SUPPLEMENTARY INFORMATION

Elemental distribution and chemical speciation of copper and cobalt in three metallophytes from the Copper-Cobalt Belt in Northern Zambia

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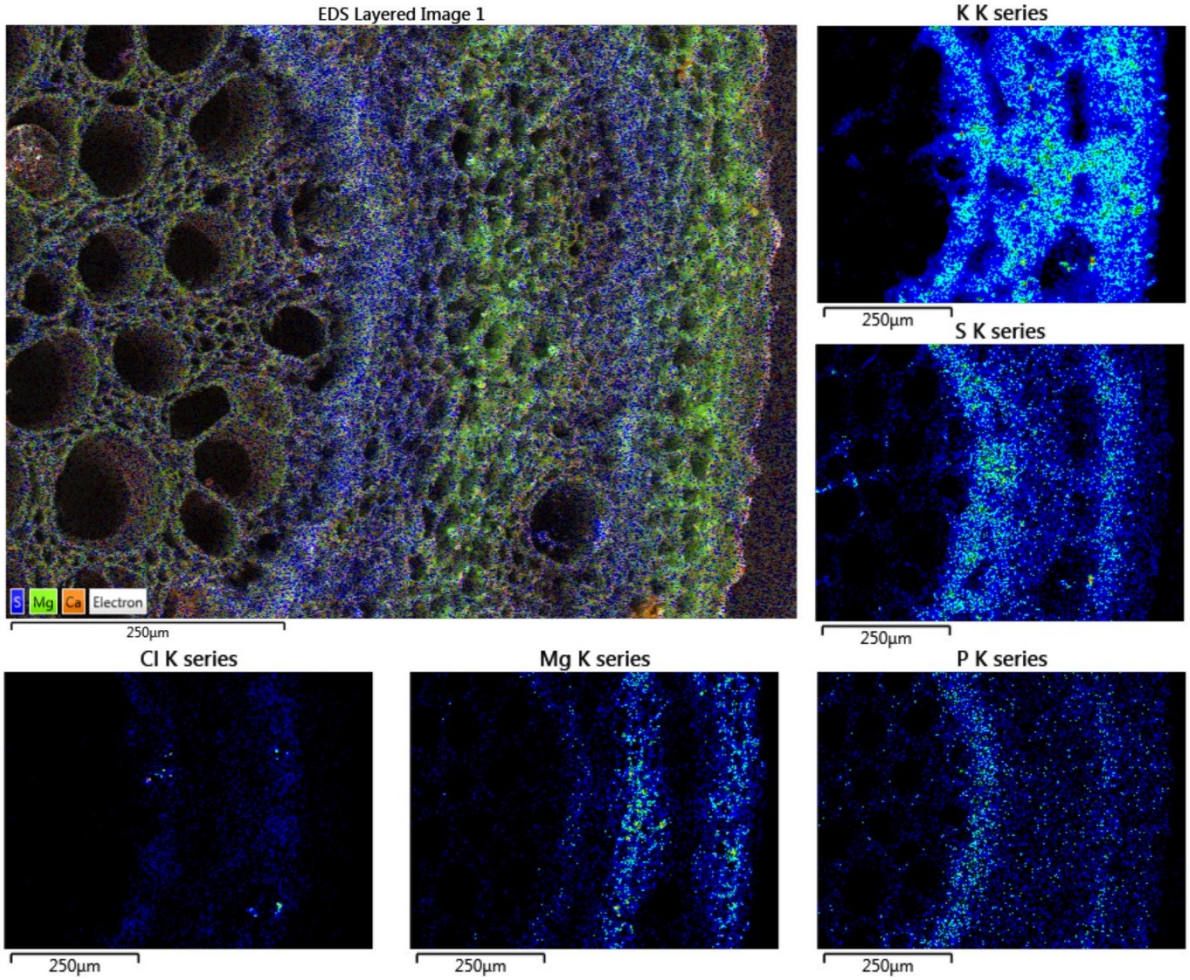
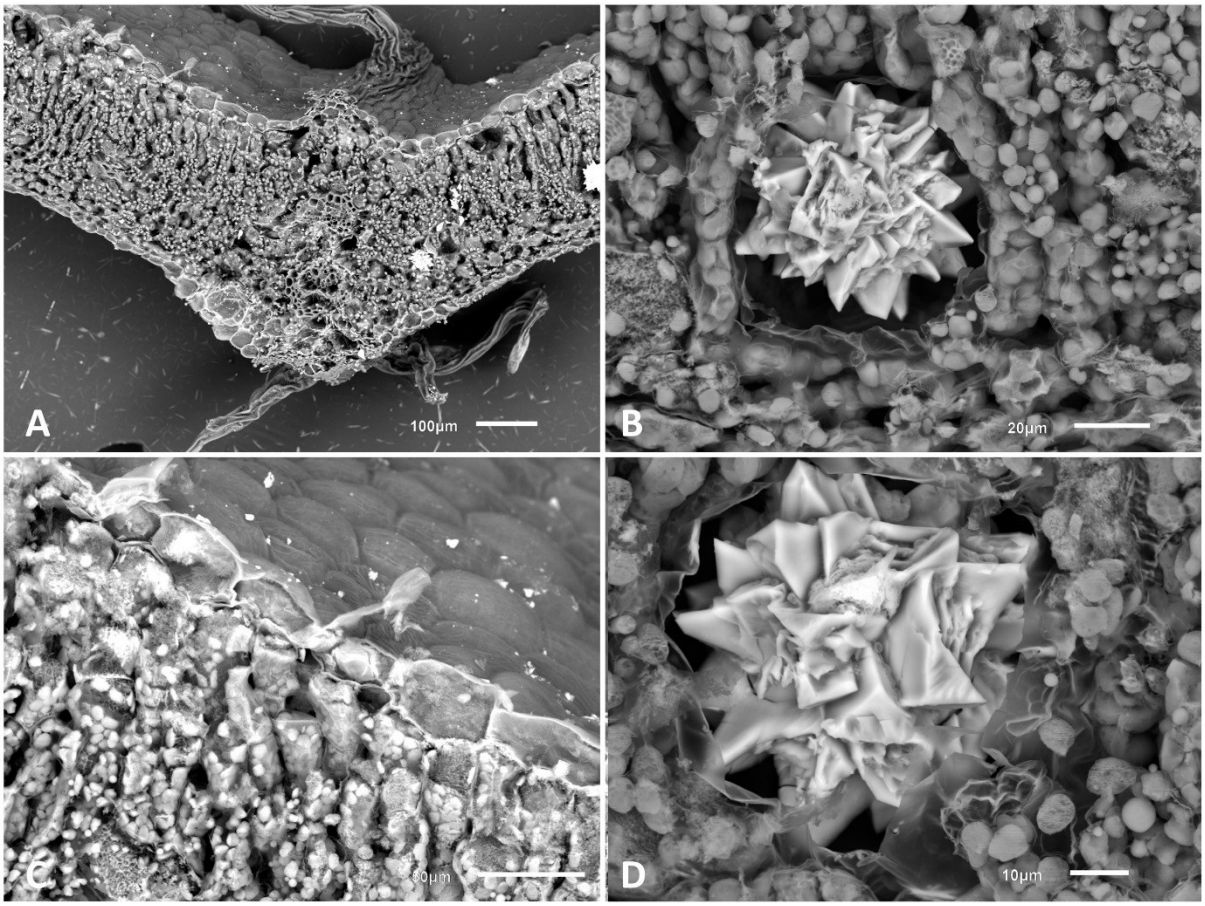
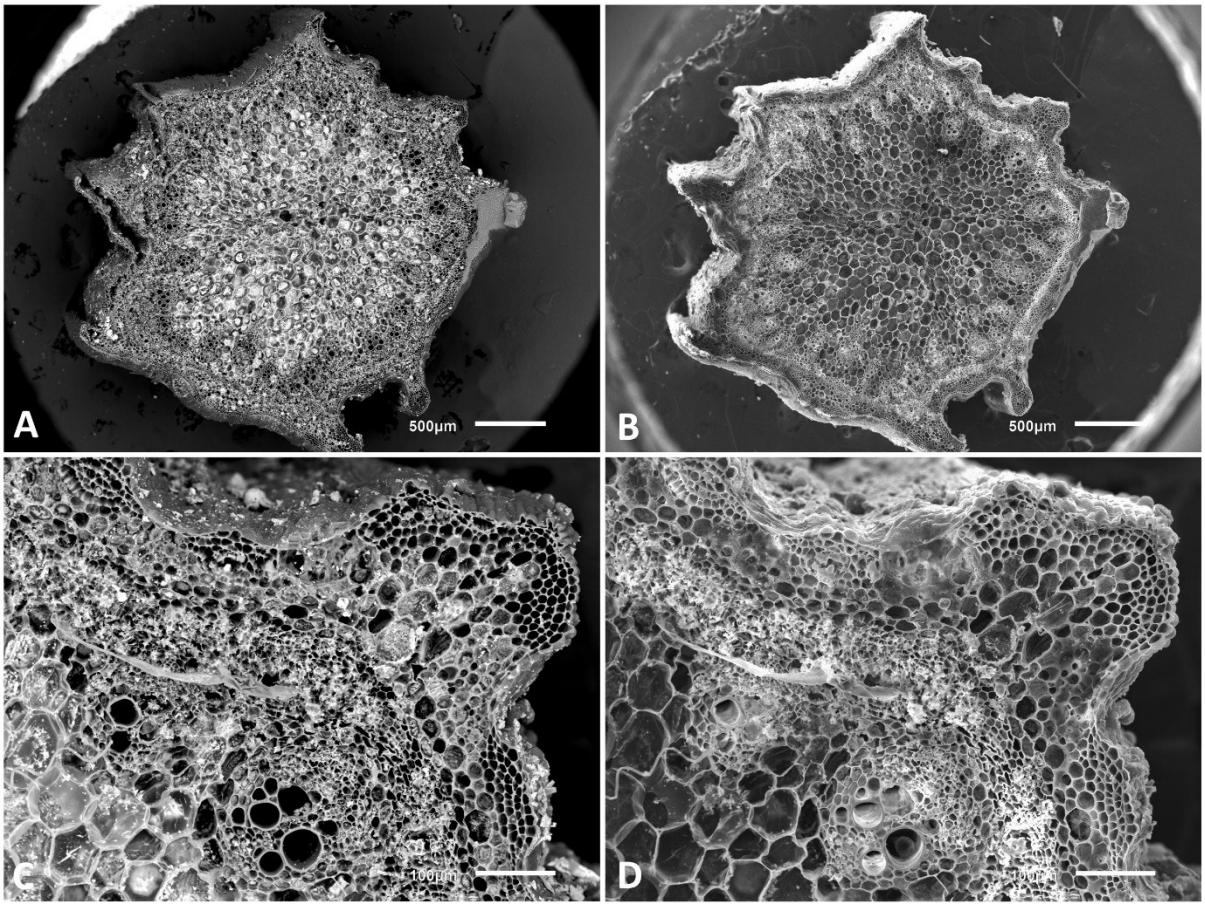


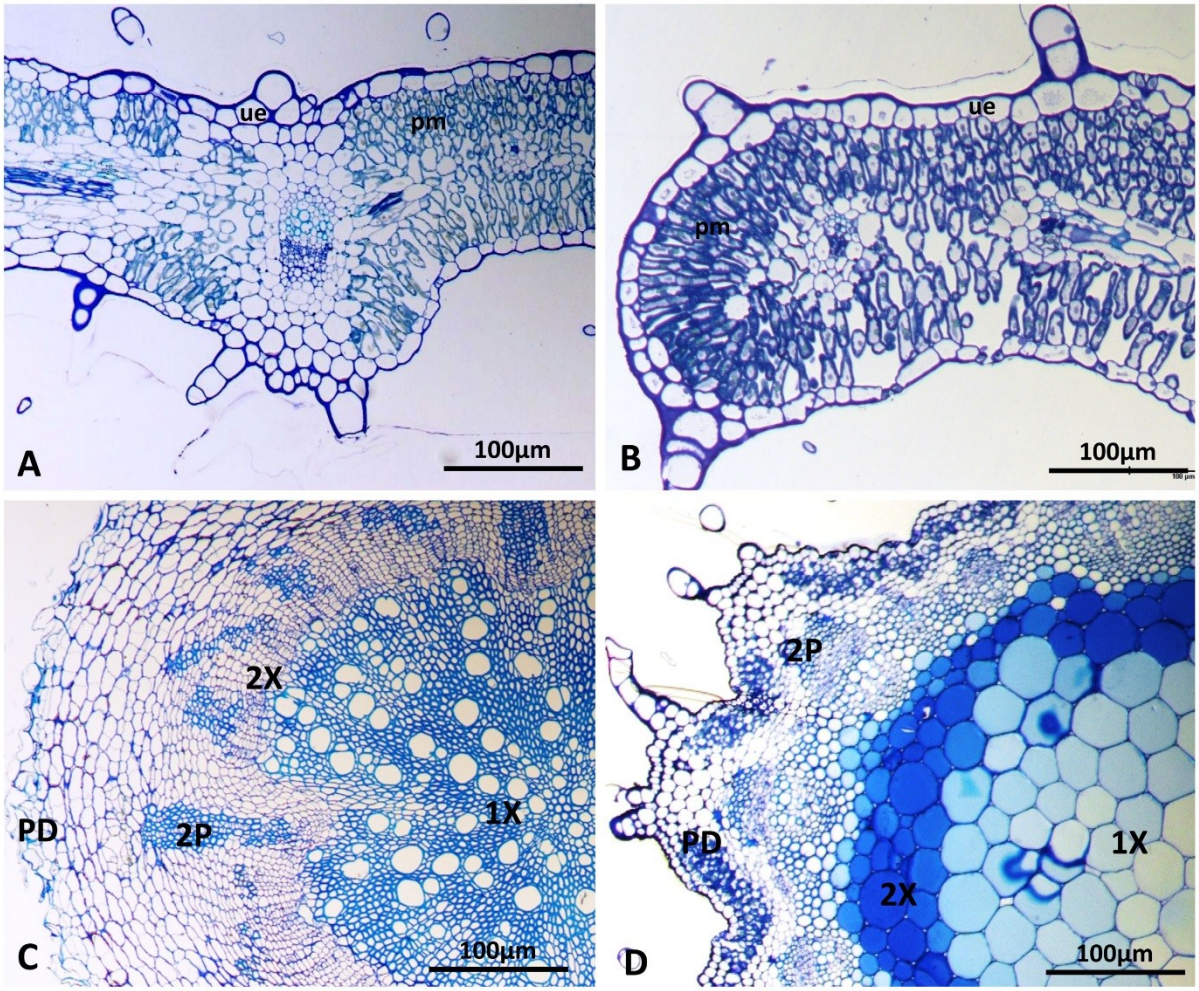
Supplementary Figure 5. Scanning Electron Microscopy with Energy-Dispersive Spectroscopy (SEM-EDS) mapping of a *Persicaria puncata* stem section.



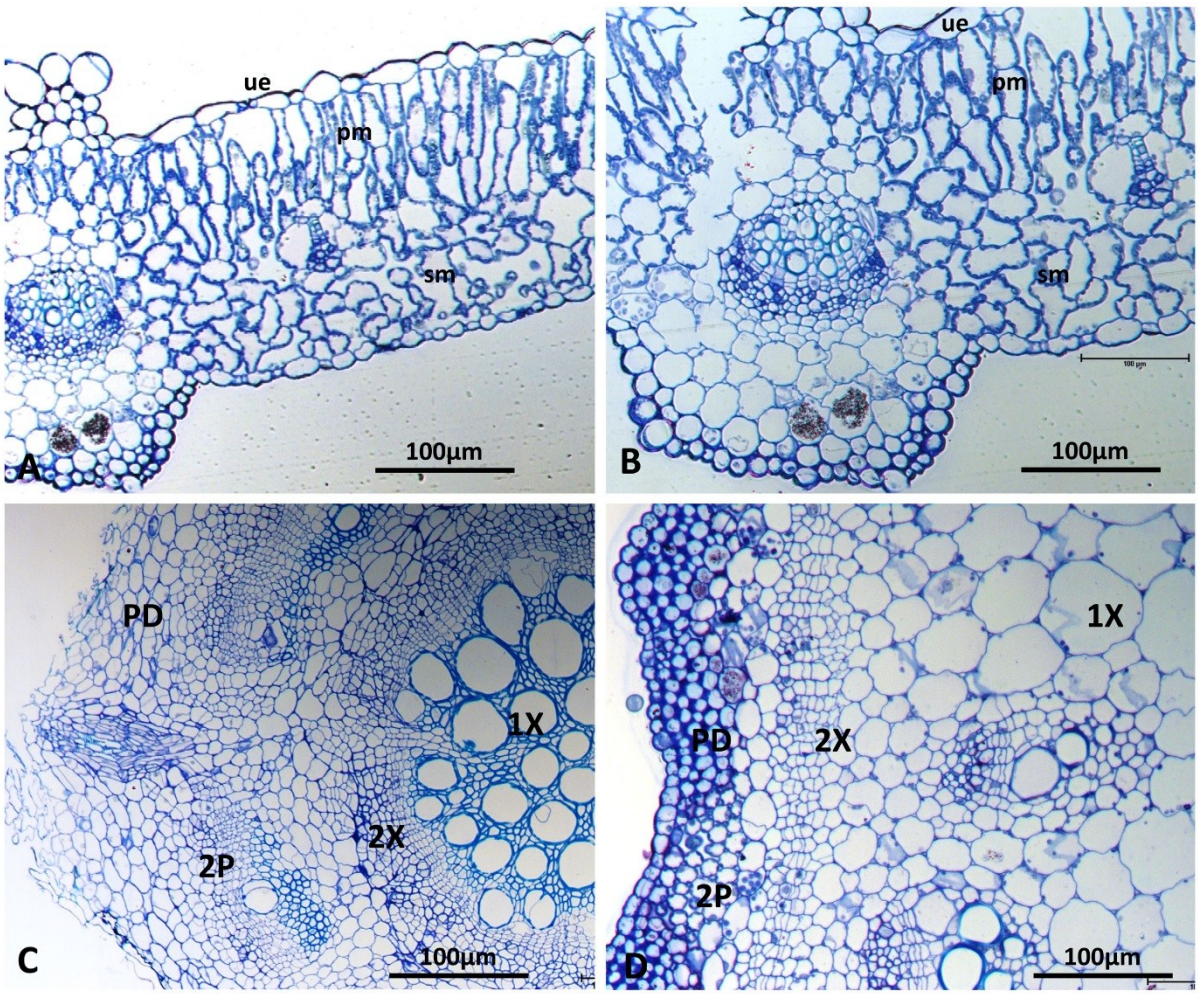
Supplementary Figure 3. Scanning Electron Microscopy images of *Persicaria puncata.* Panel A shows the foliar section, and the other panels (B, C, D) show stem sections.



Supplementary Figure 4. Scanning Electron Microscopy images of *Persicaria capitata* stem sections (panels A, B, C, D).



Supplementary Figure 1. Light *Conyza cordata* stained with Azur II and methylene blue. The top panels (A, B) show foliar sections, and the lower panels (C, D) show stem sections.



Supplementary Figure 2. Light microscopy of histological sections *Persicaria capitata* stained with Azur II and methylene blue. The top panels (A, B) show foliar sections, and the lower panels (C, D) show stem sections.

Bulk elemental concentrations in the plant tissues

In all three species the roots, stems, leaves and flowers were analysed and ranges and means provided in Table 2 (in the main text) for 12 elements. The concentrations of Co and Cu are described in the main text.

In all three species Al concentrations were variable, generally highest in the leaves (up to 1563 µg g^{-1} in *C. cordata*). Calcium (Ca) concentrations were high to extremely high in all three species, especially in the stems and leaves of *P. puncata* (up to 3.5 wt% and 2.4 wt% respectively) and in the stems and leaves of *C. cordata* (up to 1.8 wt% in either). Calcium was mainly present in the form of abundant Ca crystalline deposits (likely Ca-oxalate), visible in

the SEM images (Suppl. Fig. 3). Bulk K concentrations were also high, with up to 5.6 wt% in the stems of *C. cordata*, and up to 3.6 wt% in the stems of *P. puncata*, and 3.9 wt% in the flowers of *P. capitata*, albeit 3-fold lower in *P. capitata* stem. Magnesium (Mg) concentrations were high with up to 3.2 wt% in the leaves of *P. puncata*. Sulfur (S) concentrations were highest in *P. puncata* with a mean of 1.2 wt% in the leaves, but substantially lower in *P. capitata* (mean of 916 μg g^{-1}) and *C. cordata* (mean of 3752 μg g^{-1}). The concentrations of Fe (up to 626 μg g^{-1} in the flowers of *C. cordata*, Mn (up to 113 μg g^{-1} also in the flowers of *C. cordat*a) and Zn (up to 524 μg g^{-1} in the roots *of P. capitata*) were unremarkable. Prevailing plant Ni concentrations were higher than expected from a plant growing on a non-ultramafic soil but can be explained by the (slight) enrichment of this element in the contaminated soils (maximum of 57 μg g^{-1} in the rhizosphere of *P. capitata*). The highest concentration was in the flowers of *P. capitata* with up to 150 μg g^{-1} Ni.

Elemental distribution in the plant tissues (micro-PIXE)

The spatial distribution of 14 elements - Cu, Co and a range of macro-nutrients (Ca, K, P, S) as well as minor and trace elements, was studied in *P. capitata, P. puncata* and *C. cordata* using the nuclear microprobe. For all three species, transverse cross-sections of the roots, stems and leaves were imaged and elemental maps produced, and concentrations in different morphological structures were obtained (Tables 3, 4, 5, Figures 5, 6, 7 in the main text). The results for P, S, Cl, K, Ca and Fe are described on the basis of one representative map for roots, stems and leaves, presented in the respective figures and tables. The results for Co and Cu are described in the main text.

Roots – The concentrations of P are comparable in all three species, between 620 μg g^{-1} and 890 μg g^{-1} on average in the whole sectioned areas. The highest values were found in the phloem of *C. cordata* (3480 μg g^{-1}) and *P. puncata*, 2300 μg g^{-1} in the outer phloem. Sulfur (S) concentration is the highest in *P. puncata*, on average 4660 μg g^{-1}, about twice as high as in *P. capitata* (2480 μg g^{-1}) and almost five times more than in *C. cordata* (970 μg g^{-1}). The distribution of this element shows notable differences (Figure 5, Table 3), as follows: in *P. puncata* the highest enrichment is in the inner phloem (7340 μg g^{-1}), and the concentrations in the epidermis, outer cortex and outer phloem are very similar, of the order of 5000–5500 μg g^{-1}. In *P. capitata* the highest value was found in the cortex (6000 μg g^{-1}), twice as high as in the phloem and almost six times higher than in the epidermis. In *C. cordata* S is enriched in the wide outer ring comprising epidermis, phloem and cortex, with the concentrations in the 1100–

1800 μg g^{-1} range (Figure 5, Table 3). Chlorine concentrations are in the order of 2000 μg g^{-1} in the whole mapped areas (between 1800 μg g^{-1} and 2140 μg g^{-1}). In *P. puncata* and *C. cordata* the highest amounts are in the cortex (5000 μg g^{-1} and 5750 μg g^{-1} in the inner cortex, respectively), while in *P. capitata* the highest concentrations are in the phloem (7240 μg g^{-1}). The concentration of K is more than three times higher in *P. puncata* in comparison with *P. capitata* and *C. cordata* (3.07 wt% versus 0.87 wt% and 0.86 wt%, respectively). In all three species the highest enrichment is in the cortex and phloem (Table 3). Calcium concentrations are one order of magnitude lower, between 1440 μg g^{-1} in *C. cordata* and 1730 μg g^{-1} in *P. puncata*. The highest values are either in the epidermis (*P. puncata* and *P. capitata*) or in the cortex and epidermis (*C. cordata*). The only tissue with Fe enrichment is the epidermis, as is observed in all three species.

Stems – The highest concentrations of P are in *P. capitata* (490 μg g^{-1}), more than twice than in *P. puncata* (200 μg g^{-1}) and *C. cordata* (160 μg g^{-1}). In *P. capitata* the highest enrichment is in the phloem (1260 μg g^{-1}), while in *C. cordata* in the cambium (880 μg g^{-1}) and cortex (830 μg g^{-1}). In *P. puncata* the highest concentration was found in the cortex (450 μg g^{-1}) and slightly lower value in the epidermis (430 μg g^{-1}). Average concentrations of S in the mapped stem cross sections vary from 1890 μg g^{-1} in *P. capitata* up to 3770 μg g^{-1} and 4900 μg g^{-1} in *C. cordata* and *P. puncata*, respectively. In *P. capitata* the highest concentrations were found in the phloem, while in *P. puncata* in the meristematic cortex (9480 μg g^{-1}). In *C. cordata* the highest values are in the pith, especially in the outer pith (10 350 μg g^{-1}). Chlorine concentrations and distribution vary significantly among the species. The lowest concentrations were found in *P. puncata*, 520 μg g^{-1} in the whole mapped area, with enrichment in the pith and outer pith (760 μg g^{-1} and 800 μg g^{-1}, respectively). In *C. cordata* Cl reaches 2300 μg g^{-1} in the whole mapped area, with the highest concentration in the cortex (5230 μg g^{-1}). In *P. capitata* the respective concentration is about three times higher (6230 μg g^{-1}); the highest concentration was also found in the cortex (8670 μg g^{-1}). The highest concentration of K is in *P. puncata* (5.97 wt% in the whole mapped area), more than three times higher in comparison with *C. cordata* (1.75 wt%) and almost five times higher than in *P. capitata* (1.28 wt%). In all three species the highest concentrations were found in the cortex (Table 4). The concentration of Ca in *P. puncata* and *P. capitata* is comparable (2100 μg g^{-1} and 1700 μg g^{-1}, respectively). In *P. puncata* the highest enrichment is in the inner pith (3150 μg g^{-1}), while in *P. capitata* it is in the cortex (3440 μg g^{-1}). In both species small regions were identified, in

which calcium concentration far exceeded the values found in all other structures, reaching on average 1.94 wt% in *P. puncata* and 1.05 wt% in *P. capitata*. The average concentration of this element in *C. cordata* was much higher, 7900 μg g^{-1} in the whole stem cross section, with the highest enrichment in the pith (1.48 wt%) and the outer pith (1.86 wt%). In all three species epidermis was the only structure with high Fe enrichment (Table 4).

Leaves – The concentrations of P are very similar in *P. puncata* and *C. cordata* (1010 μg g^{-1} and 1060 μg g^{-1} in the whole area of leaf cross section). In *P. puncata* the highest concentration of this element is in the palisade mesophyll (1460 μg g^{-1}) and phloem (1330 μg g^{-1}), while in *C. cordata* the concentration in the phloem (2060 μg g^{-1}) is few times higher than in any other morphological structure. *P. capitata* is significantly depleted in P in comparison with the two former species, with the average concentration of only 340 μg g^{-1}. Sulfur concentration is the highest in *P. puncata*, with the average value in the whole leaf cross section area reaching 8000 μg g^{-1}. The highest values are in the palisade mesophyll (9070 μg g^{-1}), and slightly lower values (in the 7000–8000 μg g^{-1} range) in the xylem, phloem, spongy mesophyll and vascular bundles. The lowest concentrations were found in the lower and upper epidermis (2060 and 3100 μg g^{-1}, respectively. The concentration of this element in *C. cordata* is significantly lower (4270 μg g^{-1}), with the maximum in the minor vein vascular bundles and the lowest values in the upper and lower epidermis, and hairs (Table 5). Sulfur concentration in *P. capitata* is the lowest of all three species (2000 μg g^{-1} in the whole area of the measured cross leaf cross section), with the highest value in the main vein vascular bundles (3300 μg g^{-1}) and the lowest in the minor vein vascular bundles (420 μg g^{-1}). Chlorine concentration is the highest in *P. capitata* (7610 μg g^{-1} in the whole cross section), somewhat lower in *C. cordata* (6800 μg g^{-1}) and much lower in *P. puncata* (1920 μg g^{-1}). In *P. capitata* the highest enrichment in this element is in the upper epidermis, and the lower collenchyma, and the lowest in the spongy mesophyll (Table 5). In *C. cordata* the highest concentration is in the midrib parenchyma (2.04 wt%), while the lowest values were found in the palisade mesophyll, xylem and minor vein vascular bundles. In *P. puncata* significant enrichment in this element was in the upper epidermis, collenchyma and midrib parenchyma (in the order of 5300–5500 μg g^{-1}), while the lowest values were noted in the minor vein vascular bundles (Table 5). The highest concentrations of K were found in *C. cordata* (2.1 wt% in the whole cross section area), with the highest enrichment in the midrib parenchyma (4.48 wt%) and phloem (3.12 wt%) and the lowest concentration in the minor vein vascular bundles (0.94 wt%). Somewhat lower concentrations are in *P. puncata* (1.7 wt% in the whole cross section area). The highest values were found in the lower collenchyma and

spongy mesophyll, slightly exceeding 2.6 wt% (Table 4). The concentrations in other tissues do not show major differences, with values between 1.6 and 2.2 wt%, with the exception of the palisade mesophyll (0.9 wt%) and lower epidermis (0.72 wt%). *Persicaria capitata* is characterised by significantly lower concentration of K (1.1 wt% in the whole cross section area). Here the highest concentrations are in the midrib parenchyma (1.94 wt%) and the lowest in the palisade mesophyll and lower epidermis (0.45 wt% in both cases).

The average concentrations of Ca in *P. capitata* and *C. cordata* are similar (6260 μg $g^{-1}$and 6940 μg g^{-1}, respectively), while in *P. puncata* they are significantly lower (2290 μg g^{-1}). In the latter species the highest concentrations were found in the minor vein vascular bundles (1.06 wt%) and midrib parenchyma (0.92 wt%), The lowest concentration of this element in *P. puncata* was in the palisade mesophyll (only 174 μg g^{-1}). In *P. capitata* the highest Ca enrichment is in the minor vein vascular bundles (4.09 wt%), almost twice higher than in the lower collenchyma (2.15 wt%). The lowest value was identified in the palisade mesophyll (405 μg g^{-1}). In *C. cordata* the highest concentrations are in the hairs (upper and lower) and in the minor vein vascular bundles (Table 5). In all three species epidermis was the only structure with high Fe enrichment, and the upper epidermis always showed higher enrichment than the lower epidermis, especially in *P. puncata* (Table 5).