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Root responses to soil Ni heterogeneity in a hyperaccumulator and a non-accumulator species

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Ni heterogeneity in soil affects the morphology and root distribution patterns of Berkheya coddii and Cicer arietinum.

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We compared root responses of the Ni-hyperaccumulator plant *Berkheya coddii* Rossler with the non-accumulator plant *Cicer arietinum* L. to Ni heterogeneity in soil. We grew plants in growth containers filled with control soil, homogeneously spiked, and heterogeneously spiked soil with Ni concentrations of 62 and 125 mg kg^{-1}. Neutron radiography (NR) was used to observe the root distribution and the obtained images were analysed to reveal the root volumes in the spiked and unspiked segments of the growth container. There was no significant difference in root distribution pattern of *B. coddii* among different concentrations of Ni. Unlike *B. coddii*, the roots of *C. arietinum* initially grew into the spiked segments. However, the later developing roots did not penetrate the spiked segment suggesting an avoidance strategy. Our results indicate that, *B. coddii* does not forage towards the Ni-rich patches, although presence of Ni in soil changes its root morphology.

1. Introduction

Hyperaccumulator plants can remove significant amount of metals from soils. Therefore, they may offer a sustainable management option for the remediation of metal-contaminated sites (phytoremediation) and also an opportunity to mine naturally metal-rich soils by phytomining (Baker et al., 1994; Brooks et al., 1998; Angle et al., 2001; Li et al., 2003; Robinson et al., 2003a,b). More than three quarters of known metal hyperaccumulator plants are Ni hyperaccumulators (Brooks, 1998). *Berkheya coddii* Rossler is a summer-green perennial Ni-hyperaccumulator plant belonging to Asteraceae family that is found on ultramafic (serpentine) soils in southern Africa (Morrey et al., 1992). It is a particularly attractive species for phytoremediation and phytomining of nickel-rich soils because of its rare combination of high Ni accumulation and large biomass production. Robinson et al. (1997) reported an annual biomass production of 22 t ha^{-1} and up to 1% (w:w) Ni in the dry above-ground biomass.

The mechanisms that result in hyperaccumulation are not yet fully understood. A number of factors and mechanisms have been identified that could increase metal uptake by hyperaccumulators, including high density of uptake sites in root membranes (Lasat et al., 2000; Ueno et al., 2008), preferential root-foraging (Schwartz et al., 1999; Whiting et al., 2000; Haines, 2002) and rapid translocation mechanisms inside the plants (Pence et al., 2000; Ueno et al., 2008). The spatial distribution of heavy metals in naturally or anthropogenically contaminated soils is usually heterogeneous. Thus, preferential root proliferation into soil patches containing elevated concentrations of the target metal might be an important factor in metal acquisition by hyperaccumulator plants. Roots of the Zn hyperaccumulator *Thlaspi caerulescens* for example preferentially proliferates in response to substrate patches with high Zn concentrations (Schwartz et al., 1999; Whiting et al., 2000). Some ecotypes of *T. caerulescens* may discriminate between patches with contrasting Zn concentrations and produce more roots in patches with higher Zn concentrations (Haines, 2002). The same holds true for essential macronutrients in soil. Foraging responses to local nutrient patches have been demonstrated in a range of plant species, and in some species greater growth can be achieved in patchy habitats than in homogeneous habitats containing the same quantity of resources (Wijesinghe et al., 2001; Hutchings and John, 2004). Foraging nutrients may aid the survival of plant in environments with limited resources. Lynch and Brown (2001) showed

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that root architectural traits that enhance topsoil foraging appear to be particularly important for genotypic adaptation to low phosphorus soils. However, root proliferation in nutrient-rich patches may not be cost-effective if the patch vanishes or if a competitor plant occupies it more rapidly (vanVuuren et al.,1996; Leyser and Fitter, 1998).

Contrary to positive foraging towards metal-rich patches, some plants might avoid parts of the soil as a mechanism to tolerate the presence of undesired conditions in their environment. Hairiah et al. (1995) reported avoidance of the acidic subsoil by *Mucuna pruriens*. A similar mechanism was found to be responsible for drought-avoidance of some traits of *Cicer arietinum* L. (Gaur et al., 2008). Menon et al. (2007) showed that root growth of Lupin was significantly reduced in the contaminated part of the soil with boron and zinc. There is little information on the mechanism of this avoidance and it is not known if any signalling mechanism is involved.

Hitherto, most studies have focused on the root growth and development of hyperaccumulator species in the Brassicaceae family in soil containing metal-contaminated patches (Schwartz et al., 1999; Whiting et al., 2000; Haines, 2002; Podar et al., 2004; Dechamps et al., 2008). The root development of *B. coddii* in heterogeneously contaminated soil has yet to be investigated. It is unclear whether *B. coddii* behaves in the same way as the Brassicaceae family.

One barrier in studying root development in soil is the technical difficulties in accessing the roots without disturbance (Hopmans and Bristow, 2002; Pierret et al., 2005). Conventional methods such as transparent rhizotrons or rhizoboxes for studying root systems are destructive, tedious, and difficult to interpret. Among the few available non-destructive methods, neutron radiography (NR) has proved to be an efficient tool to image living roots in situ (Willatt et al., 1978; Furukawa et al., 1999; Menon et al., 2007; Moradi et al., 2009; Oswald et al., 2008; Tumlinson et al., 2008).

The goal of this study was to investigate the root growth of the Ni hyperaccumulator *B. coddii* in response to heterogeneously spiked soil with Ni in two different concentrations in comparison with a non-accumulator plant, *C. arietinum* L. We used neutron radiography combined with an image analysis tool to monitor root growth and to quantitatively calculate root allocations in various parts of the growth container.

2. Materials and methods

2.1. Pot experiment

A sandy soil (see Table 1 for selected properties) was used in a pot experiment to determine the suitable Ni concentrations in soil that would result in the greatest growth and Ni uptake by *B. coddii* Roessl. *B. coddii* was grown in soil spiked with Ni (as $NiCl_2$) at concentrations of 0, 31, 62, 125, 250, and 500 mg kg^{-1} soil (900-ml plastic pots, 3 replicates). The pots were irrigated with Hoagland's nutrient solution (Hoagland and Arnon, 1938) and kept in a climate chamber for 5 weeks with a daily light cycle of 16 h light/8 h darkness, constant humidity (75%) and controlled temperature (16/23 °C night/day). After 5 weeks, the plants were harvested and the shoot biomass dried at 65 °C until a constant weight was obtained, then ground and digested with 15 ml HNO_3 (65%) at 150 °C for 1 h in Teflon tubes on a heating block DigiPREP MS (SCP Science, QC, Canada). Samples were analysed for Ni using ICP-OES (Vista-MPX Varian, Australia). For quality assurance, certified reference material from the Community Bureau of Reference BCR (No. 62, *Olea europaea*) was digested and analysed using ICP-OES. We obtained recovery of 89% for Ni in the certified reference plant materials compared to the certified and reported values by the Community Bureau of Reference BCR.

Table 1
Selected soil physical and chemical properties.

pH	CEC (cmol(+) kg^{-1})	EC (dS m^{-1})	Texture	Bulk density (g cm^{-3})	Porosity (% v/v)	Provenience
6.4	12	0.12	Sandy	1.3	36	Eiken, Switzerland

Based on the results obtained from the pot experiment here, we chose the Ni concentrations of 62 and 125 mg kg^{-1} in soil (relevant to *B. coddii*) and then grew plants in growth containers made of aluminium for the NR experiment.

2.2. Plant growth in growth containers for NR experiment

B. coddii (a Ni hyperaccumulator) and chickpea (*C. arietinum* L., a non-hyperaccumulator) were used; both of these species grow rapidly and have roots that are easy to delineate using neutron radiography. Aluminium containers with inner dimensions of 17 × 15 × 1.5 cm were used. The containers were made of aluminium due to its low neutron attenuation and therefore high transparency. 30 containers were filled with the sandy soil. For each plant species, there were five treatments, each with three replicates: control soil, heterogeneously spiked soil with 62 and 125 mg kg^{-1} Ni, and homogeneously spiked soil with 62 and 125 mg kg^{-1} Ni (see Fig. 1). The average bulk density of the packed soil was 1.3 g cm^{-3} and the top 2 cm of the containers were left unfilled to facilitate irrigation. The seeds of *C. arietinum* were sown directly onto the soil surface at the middle of the containers and one-week old seedlings of *B. coddii* were transplanted into the containers. Before imaging, the plants were grown for 5 weeks in a controlled environment chamber at 16–19 °C and a daily photoperiod of 16 h generated by fluorescent lighting (0.4–0.6 lumen cm^{-3}). Plants were irrigated with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1938) and maintained at a soil water content of 20%. Irrigation was stopped 3 days prior to imaging to increase the contrast between soil and roots in the neutron radiographs. Neutron radiographs were taken 3 times over the course of the experiment. After the NR experiment was finished, one side of the containers was opened and the soil subdivided into 6 sections (Fig. 2). Each section was sieved using a 2-mm mesh sieve and the roots were carefully separated from the soil and washed with deionised water. The dry mass of the roots in each section were determined gravimetrically after drying the roots at 65 °C for 48 h.

2.3. Neutron radiography set up

The experiments were performed at the cold neutron radiography facility (ICON) at Paul Scherrer Institut (PSI), Villigen, Switzerland. The neutron radiography set up is explained in detail by Moradi et al. (2009). A CCD camera detector with an array of 1024 × 1024 pixels in conjunction with a neutron-sensitive ^{6}Li based scintillator screen (Applied Scintillation Technologies, UK) was used giving a resolution of 110–170 μm in the digital images.

2.4. Image analysis

Image analysis was carried out using the algorithm of Menon et al. (2007) to correct beam variation, segment the roots from the soil, and compute root volume in each of 6 segments of the growth containers in NR images (Fig. 2). All other calculations above were carried out using Matlab.

All statistical analysis were carried out using Matlab (ANOVA). Data that were log-normally distributed and differences at $P < 0.05$ level were considered significant.

3. Results

3.1. Pot experiment with B. coddii

There was no significant difference in the shoot biomass among the treatments. The average shoot biomass was 1.5 ± 0.7 g. Fig. 3 shows Ni concentration in dry shoot biomass of *B. coddii* grown in various treatments. Ni concentrations ranged from 25 ± 14 mg kg^{-1} in dry above-ground biomass in the control treatment to 3303 ± 443 in BK 500. There was no significant difference between Ni concentrations in shoots of treatments BK 31 and the control. However, a sharp increase in shoot Ni concentration was observed in the soil Ni concentrations ranged from 62 to 250 mg Ni per kg^{-1} soil which indicated that *B. coddii* was most effective in extracting Ni in this range of the soil Ni concentrations under our experiment conditions. The highest Ni concentration occurred in the shoots of BK 500 and BK 250 treatments (there was no significant difference between the two). We chose soil Ni concentrations of 62 and 125 mg kg^{-1} for our experiment in heterogeneously spiked media. The shoot biomass of *B. coddii* in these concentrations of Ni was not significantly different than the control while there was a sharp response by *B. coddii* in Ni uptake compared to the control soil.