Electronic Supplementary Material (ESI) for Metallomics.

Electronic Supplementary Information

X-ray fluorescence spectroscopy (XRF) applied to plant science: challenges towards in vivo analysis of plants

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Equation S1. TR=E(Ws-Wr)/100S(1000-Ws)

Equation employed to assess the transpiration rate (TR) in soybean leaves according to Li COR™ infrared gas analyzer[1], where: E is air flow rate (µmol s^{-1}), Ws and Wr are sample and reference water mole fractions (mmol H_2O (mol air)$^{-1}$), and S is leaf area (cm^2).

Equation S2. PR=(F(Cr-Cs(1000-Wr/1000-Ws))/1000 S

Equation employed to assess the photosynthetic rate (PR) in soybean leaves according to Li COR™ infrared gas analyzer[1], where: F is transpiration (mol H2O m^{-2} s^{-1}), Cr and Cs are the is reference and sample mole fraction of CO2 (µmol CO_2 mol^{-1} air), and Ws and Wr are sample and reference water mole fractions (mmol H_2O (mol air)$^{-1}$), and S is leaf area (cm^2).

Equation S3. SC=1/(1/((g_{tw})-(k_f/g_{bw}))

Equation employed to assess the stomatal conductance to H_2O (SC) in soybean leaves according to Li COR™ infrared gas analyzer[1], where: g_{tw} and g_{bw} are the total and boundary layer conductance to water vapor (mol H2O m^{-2} s^{-1}), and k_f is a factor to estimate K of the fraction of stomatal conductance of one side of the leaf to the other.

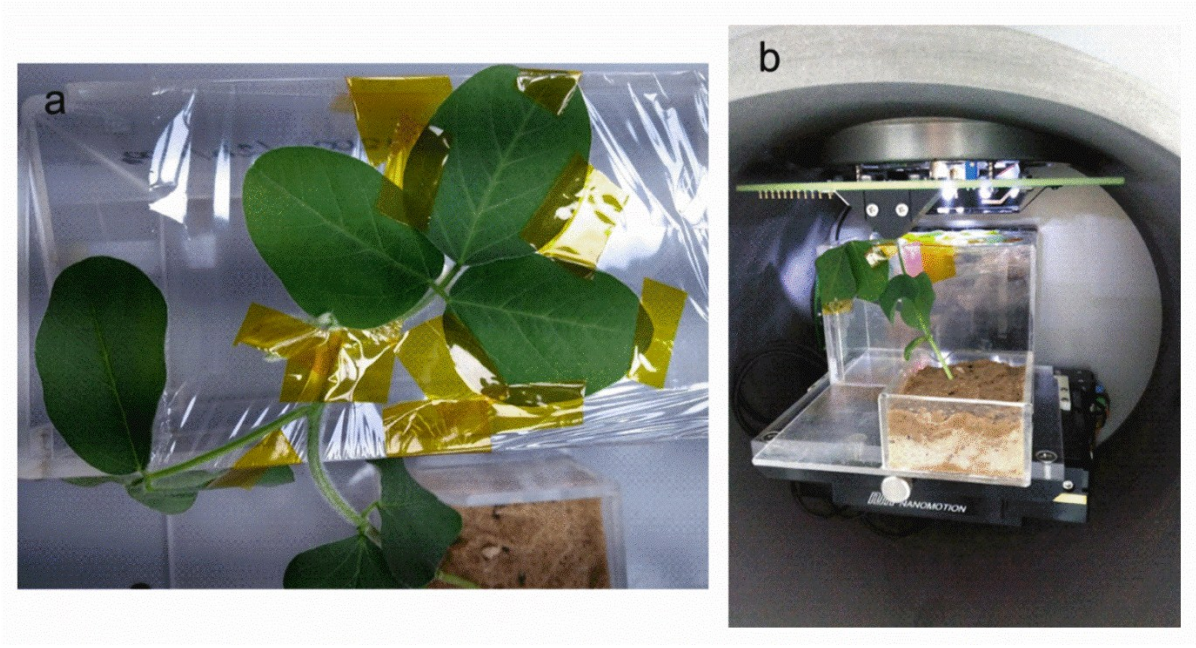

Figure S1. Representation of radiation experiment (case 1). Figure (a) show how leaves of soybean plants were fixed in the sample holder. Figure (b) shows how the experiment was carried out.

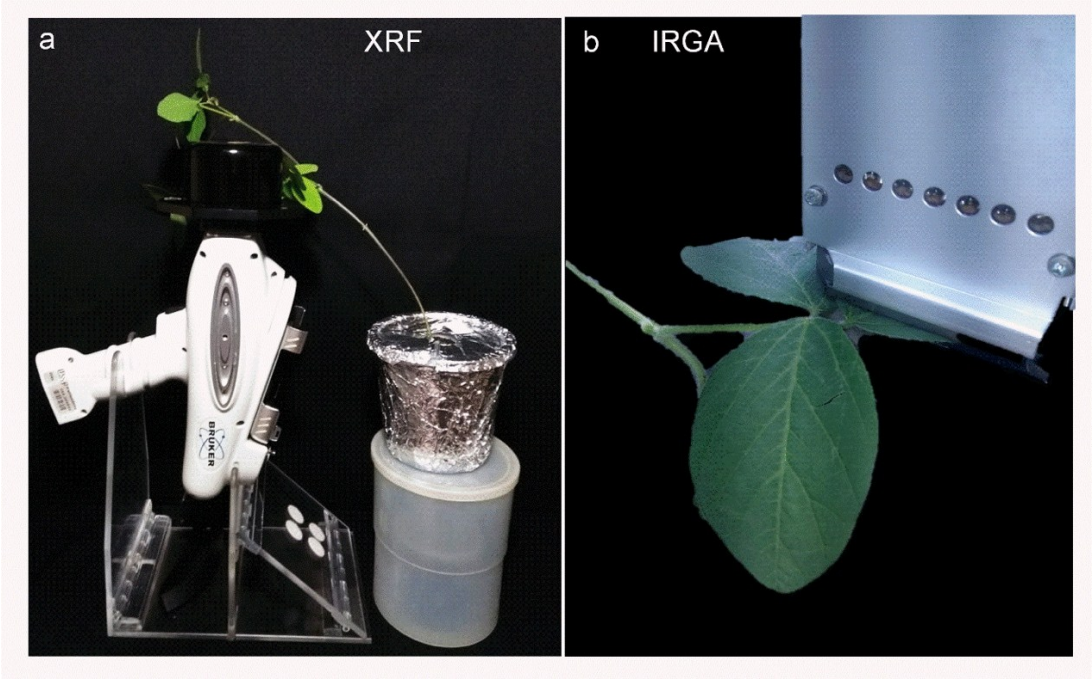

Figure S2. Experimental setups for the X-ray fluorescence (a) and Infrared Gas Analyzer (IRGA) (b) *in vivo* analysis. The X-ray fluorescence spectrometer determined Zn and Mn concentrations while the IRGA acquired the transpiration rate of soybean leaves.

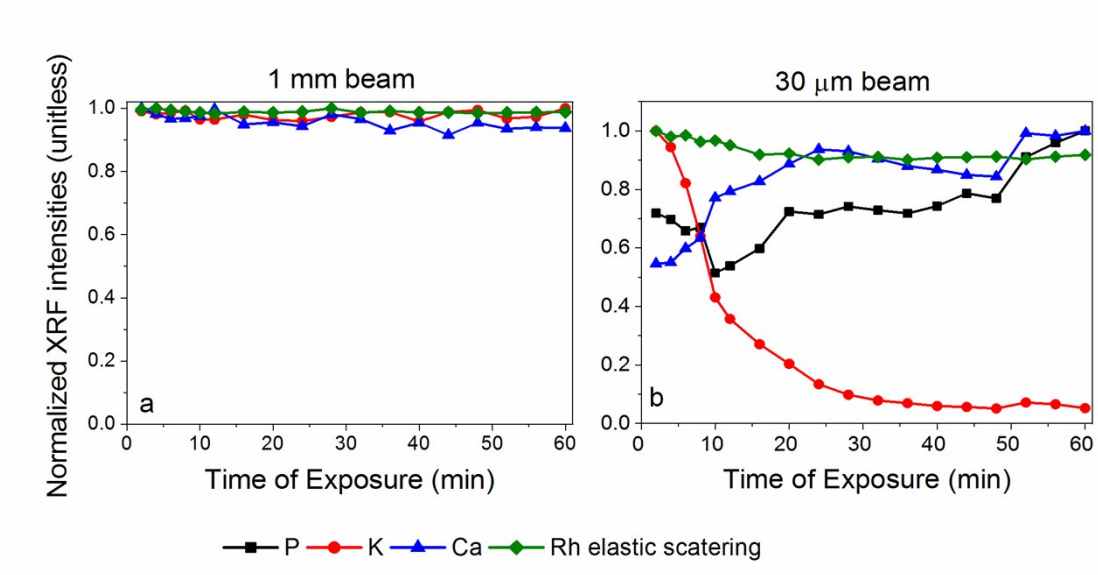

Figure S3. Biological replicate of the *in vivo* monitoring of normalized P, K, Ca, and Mn XRF intensities in soybean leaves exposed to (a) 1 mm collimated X-ray beam and (b) 30 μm focusing polycapillary during 60 min by employing a Rh X-ray tube at 45 kV and 900 μA. All elements were normalized by their corresponding maximum intensity. Note a variation on the elemental composition caused by the 30 μm focusing polycapillary X-ray beam.

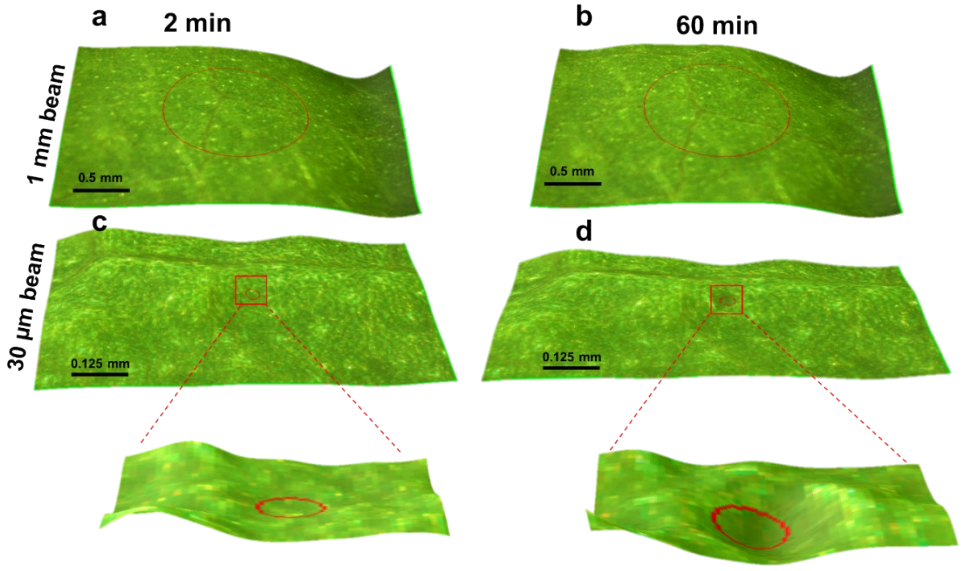

Figure S4. 3D photographs of soybean leaves irradiated by 1 mm collimated X-ray beam for (a) 2 min and (b) 60 min; Normal and high magnification of leaf irradiated by 30 µm focused X-ray beam for (c) 2 min and (d) 60 min. The red circle represents the X-ray beam location. Tissue damage was observed only at the leaf irradiated by the 30 µm beam for 60 min. The images were transformed in 3D using the ImageJ software. Magnification: (a) and (b) - 70-fold; c) and (d) 200-fold for normal, and 400-fold for high magnification

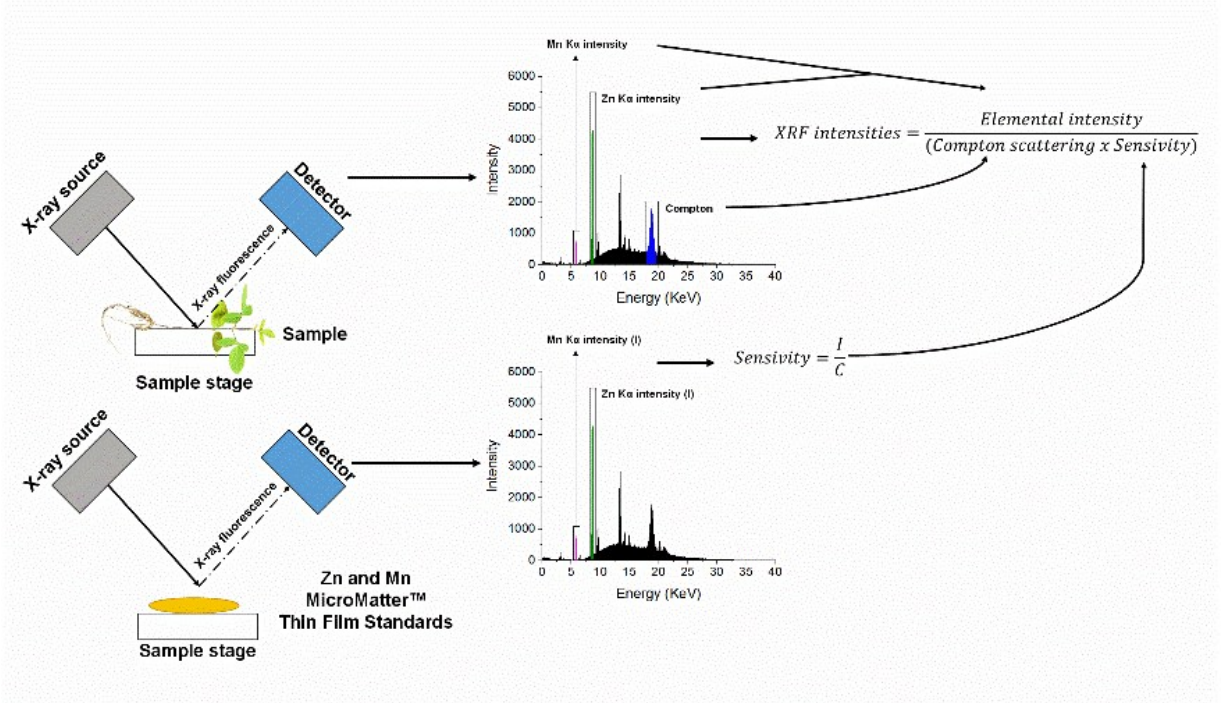

Figure S5. Representation of XRF data treatment steps.

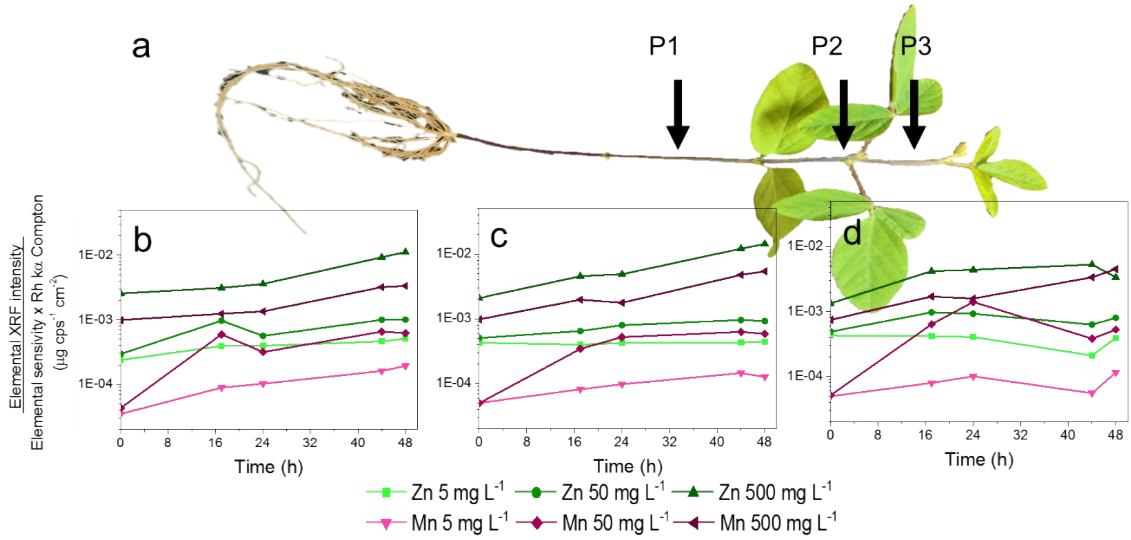

Figure S6. Biological replicate of time-resolved *in vivo* Mn and Zn XRF intensities at the stem of soybean plants exposed to hydroponic solutions containing 5, 50 and 500 mg L^{-1} of Mn and Zn for 48 h; (a) picture of presenting location of the measurements at the stem; Mn and Zn XRF signals at (b) P1 (basal), (c) P2 (middle), and (d) P3 (apical) points. The elemental intensity increased as a function of concentration and time. A root to shoot dilution effect was observed.

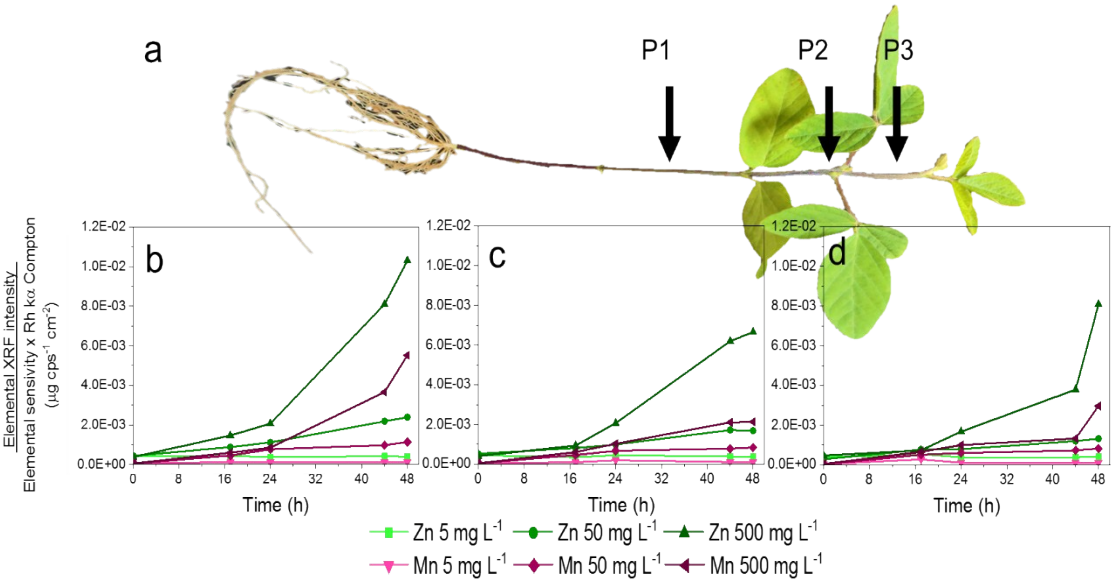

Figure S7. Linear scale plot of the XRF Mn and Zn intensities of soybean stems presented in Figure 3.

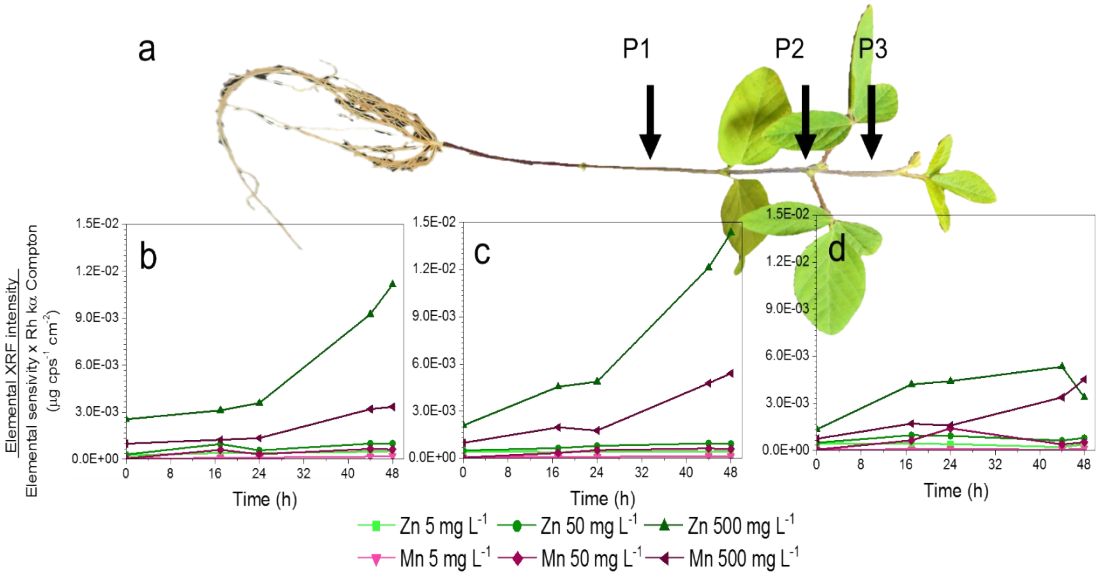

Figure S8. Linear scale plot of the XRF Mn and Zn intensities of soybean stems presented in Figure S5.

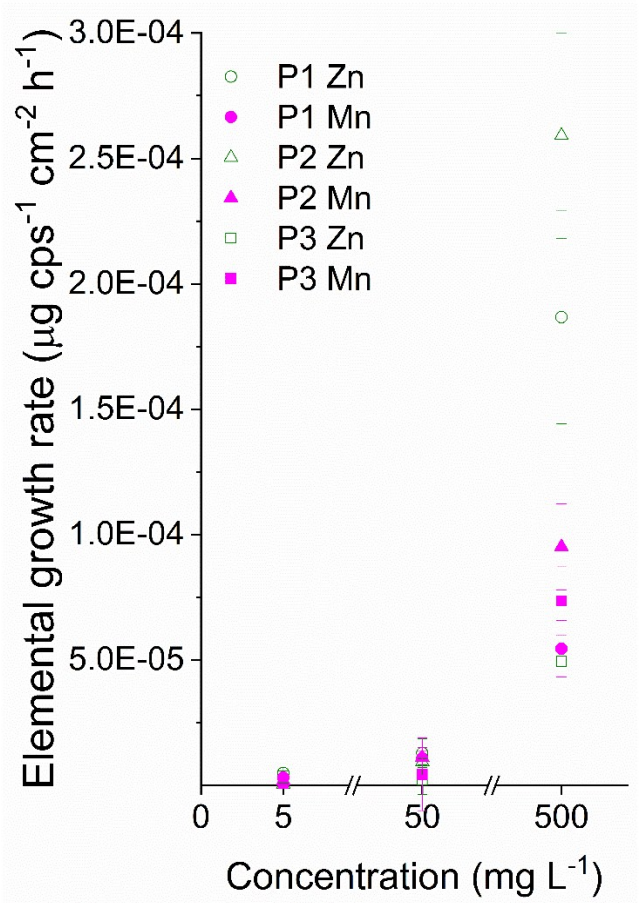

Figure S9. Elemental growth rate at the stem as a function of exposure concentration for the replicate shown in Figure S6. The concentration of Zn and Mn increased accordingly to the concentration of the solution. This effect was more evident in the lower parts of the stem (P1>P2>P3).

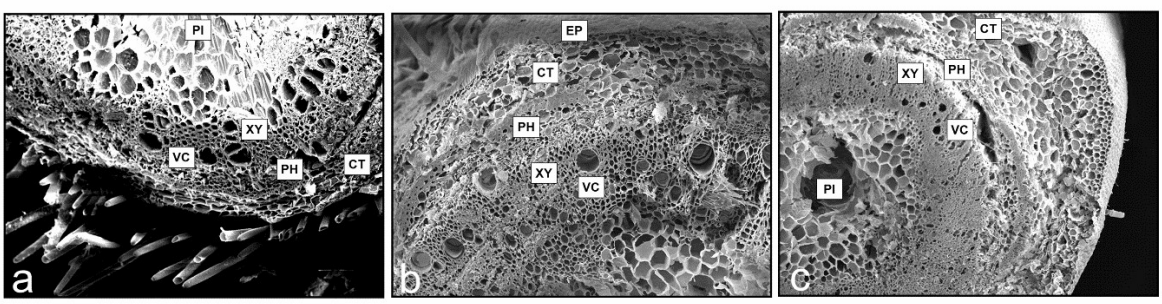

Figure S10. Scanning electron micrographs of *Glycine max* (L.) Merrill. A-C Cross-sectional planes at different heights along the stem. A– Apical region. B. Median region. C – Basal region where the trichomes are absent, the secondary xylem well developed, and the fistulated pith comparing to region P1. CT – Cortex; EP – Epidermal; PH – Phloem; Pi – Pith; VC – Vascular Cambium; Xy – Xylem.

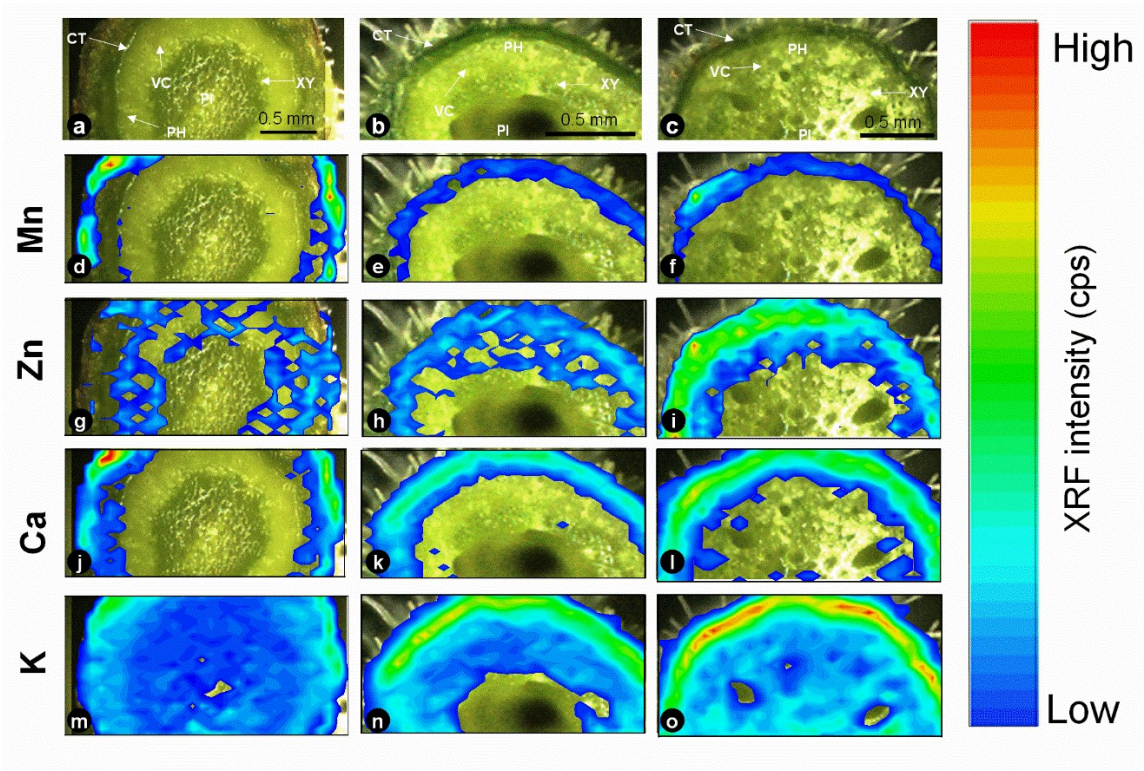

Figure S11. XRF maps of free-hand cross-sections of *Glycine max* fresh tissue for Mn, Zn, Ca and K at the stem of soybean plants exposed to hydroponic solution containing 500 mg L^{-1} of $ZnSO_4$ and $MnSO_4$ for 48h; (a-c) presents pictures of the mapped cross-sections, (d-f) shows the Mn spatial distribution in P1, P1, and P3, respectively. Subsequently, Zn (g-i), Ca (j-l), and K (m-o) are reported. Note that Zn and Mn assume different transport routes within the stem, which are similar to K and Ca, respectively. CT – Cortex; EP – Epidermal; PH – Phloem; Pi – Pith; VC – Vascular Cambium; Xy – Xylem.

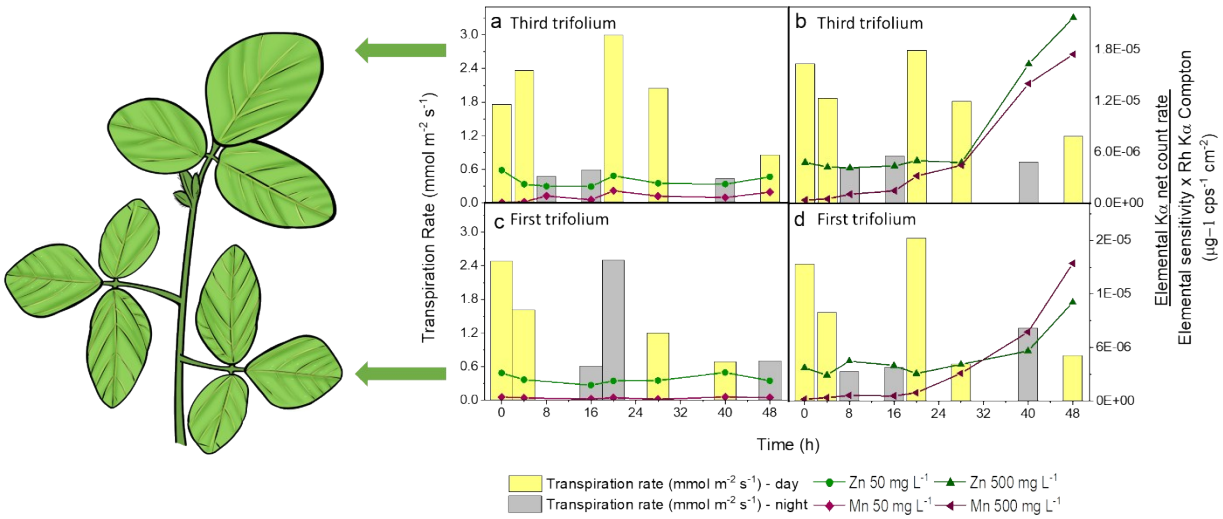

Figure S12. Biological replicate of Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis). Transpiration rate monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. Besides, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

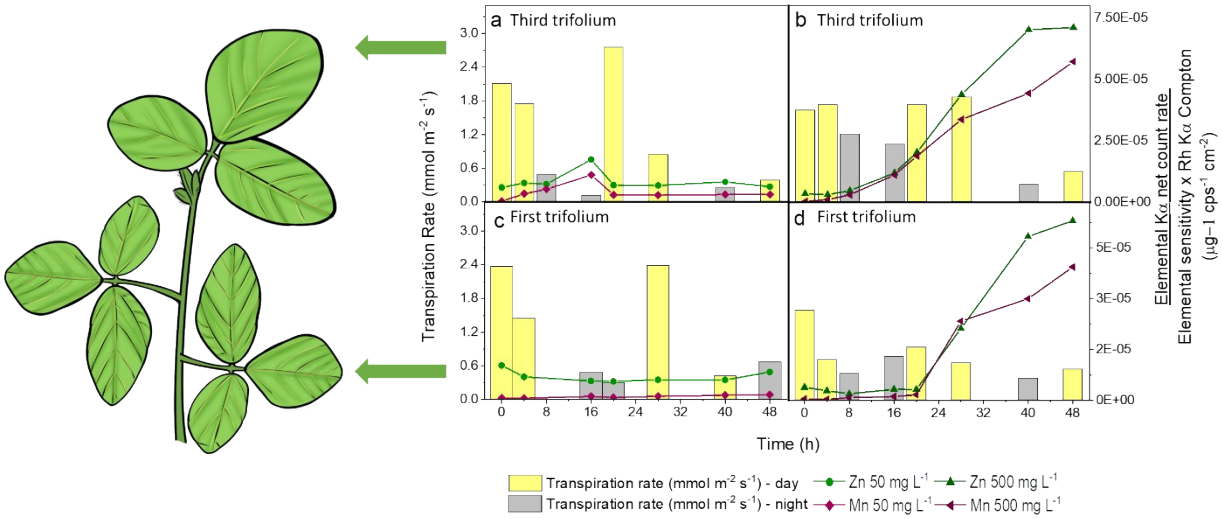

Figure S13. Biological replicate of Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis). Transpiration rate monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. In addition, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

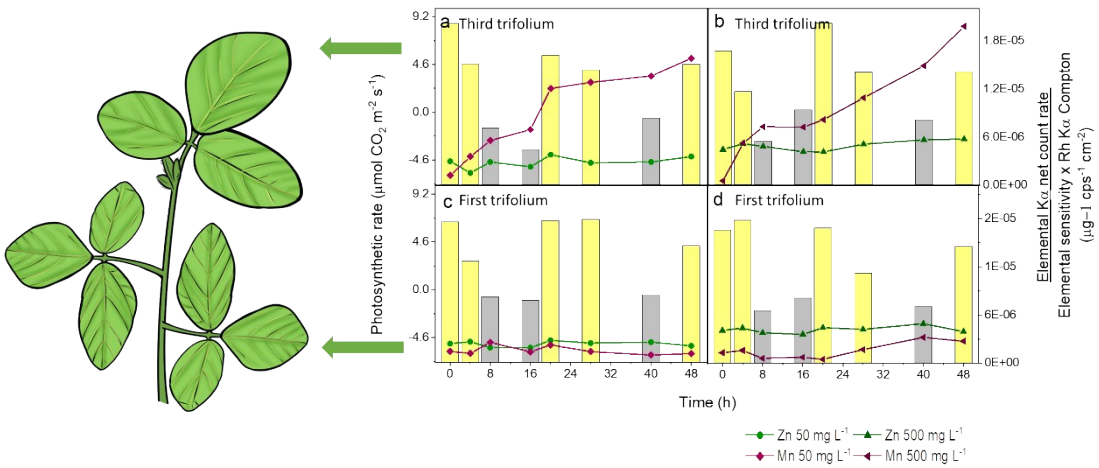

Figure S14. Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis), and photosynthetic rate monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. In addition, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

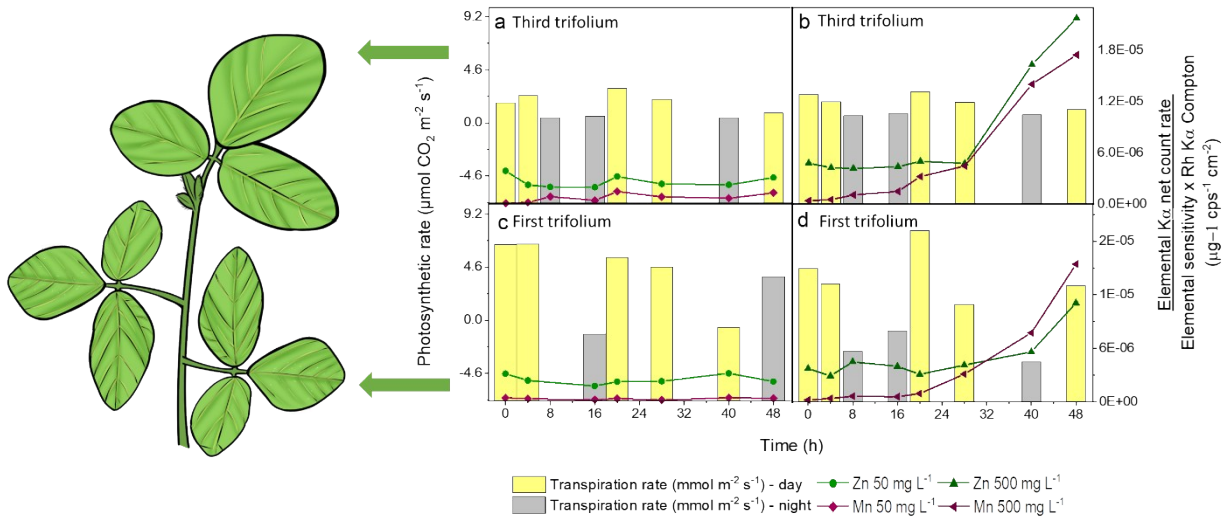

Figure S15. Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis), and photosynthetic rate monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. In addition, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

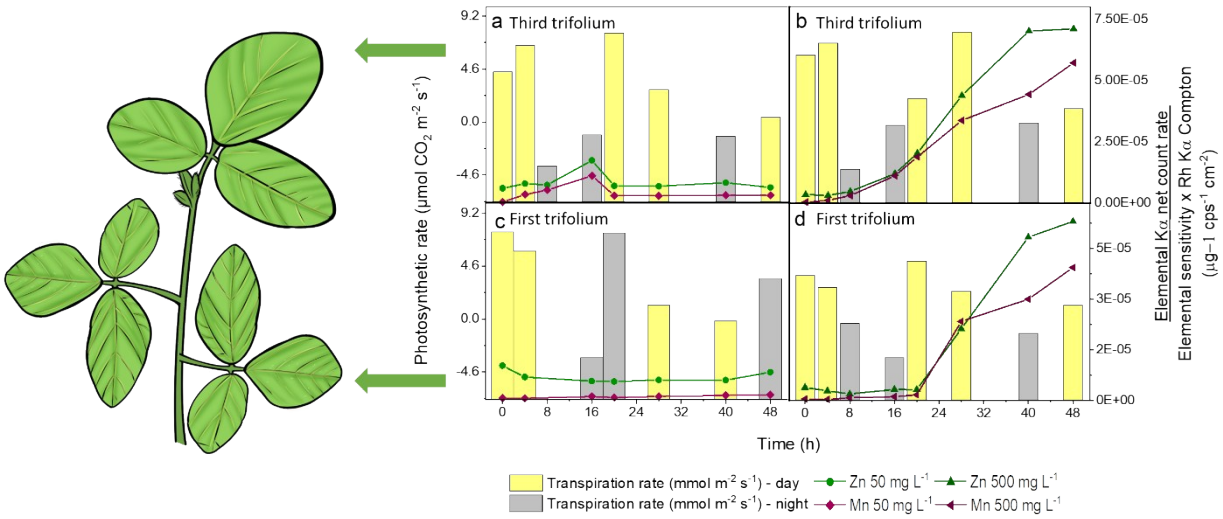

Figure S16. Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis), and photosynthetic rate monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. In addition, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

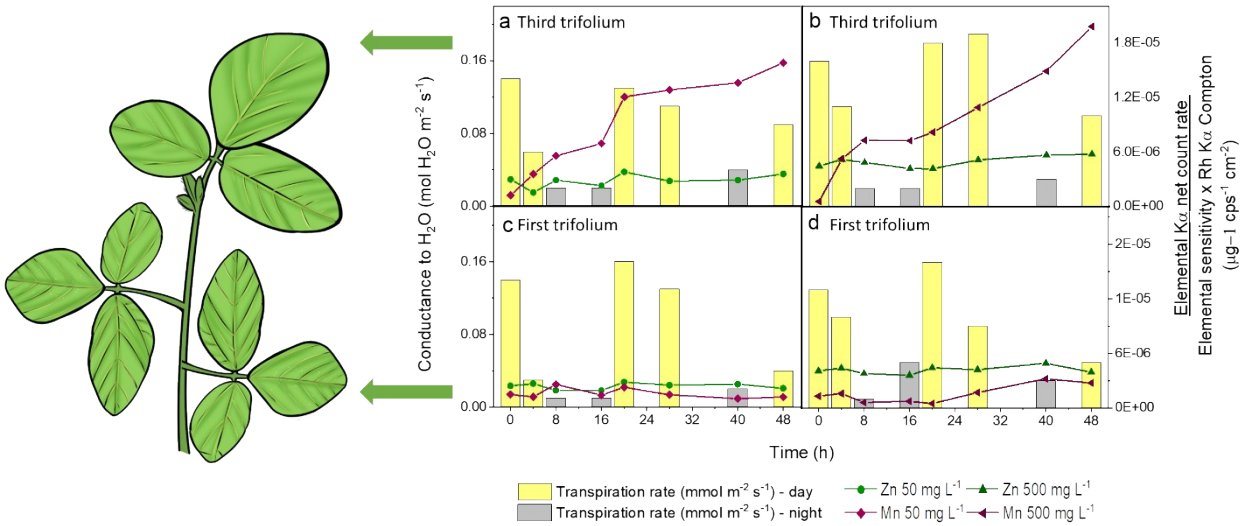

Figure S17. Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis), and stomatal conductance to H_2O monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. In addition, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

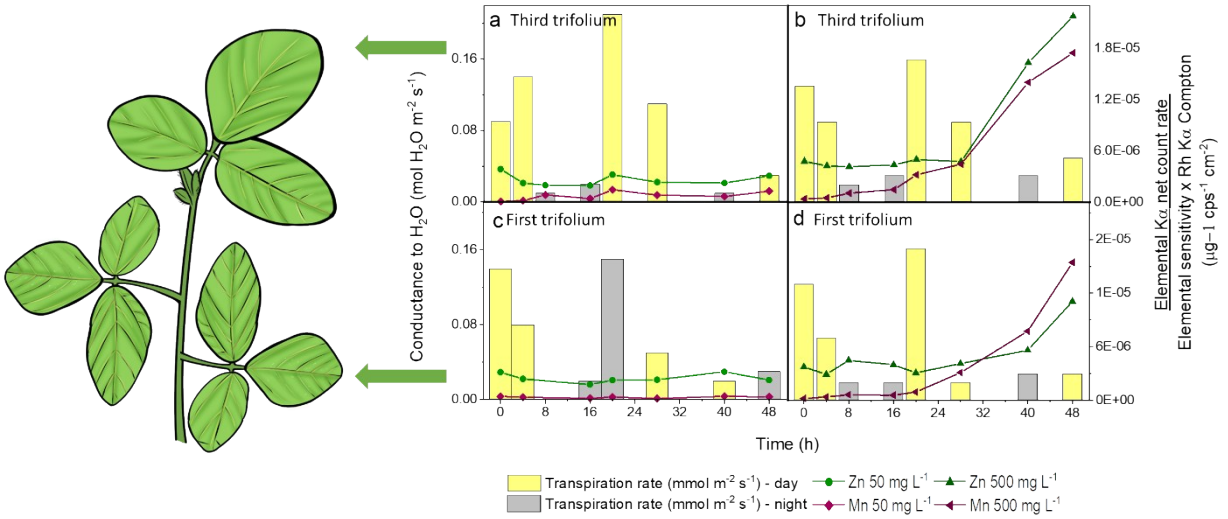

Figure S18. Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis), and stomatal conductance to H_2O monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. In addition, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

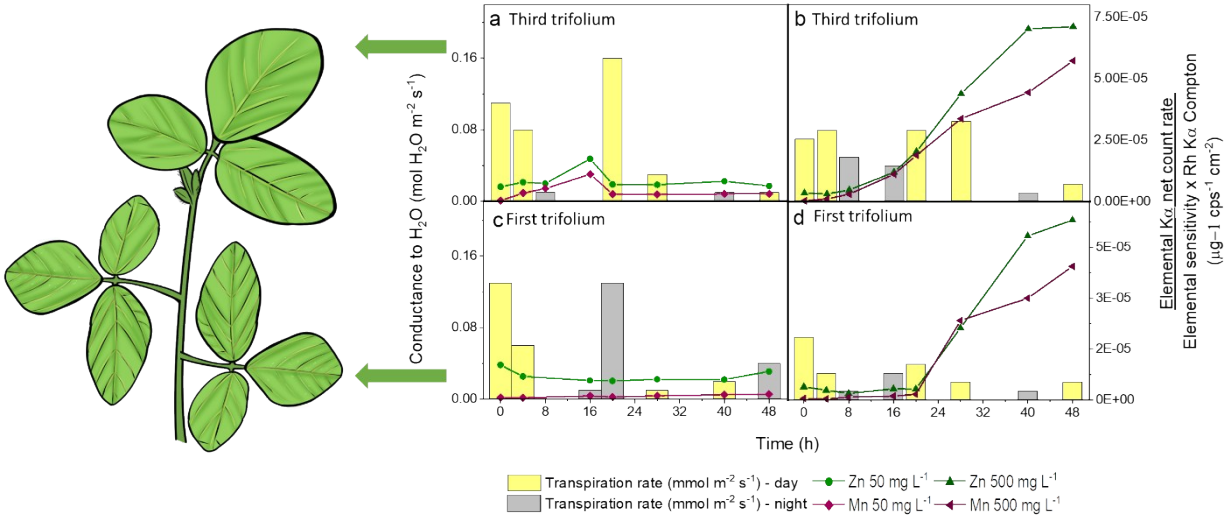

Figure S19. Mn and Zn XRF intensities recorded for soybean leaves by handheld XRF spectrometer (right y-axis), and stomatal conductance to H_2O monitored in the same leaves using infrared gas analyzer (IRGA, left y-axis). The plant roots were exposed to Mn and Zn. (a) and (b) show the data recorded at the third trefoil of plants exposed to 50 and 500 mg L^{-1}, respectively. In addition, (c) and (d) show the data recorded at the first trefoils, as shown by the arrows in the draw, of plants exposed to 50 and 500 mg L^{-1}, respectively.

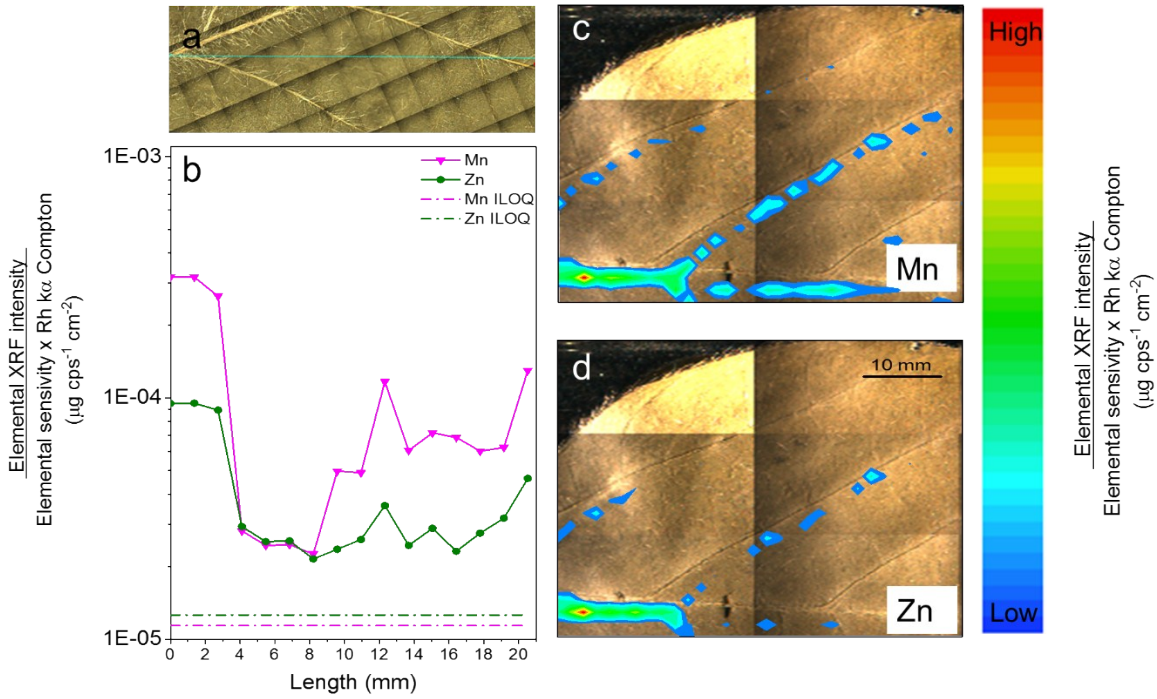

Figure S20. Biological replicate of XRF maps showing spatial distribution of Mn and Zn at soybean leaves; (a) picture of the area probed by linescan strategy, (b) Mn and Zn count rate determined through line scan in plants treated with 50 mg L^{-1}, respectively; Chemical images revealing the spatial distribution of (c) Mn and (d) Zn at leaves of plants treated with 500 mg L^{-1}. The element count rate was higher at veins partially due to the larger thickness of this structure.

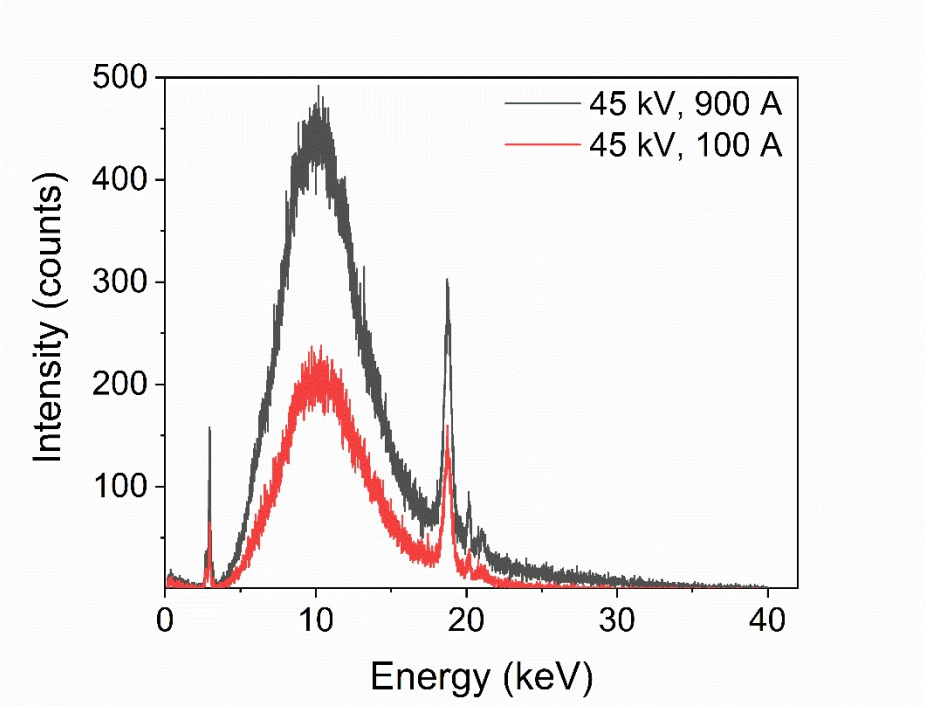

Figure S21. Comparison between X-ray fluorescence scattered spectra recorded in an acrylic sample using a 30 µm beam at 45 kV combined to 900 (black line) and 100 µA (red line).

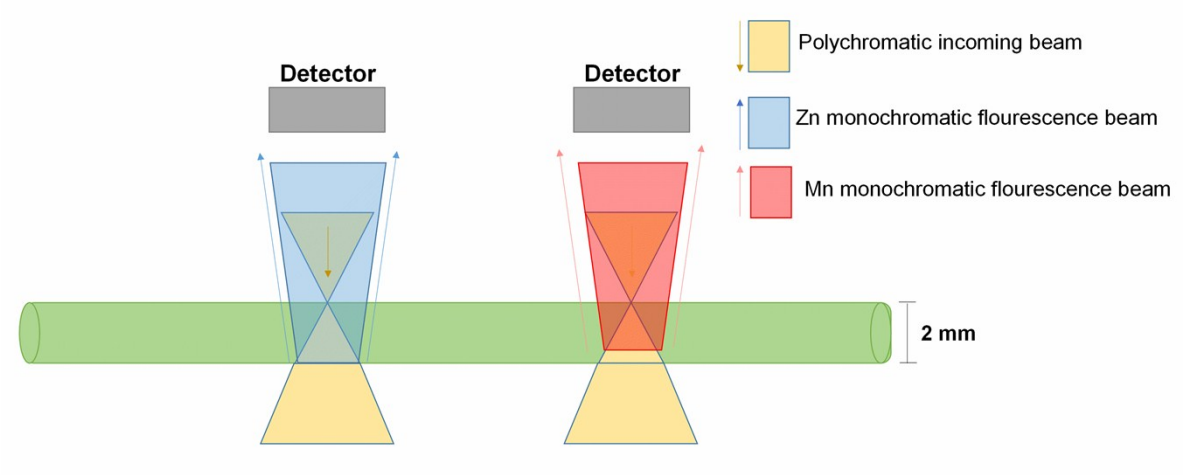

Figure S22. Representation of Zn and Mn interaction with the X-ray during X-ray fluorescence analysis. The blue polygon and arrows indicate the Zn monochromatic energy of the 2 mm soybean stem, while the pink polygon and arrows indicate the same phenomena for Mn. Note that Mn photons would hardly escape from depths below 1.5 mm, while the photons emitted by Zn would come from depths even higher than 2 mm.

REFERENCES

1. LI-COR, *Using the LI-6400: Portable Photosynthesis System*, Version 5, LI-COR Biosciences, Lincoln, 2004.