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Exceptionally High Ni Concentration in Phloem of Roots of Nickel-hyperaccumulating *Berkheya zeyheri* subsp. *rehmannii* var. *rogersiana*

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Hyperaccumulation is an unusual plant response to metaliferous soils. Such soils, like those derived from ultramafic rocks, are characterized by elevated concentrations of heavy metals including Ni, Cr, Zn and Cd. Most of the plants growing on these metal-rich soils exclude metals from their shoots as excessive accumulation of heavy metals is toxic to the majority of plants. However, about 2% of plants on metaliferous soils take up and accumulate large quantities of metals in their shoots: a phenomenon known as hyperaccumulation. In a previous study [1] significantly higher concentrations of Ni were found in leaves of the Ni-hyperaccumulator, *Berkheya zeyheri* subsp. *rehmannii* var. *rogersiana* compared to those of its non-hyperaccumulating counterpart *Berkheya zeyheri* subsp. *rehmannii* var. *rehmannii.* The present study is an extension of the previous one and compares the distribution and concentration of Ni, as well as other elements, in the roots of the two varieties. The Ni-hyperaccumulator (H) was collected from Songimvelo Game Reserve and the non-hyperaccumulator (NH) from Agnes Mine, Mpumalanga, South Africa.

Roots were sampled in the root hair region. To correlate elemental distribution with anatomical features, samples were processed for anatomical studies using standard resin-embedding procedures. Elemental distribution and concentration were determined using a nuclear microprobe on root samples parallel to those taken for anatomy. These were sectioned, cryofixed and subsequently freeze-dried. Two complementary techniques, particle induced X-ray emission (PIXE) and proton backscattering (BS) were performed simultaneously. Anatomical features of roots of the H and NH are shown in Fig.1a and d respectively. Tissue composition is similar except that in the cortex of the H sclereids are also present (Fig.1a). Average concentrations of selected elements in the analyzed cross sections are given in Table 1 and representative distribution maps of Ni and Ca are shown in Fig.1b,c,e,f. The average Ni concentration was significantly higher in the H (6430 mg/kg; ca.35 times higher) compared to the NH (Table 1). The highest Ni enrichment (up to 2.5 wt.%) occurred in the phloem of the H (Fig.1b) with lower enrichment in tissues contiguous with it and in the cortex (Fig.1b). In contrast in the NH, the average Ni concentration was significantly lower (180 mg/kg, Table 1) with the highest Ni enrichment external to the cortex in the exodermis/epidermis and root hairs (Fig.1e). The average concentration of Ca was higher in the H (1.75 wt.%, Table 1) compared to the NH (1.56 wt.%, Table 1). The highest Ca enrichment areas in the H mirrored those of Ni (phloem, tissues contiguous with the phloem and cortex, Fig.1c) while in the NH this was confined mainly to the exodermis/epidermis with lower Ca enrichment in the cortex and root hairs (Fig.1f). With regard to the other main nutrient elements, the average concentration of K and S was higher in the H compared to the NH while the average concentration of P was higher in the NH (Table 1). K enrichment was restricted mainly to the cortex in both varieties and S and P to tissues in close association with the phloem (endodermis and vascular cambium) in the H and NH. Minor or trace elements (Fe, Cu, Zn) were confined to the outermost tissues (epidermis/exodermis) in both varieties.

The extremely high concentration of Ni in the phloem of the H suggests that this tissue may be involved in Ni transport. The known high mobility of Ni in the phloem would enhance such a role

[2]. Ca is also concentrated in the phloem tissues indicating possible co-distribution with Ni. This would be of relevance as it has been shown that Ni is able to use Ca transport proteins to facilitate its uptake and movement in plants [3].

References:

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Table 1. Average concentrations of selected elements (mg/kg) in roots of *Berkheya zeyheri* var. *rogersiana* (H) and *Berkheya zeyheri* var. *rehmannii* (NH).

Root	P	S	Cl	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu	Zn
H	240	13630	4200	80200	17500	1320	85	790	220	44700	6430	180	80
	(43)	(570)	(90)	(800)	(240)	(40)	(16)	(20)	(40)	(700)	(140)	(17)	(7)
NH	550	3760	15460	62900	15620	490	44	340	420	20800	180	37	113
	(40)	(160)	(270)	(440)	(190)	(15)	(10)	(13)	(30)	(390)	(9)	(4)	(7)

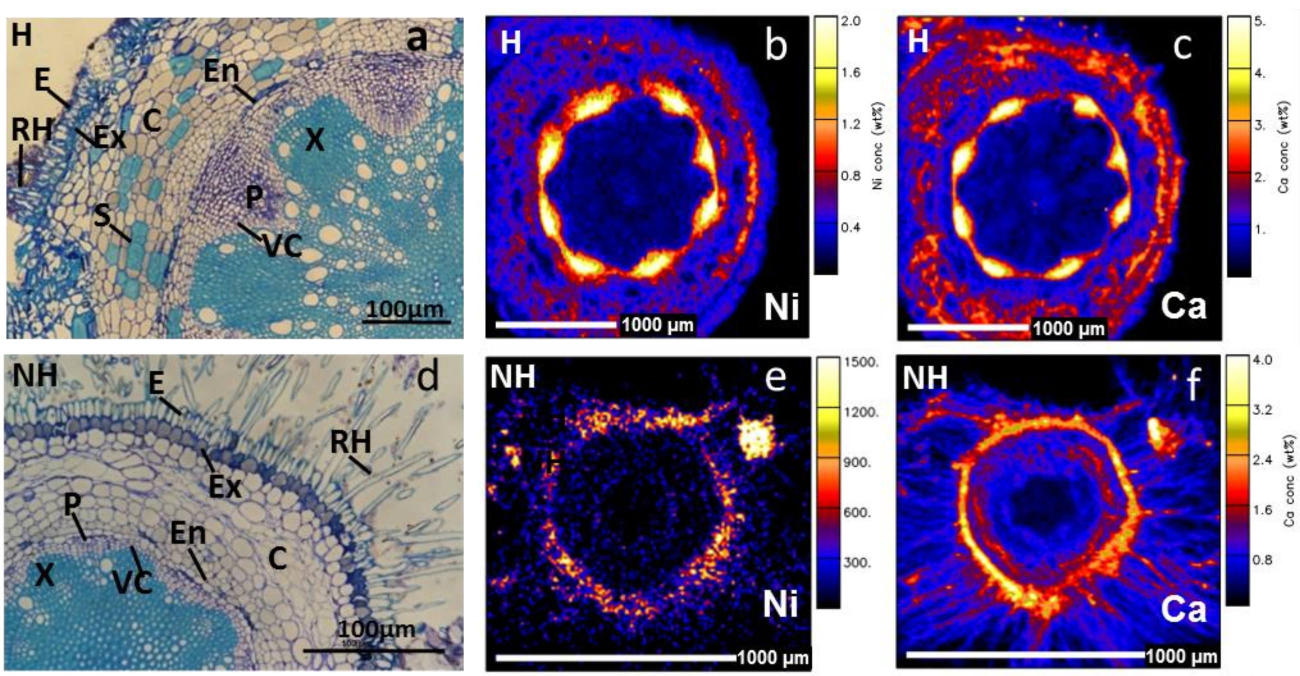

Figure 1. Light micrographs of anatomical features (a,d) and quantitative elemental maps of Ni and Ca distribution in root sections of Ni-hyperaccumulating (H) and non-hyperaccumulating (NH) varieties of *Berkheya zeyheri*. Concentration scale in wt.% (b,c,f) or in mg/kg (e). C,cortex; E,epidermis; Ex,exodermis; P, phloem; RH, root hair; S, sclereids; VC, vascular cambium; X, xylem.

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