THE EFFECT OF COCROPPING ON METAL SEQUESTRATION IN *BRASSICA JUNCEA*

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ABSTRACT

Some plant species known as hyperaccumulators have adapted to soils high in metals. These plants sequester nonessential levels of metals within their tissues, isolating them from regular plant metabolic activities that would otherwise prove toxic to plant physiology. An ideal hyperaccumulator should accumulate a target metal, have high biomass in which metal sequestration occurs, and be easily grown without disturbance of existing ecosystems (Alaboudi et al. 2018). A known hyperaccumulator with these ideal traits is *Brassica juncea (B. juncea).* It is known to hyperaccumulate a variety of metals including Pb, Mn, Zn, Hg, Ni, and Cr (Bouquet *et al.* 2017; Goswami and Das, 2015; Rathore *et al.* 2017). The *Brassica* species sequesters metals through mechanisms such as binding metals to cell walls and moving chelated metals into vacuoles and trichomes (Hall 2002). Moreover, phytoremediation efficiency can be impacted by modifications to soil chemistry from non-hyperaccumulator species growing simultaneously in the same soil, often referred to as cocropping. A side effect of cocropping is altering the balance of soil nutrients, which can impact the hyperaccumulation efficiency of soil phytoremediators. The legume *Lupinus perennis (L. perennis)* is known to release organic acids from its root system, changing the soil rhizosphere chemistry, thereby allowing for greater trace metal uptake into the root systems of nearby plants in nutrient-deficient soils. (Kamh *et al.* 1999; Wiche *et al.* 2016). We evaluated the effect of cocropping *B. juncea* with *L. perennis* using microbeam synchrotron X-ray fluorescence (μsxrf), measuring the concentration and location of trace metals within both the monocropped and cocropped *B. juncea* stem and leaf tissues. Although cocropping of *B. juncea* with *L. perennis* had minimal impact on the ability of *B. juncea* as a whole plant to hyperaccumulate, the sequestration patterns of certain metals in whole leaf tissue were affected.

MOTIVATION

Soils in urban environments frequently contain high levels of metals due to anthropogenic activities which impact soils around the world and the resulting trophic transfer of these metals poses a risk to humans. Effective methods of soil phytoremediation are needed.

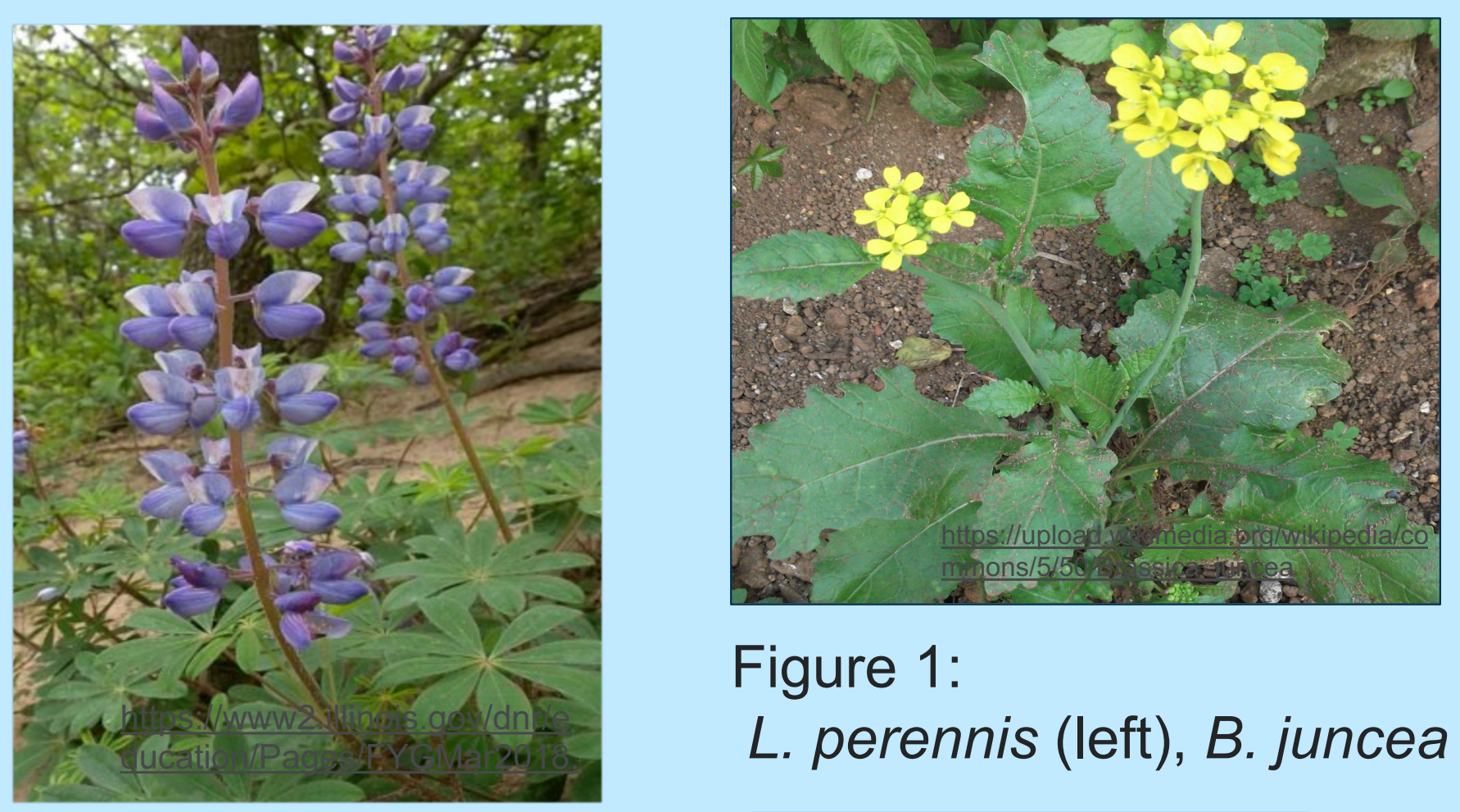

Figure 1:
L. perennis (left), *B. juncea* (right)

METHODS

- *L. perennis* and *B. juncea* plants were grown in pH 7 soil in similar growing conditions for 8 weeks.
- Two sets of *B. juncea* plants were monocropped and two sets of plants were grown in conjunction with *L. perennis* as a cocropped treatment.
- Elemental analysis of the mono and cocropped *B. juncea* plants was accomplished via Microbeam synchrotron X-ray fluorescence (μsxrf) using a 4-element silicon drift detector with an incident beam energy of 12KeV.
- Whole plant analysis was done with above ground plant samples that were dried and finely ground . The fine powder was placed on Kapton tape to measure whole plant elemental abundances. Stem analysis was done using 40 micrometer cross-sections mounted on Kapton film. Whole leaf analysis was done using in vivo leaf samples that were halved along the primary vein and placed on Kapton tape.
- Morphology of mono and cocropped *B. juncea* stem cross sections were evaluated by secondary electron imaging with a JOEL NeoScope 6000Plus SEM

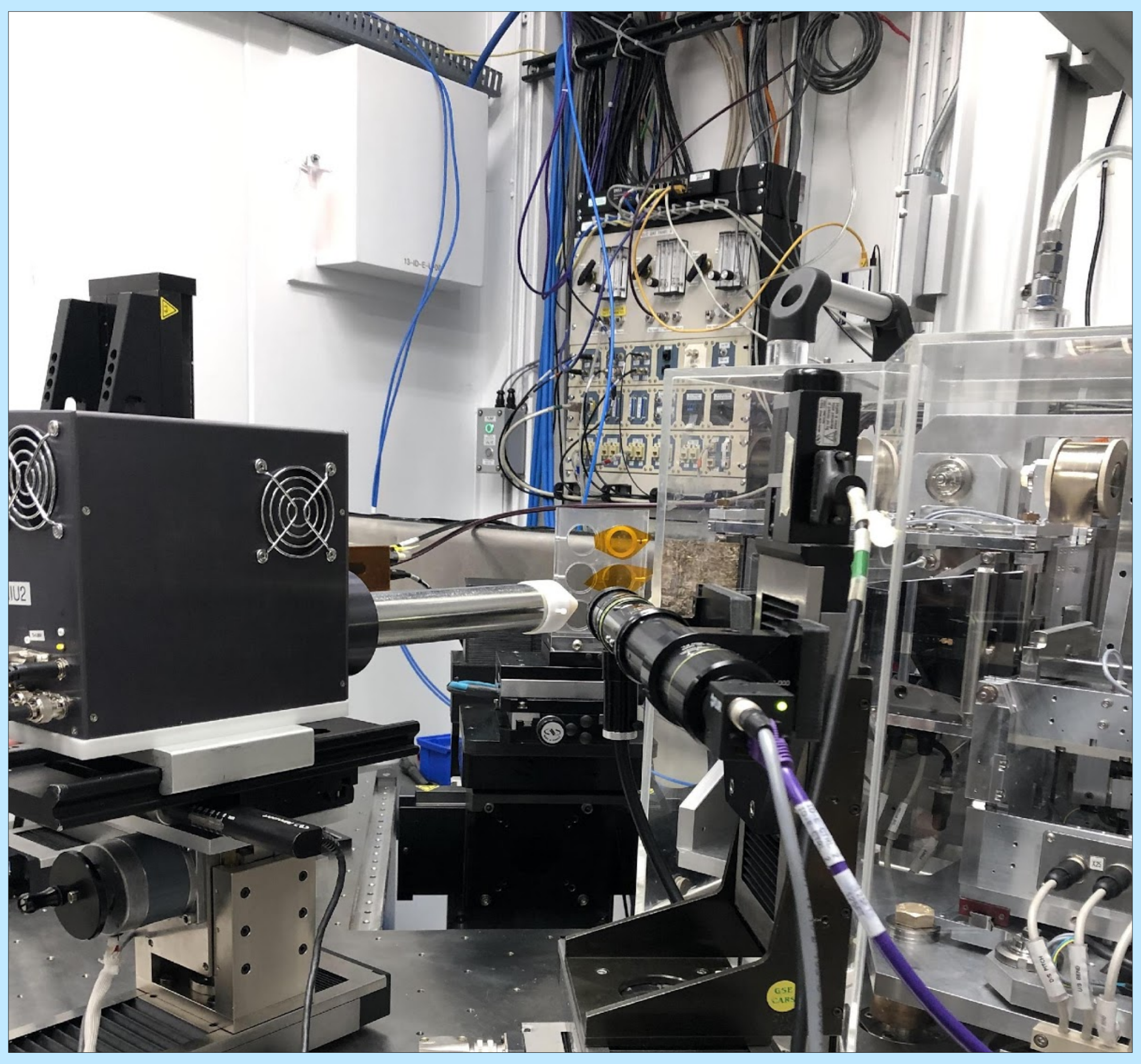

Figure 2 : Beamline 13 ID-E at GSECARS

RESULTS

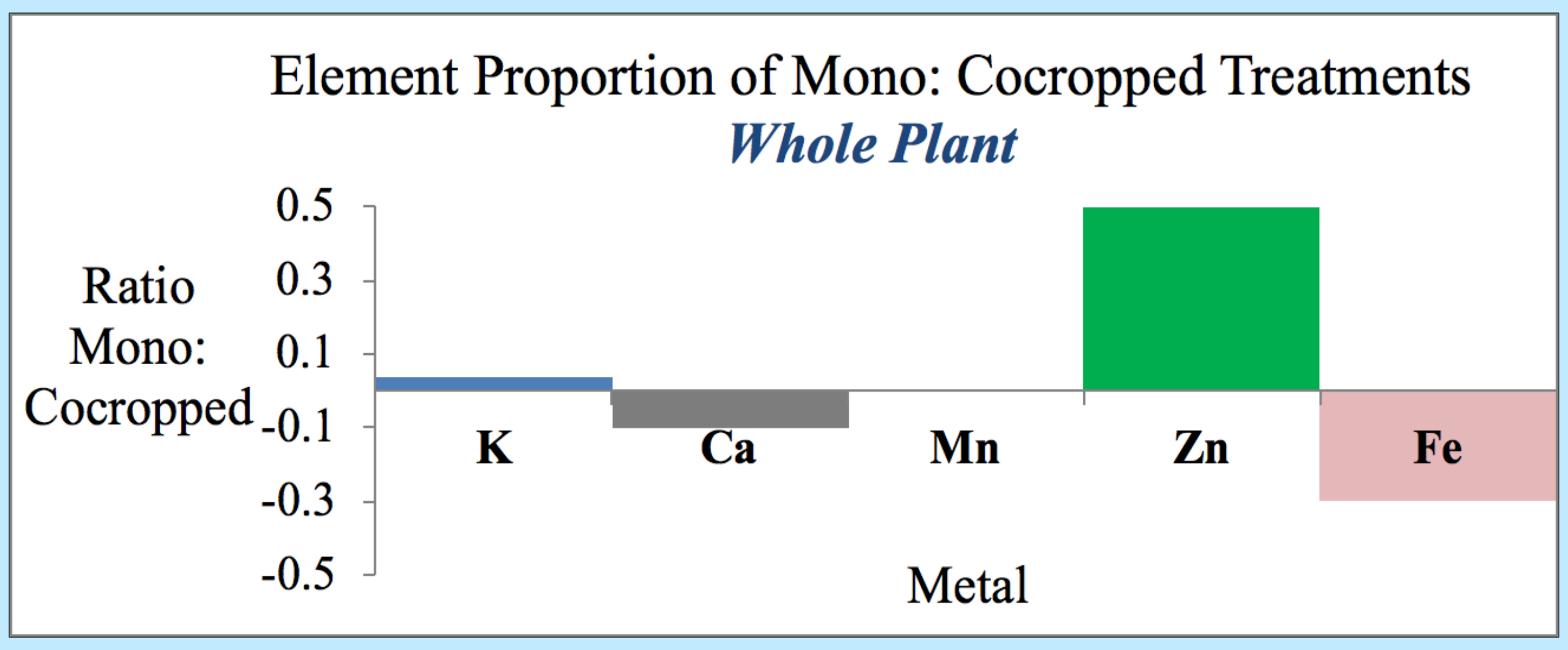

Figure 3: Comparative proportions of elemental abundances in cropping treatments of dried, crushed above ground whole plant tissue. Ratio is of K-alpha fluorescence counts.

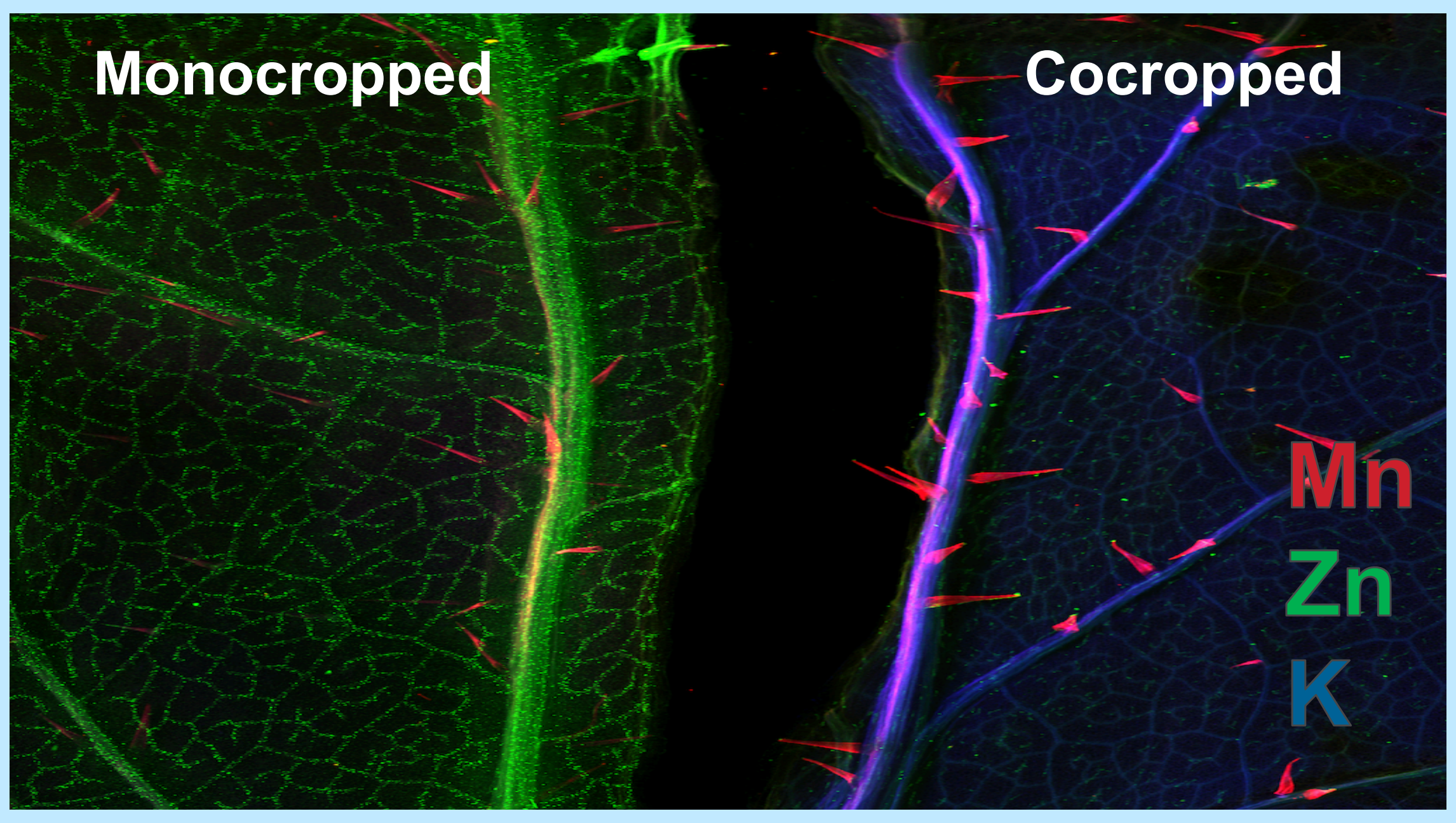

Figure 5: *B. juncea* whole leaf μsxrf 2D mapping images (10mm x 10mm) of K, Mn, and Zn. Monocropped (left) and Cocropped (right)

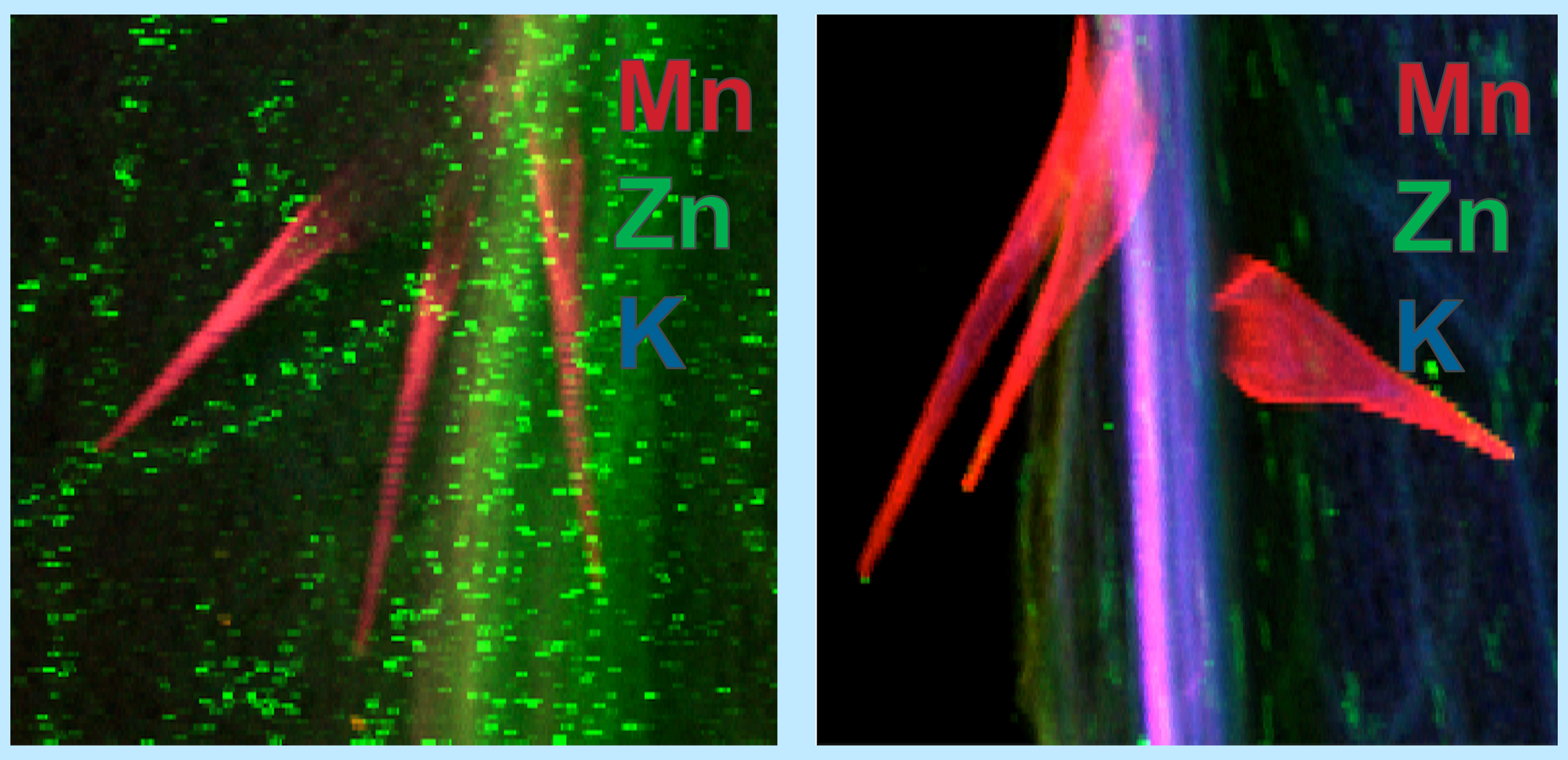

Figure 6: Metals found in trichomes (red) and surrounding vascular tissue (blue, green). Monocropped (left) and Cocropped (right)

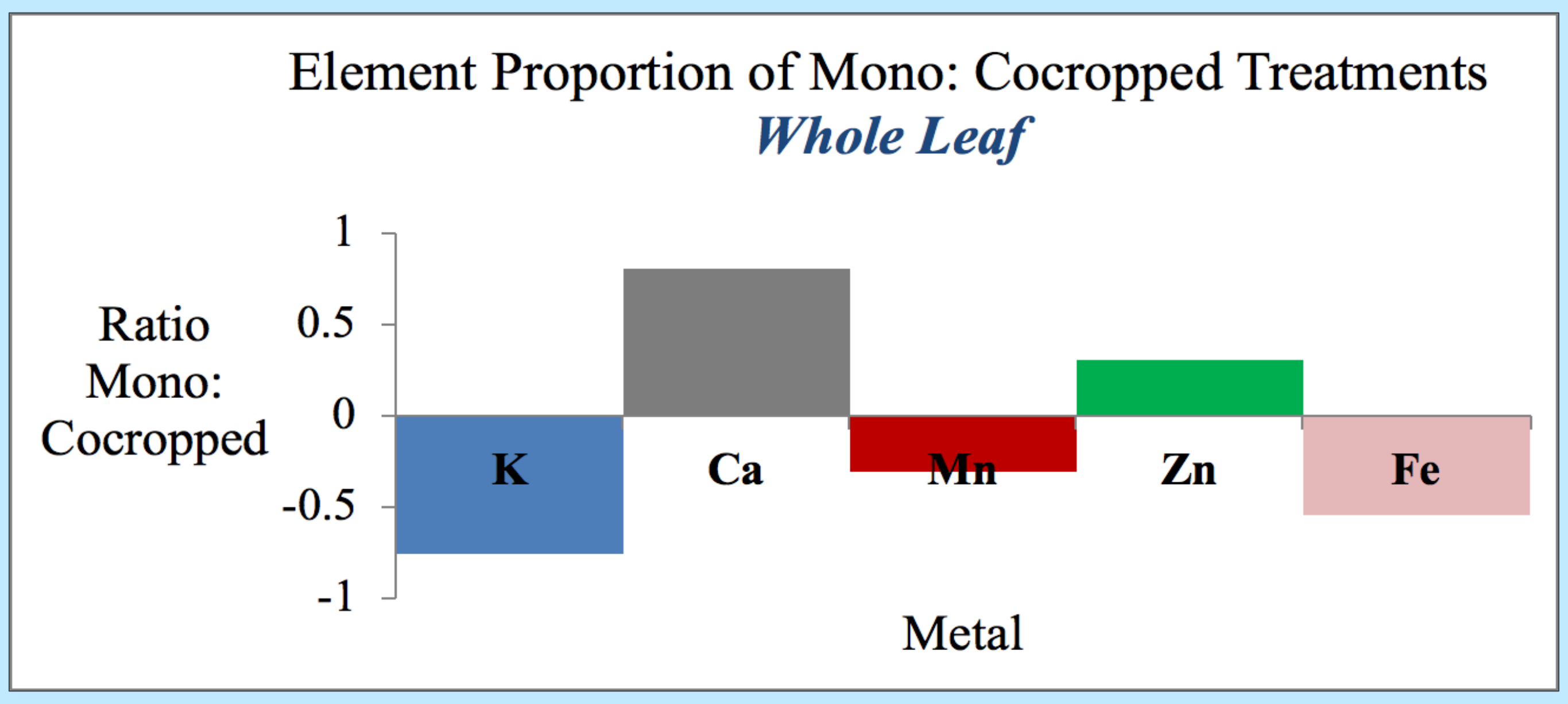

Figure 4: Comparative proportions of elemental abundances in cropping treatments of in vivo whole leaf tissue. Ratio is of K-alpha fluorescence counts.

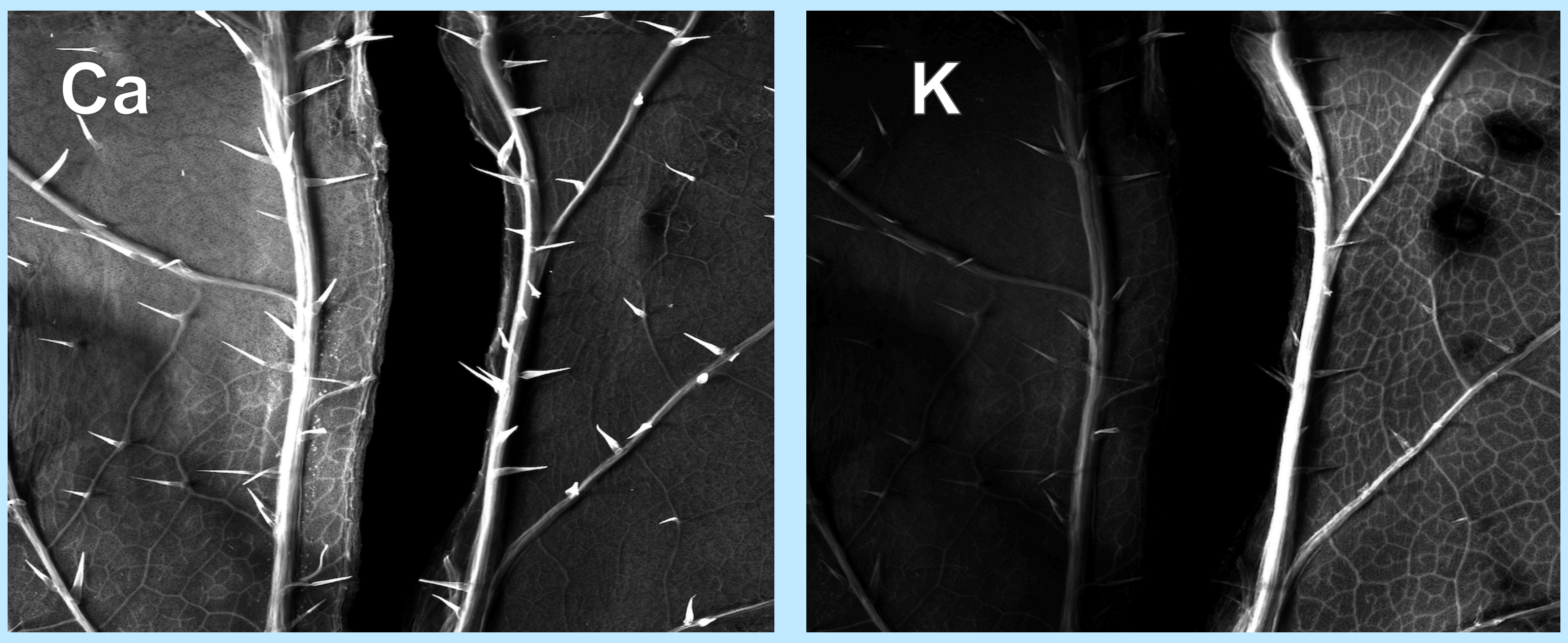

Figure 7 : *B. juncea* whole leaf μsxrf 2D mapping (10mm x 10mm) of Ca and K distributions. Images developed at same scale. Monocropped (left) and Cocropped (right)

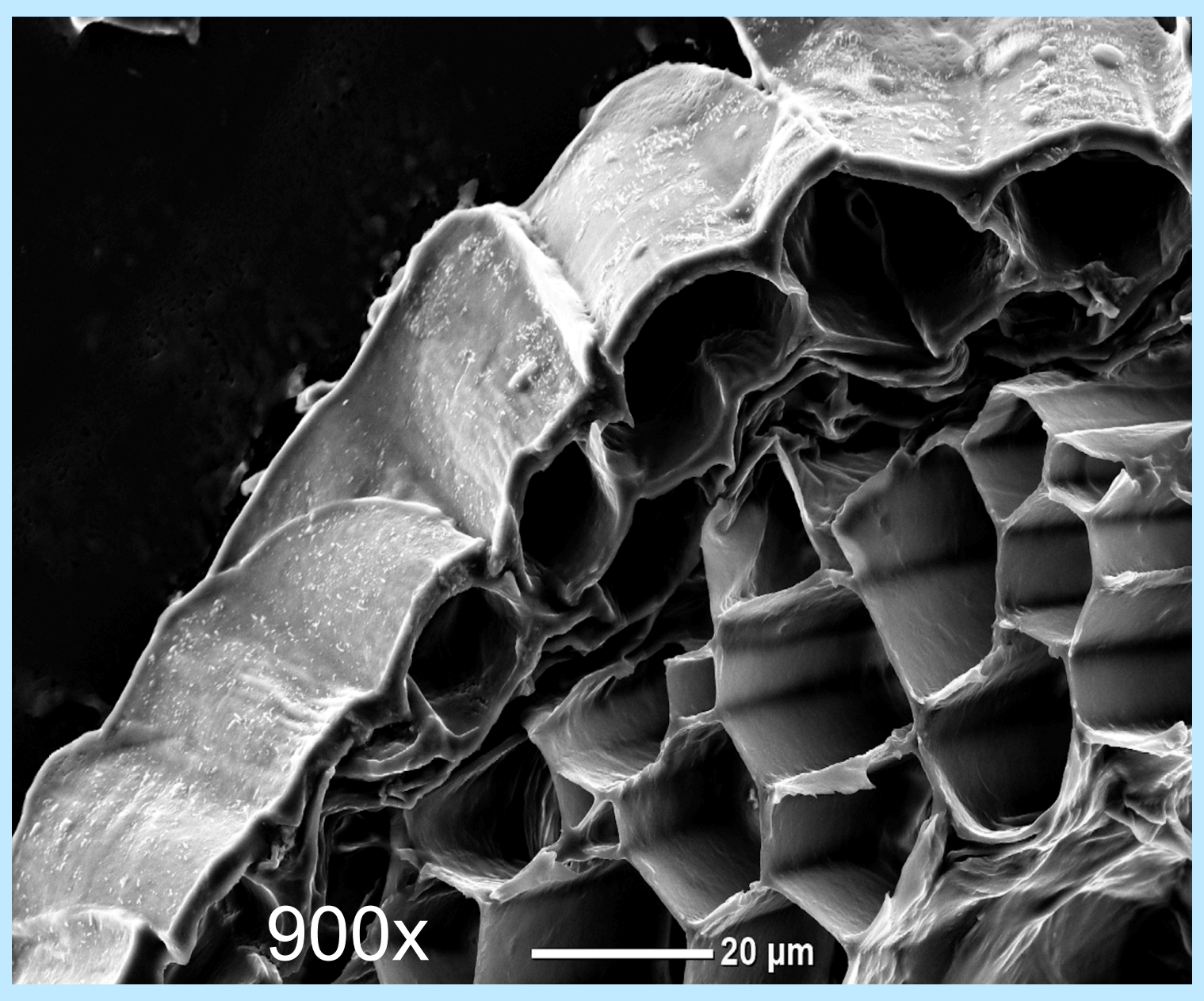

Figure 8: SEM image of *B. juncea* sectioned stem vascular morphology

CONCLUSIONS

Whole plant elemental abundances in *B. juncea* shows little difference between treatments. There appears to be minimal impact on the ability of *B. juncea* as a whole plant to hyperaccumulate the measured metals. When comparing whole plant to whole leaf tissue, the proportions change. When metals enter the plant some elements are found in greater quantity in leaf tissue (see fig 3, 4). In whole leaf tissue there are differences in metal abundance and sequestration patterns when comparing treatments. In whole leaf tissue there is proportionately greater Zn and Ca in *mono* versus cocropped treatments, however, there are greater levels of K, Mn, and Fe in *co* vs monocropped treatments (see figures 4,5,6,7). Trichomes had elevated Mn in both treatments, a possible sequestration site for this element. The variation in deposition amounts and patterns may be due to *L. perennis'* releasing organic acids from its root system, lowering the rhizosphere pH. When *L. perennis* is used in cocropping its presence would affect the rhizosphere pH of *B.juncea*. Because of this lower rhizosphere pH, metal complexes may form differently in mono versus cocropped treatments, possibly leading to varied metal transport pathways and sequestration sites through the *B. juncea* tissues (see figure 8) resulting in the varied sequestration patterns observed.

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