmigona complanata)* (Figure 2), will be used as a part of the restoration of the impaired Spring Brook Creek #1 in Wheaton, IL. The water willow will be used for stream bed stabilization and the freshwater mussels will be reintroduced into their natural habitat. The aim of this investigation was to determine if water willow and mussels helped in the reduction of the nutrient load of Spring Brook #1 creek. Water willow was grown in inert growth substrate and regularly watered in three sample groups: 0 ppm, 3 ppm, and 15 ppm phosphoric acid. Mussels were harvested from the stream just before analysis without being placed in different environments. Phosphorus and other elemental concentrations and distributions in both water willow and freshwater mussel mantle tissue were measured by synchrotron X-ray fluorescence (sXRF) at beamline 2-ID-E at the Advanced Photon Source, Argonne National Lab. There was no significant difference in tissue phosphorus levels in any of the treatments of stem and leaf in the water willow. A Pearson correlation coefficient R2=0.09 indicated that water willow tissue phosphorus concentrations were not correlated to increasing environmental phosphorus levels. Mussel mantle tissue did have multiple elements present. These results indicate sequestration of phosphorus is not present in species tested.

Figure 1. Water willow

INTRODUCTION

The ecological health of urban streams has recently gained interest alongside the increased spread of suburban and urban communities. Spring Brook #1 creek in Wheaton (figure 3) is an impaired stream due to increased nutrient load. Phosphorus levels are considered to have the greatest effect on the maintenance of healthy streams. (EPA, 2007) Local mitigation efforts are directed by the Forest Preserve District of DuPage County (Jessi DeMartini, FPDDC, personal communication, October 2014). The stream restoration plan, both physical and biological is, in part, a response to the water quality of the wastewater effluent from the Wheaton Sanitary District which regulates phosphorus effluent at about 3 ppm (3000 µg/L) (Sue Baert, Wheaton Sanitary District, personal communication, October, 2014). Further reduction of phosphorus may support sustainable creek ecology. The use of water willow for bed stabilization and the reintroduction of native freshwater mussels, in combination with the physical restoration efforts, is hoped to result in an improved stream ecology.

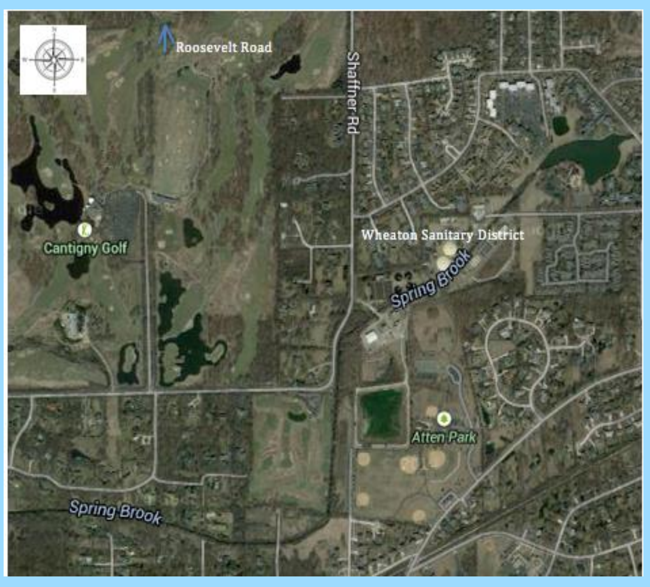

Figure 3. Spring Brook #1 in Wheaton, IL.

Figure 2. White heelsplitter

METHODS

Sample Preparation:

- Water willow plants were grown in greenhouse conditions in a vermiculite/peat moss growth medium. Three groups of plants were watered with a phosphoric acid solution of differing concentrations: 0 ppm, 3 ppm, and 15 ppm. Solution was applied with equal saturation for 12 weeks. In vivo samples of stem and leaf were prepared for analysis by sectioning with a razor blade. The samples had variable thickness. Freshwater mussels were harvested from Spring Brook creek #1 and kept live for sampling. Mussel in vivo mantle tissue was extracted for analysis. Stem, leaf and mussel tissue was placed on Kapton film for imaging.

Elemental Analysis:

- Sample analysis was completed at 2-ID-E x-ray microprobe beam line at the Advanced Photon Source at Argonne National Laboratory (see figure 5). The incident X-ray beam energy was 10.2 keV, that produces a focused beam size of 0.5µm x 0.4µm. Samples were raster scanned and the characteristic X-ray fluorescence spectra were used to determine the elemental composition of samples. X-ray fluorescence counts were converted to µg/cm² concentration using a calibration standard.

Figure 13: Students preparing samples in lab.

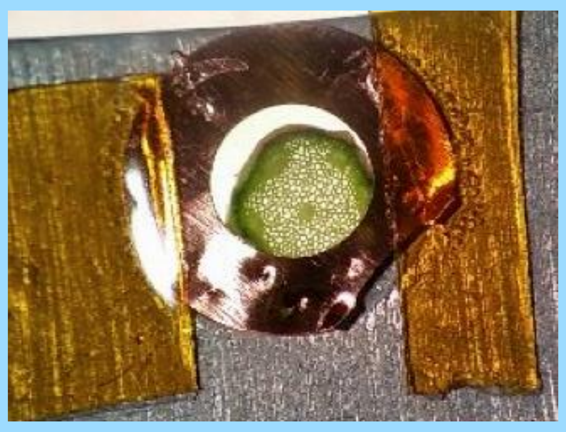

Figure 4: Sectioned stem sample

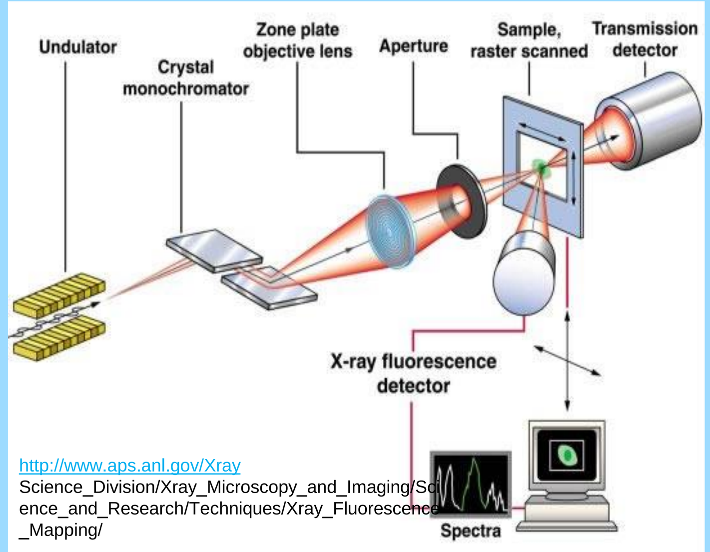

Figure 5. Schematic of X-ray fluorescence and detection at beamline 2-ID

DATA

Stem – Treatment - 3 ppm added Phosphorus

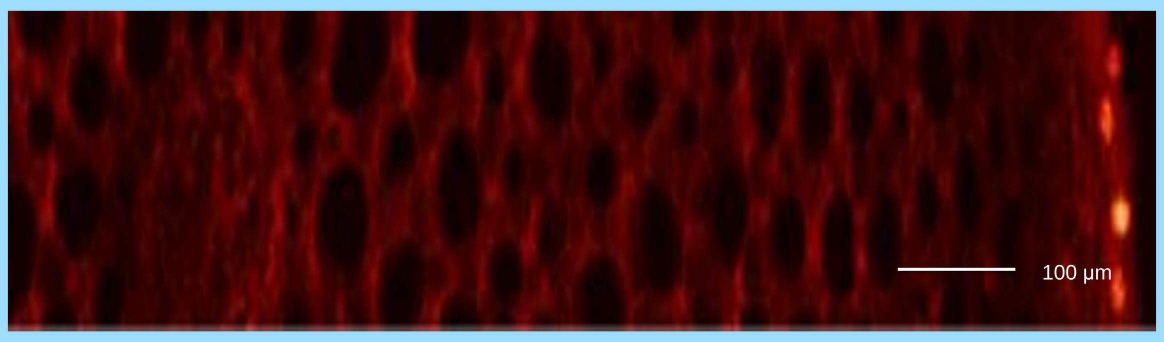

Figure 6. Phosphorus (red) distribution in stem. Image developed from sXRF fluorescence counts. Greater concentration shown at brighter regions.

Leaf – Treatment - 3 ppm added Phosphorus

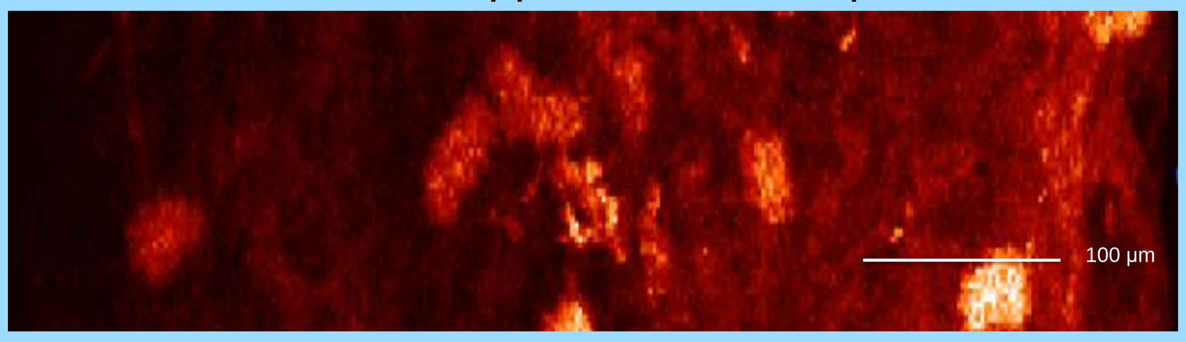

Figure 8. Phosphorus (red) distribution in leaf. Image developed from sXRF fluorescence counts. Greater concentration shown at brighter regions.

Mussel Mantle

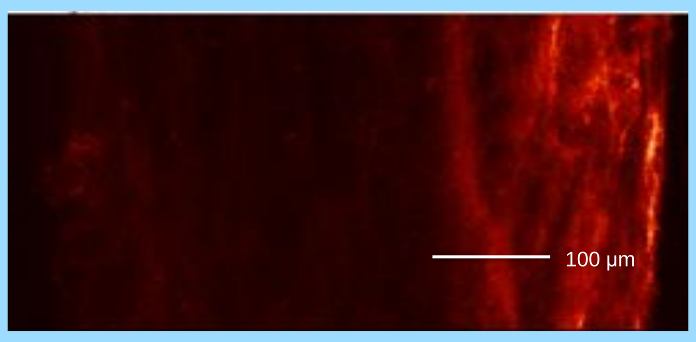

Figure 10. Distribution of phosphorus (red) in mussel tissue developed from sXRF fluorescence counts.

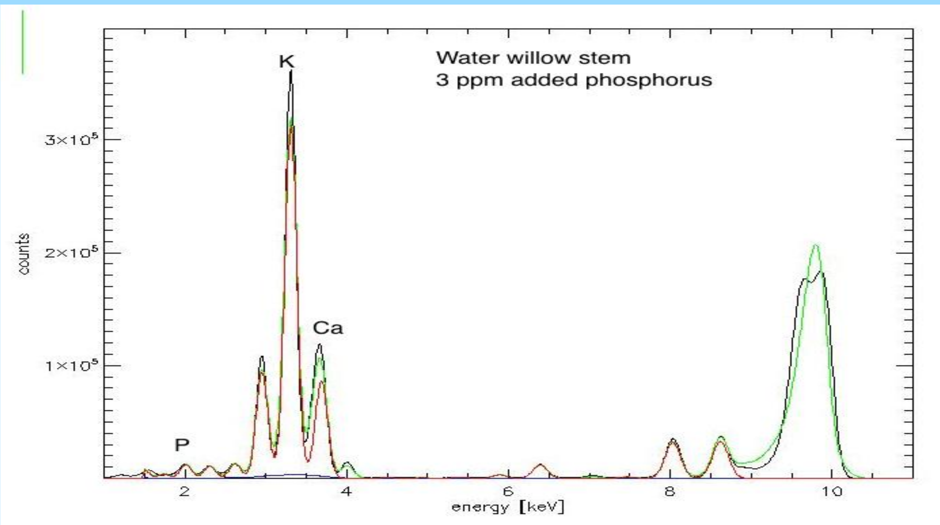

Figure 7: X-ray energy fluorescence spectrum of total scan of stem in 3 ppm phosphoric acid. Linear scale

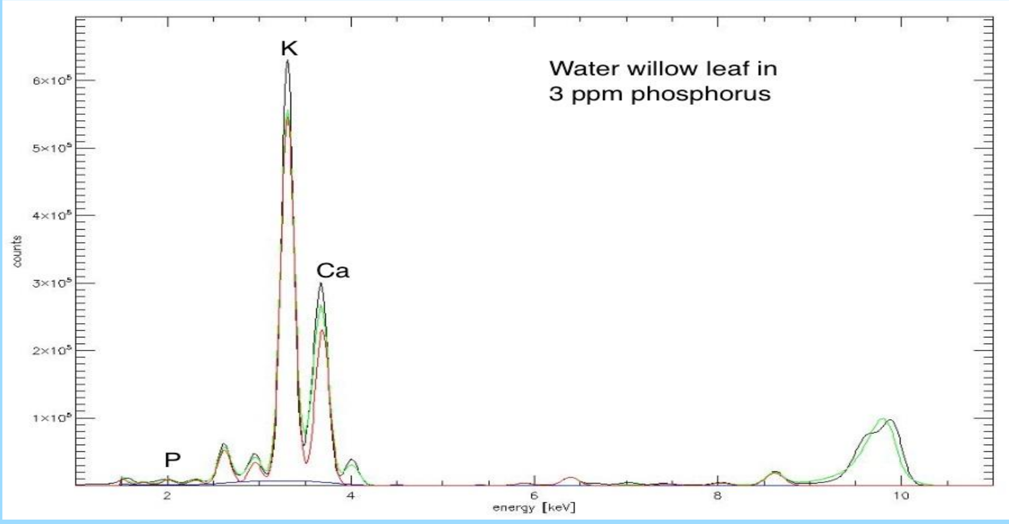

Figure 9: X-ray energy fluorescence spectrum of total scan of leaf in 3 ppm phosphoric acid. Linear scale

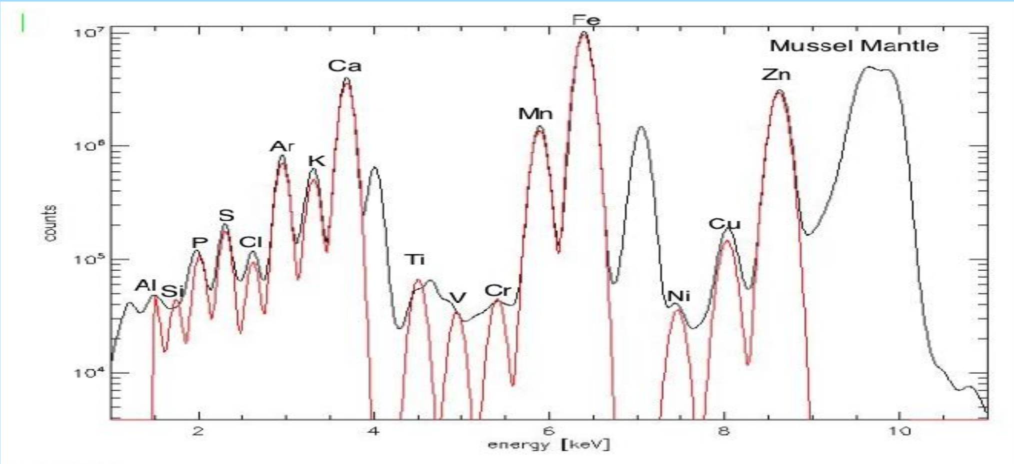

Figure 11: X-ray energy fluorescence spectrum of total scan of mantle tissue. Log Scale

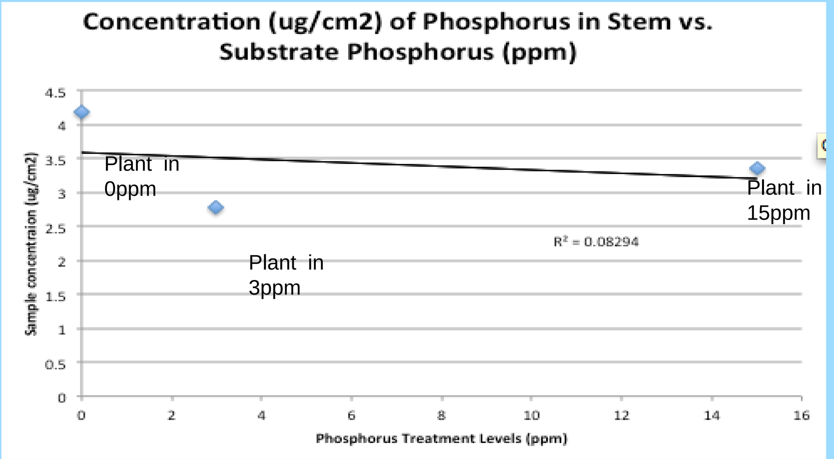

Figure 12: Linear regression used to determine correlation between measured stem phosphorus levels and phosphorus levels in growth medium substrate. R^2=0.08

DISCUSSION

Phosphorus levels in Spring Creek #1, as sampled by the Illinois EPA and First Environmental Laboratories, Inc. in Naperville, IL, range between 20 and 2000 micrograms per liter. Factors affecting this variation involve phosphorus cycling and myriad stream biota interactions in different seasons and environmental conditions. Phosphorus tissue concentrations in all treatments of water willow were very low compared to elements such as K and Ca (Figure 8). Phosphorus deposition in stem and leaf tissue (figures 6 and 8) is consistent with cell positions and is used by every organism's cells in basic metabolic processes such as respiration and photosynthesis. There is no correlation between phosphorus levels in stems and the phosphorus in the growth substrate as shown by a Pearson's coefficient value of 0.08 (figure 12). The areas higher concentrations of phosphorus in leaf tissue may be due to an aphid infection. There is no significant difference in phosphorus tissue levels between stems and leaves in the three treatment levels. When compared to other detected elements there are low levels of phosphorus in mussel mantle tissue. The uneven distribution may be due to sample tissue placement. High concentrations of calcium phosphate compounds are found in Australian fresh water mussels mantle granules (Byrne, et al., 2000). Although similar to Australian freshwater mussels in the presence of multiple elements in mantle tissue, there is no evidence of granules in the specie's tissue we imaged.

CONCLUSIONS

Although water willow is known to be an excellent stream bed stabilizer, its role in maintenance of healthy stream ecology by means of element bioaccumulation is unknown. This data shows there is no correlation between greater levels of phosphorus in plant substrate and phosphorus deposition in stem or leaf, therefore, we conclude that water willow does not act as a bioaccumulator of phosphorus. Environmental phosphorus levels also do not seem to have influence on increasing concentration of the other elements detected. Freshwater mussels are known to be an integral part of the filtration of stream waters and many species bioaccumulate elements, including phosphate species, in the mantle tissue. Our mussels did not show increased phosphorus in the mantle.

ACKNOWLEDGEMENTS

This research was made possible through the Exemplary Student Research Program, supported by Argonne National Laboratory's Educational Programs (CEPA), the APS User Office and Argonne National Laboratory; a U.S. Depart. of Energy laboratory managed by UChicago Argonne, LLC . Thanks to the following for their support: David Vine, Jessi DeMartini of Urban Streams of FPDDC, John Jakes of the US Forest Service, Sue Baert of Wheaton Sanitary District, Larry Creekmur of Country Road Greenhouse, Rochelle, IL, and Karen Beardsley of GEHS.

Figure 14. Thank You, David!

REFERENCES

Byrne, Maria, and Vesk, Peter A. "Elemental composition of mantle tissue granules in *Hyrideola depressa* from the Hawkesbury-Nepean River system, Australia: inferences from catchment chemistry." Marine Freshwater Research 51 (2000):183-92.

Correll, David L. "The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review." Journal of Environment Quality 2 7.2 (1998):261.

EPA, Region 10, Public Affairs Unit. "Advanced Wastewater Treatment to Achieve Low Concentration of Phosphorus." report(2007):

Fritz, Ken M. "Substratum Stability Associated with the Riverine Macrophyte Justicia americana." Freshwater Biology 48:1630-1639.

Illinois Environmental Protection Agency Bureau of Water, Permit Section. (2013). Water Quality Certification to Discharge into Waters of the State: Fact Sheet for Antidegradation Assessment for Illinois State Toll Highway Authority. (IEPA Log No. C-0396-12), Springfield, Illinois.