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Synchrotron-based X-ray fluorescence spectroscopy unfolding the pathway of Zn and Mn absorption by coffee leaves

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The foliar fertilisation with zinc (Zn) and manganese (Mn), essential micronutrients on vegetal metabolism, contributes to increasing crop species' productivity. Due to their widespread deficiency in weathered soils, this is particularly important in Brazil, the most important producer of coffee worldwide. Although foliar-based strategies often regard the use of chelates, such as EDTA, their uptake movement and tissue-partitioning dynamics in coffee leaves are not understood. In this regard, the apical leaves of 6-month-old coffee (*Coffea arabica*) plants were herein harvested 48-h past the exposure either to Zn: Mn-EDTA or Zn: Mn-IDHA solutions (1:1 Zn: Mn atomic ratio), then cryofixed through rapid plunging in supercooled isopentane, cross-sectioned in a cryo microtome at -25°C , and measured through high-resolution X-ray fluorescence spectroscopy yielded by a ca. $0.15 \times 0.15 \mu\text{m}$ X-ray beam at Zn K-line excitation energy (9750 eV) at the Tarumã endstation of the Carnaúba beamline at the Sirius Brazilian Synchrotron Light Source, Brazil. The results revealed that the Zn and Mn signals throughout the whole leaf-cross sections were ca. 2-fold higher for the Zn: Mn-EDTA compared to Zn: Mn: Mn-IDHA complex. It also displayed reveals Ca hotspots in the palisade parenchyma, likely related to calcium oxalate crystals usually found in this tissue. Moreover, a clear Zn: Mn gradient exhibited from the fertilised regions up the vascular bundles pinpoint their translocation towards other organs. Curiously, regardless of the applied compounds, the Zn and Mn intensities were mostly found surrounding the cells, thereby suggesting its transport takes place solely through the apoplastic pathway, i.e., between the cell wall and the cell plasmalemma. These findings unveil that apoplast might play an important role in the foliar absorption of nutrients by controlling the leaf cell's ionic balance.

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