Environmental Pollution 157 (2009) 2189–2196

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

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.

ARTICLE INFO

Article history:
Received 13 March 2009
Received in revised form
9 April 2009
Accepted 9 April 2009

Keywords:
Berkheya coddii
Cicer arietinum
Neutron radiography
Nickel hyperaccumulator
Root growth
Root response

ABSTRACT

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 summergreen 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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doi:10.1016/j.envpol.2009.04.015

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.

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.

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

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.

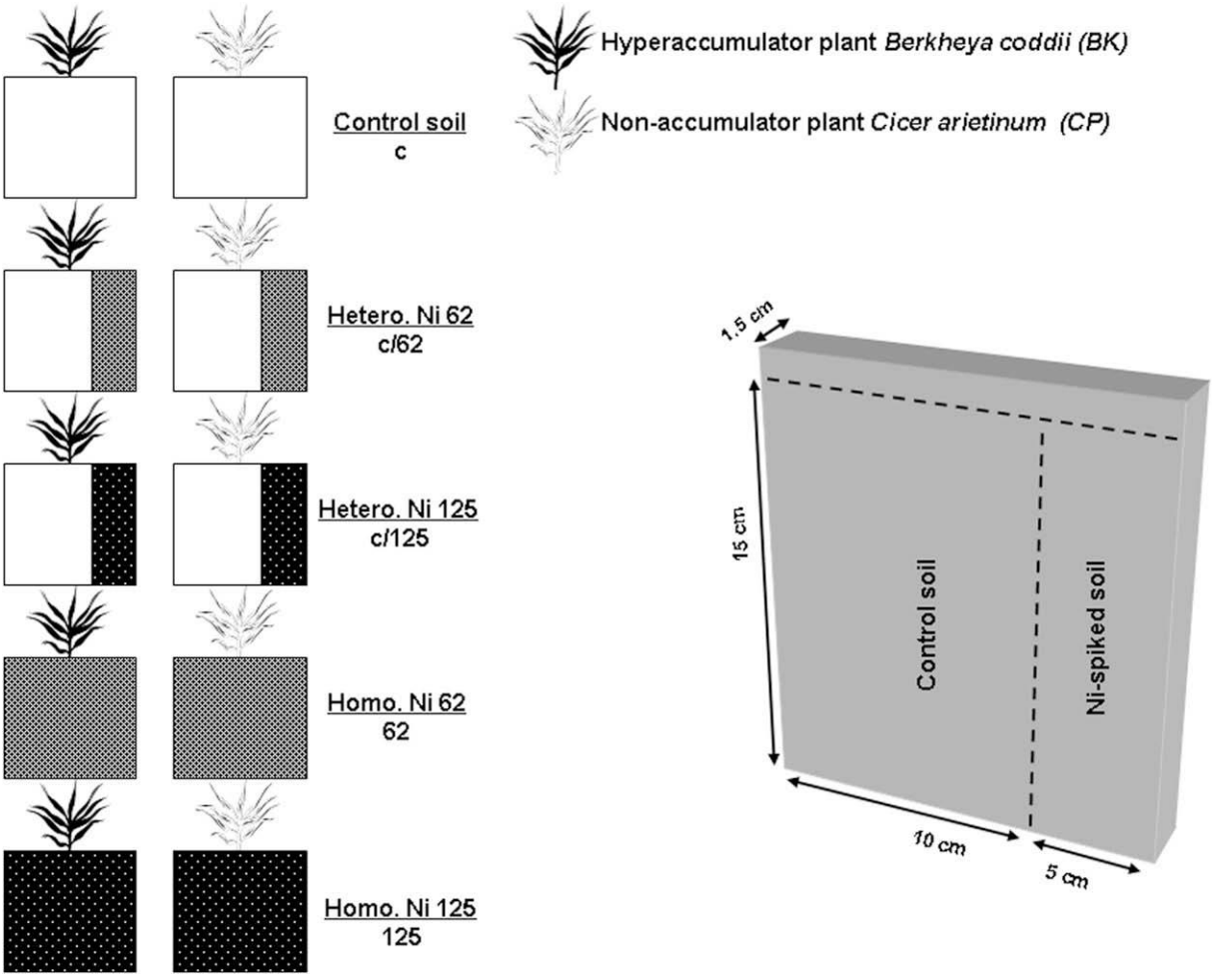

Fig. 1. Experimental design of the NR experiment investigating root responses of Ni hyperaccumulator *Berkheya coddii* and non-accumulator *Cicer arietinum* to Ni heterogeneity in soil. A sandy soil (c) was spiked with 62 and 125 mg kg^{-1} nickel.

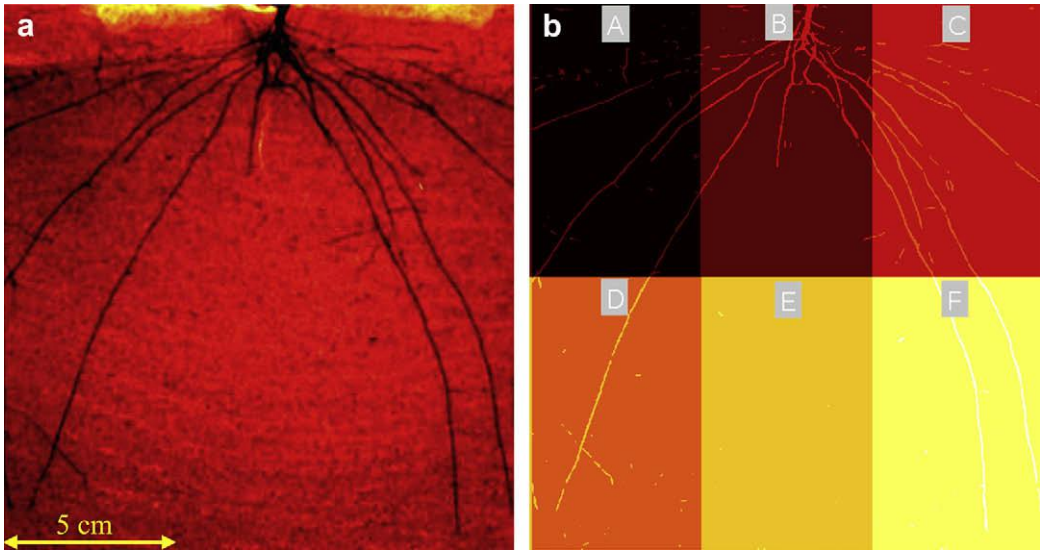

Fig. 2. Neutron radiograph of *Cicer arietinum* roots in the aluminium growth container (a) and the corresponding segmented roots in 6 subdivisions of the container (b).

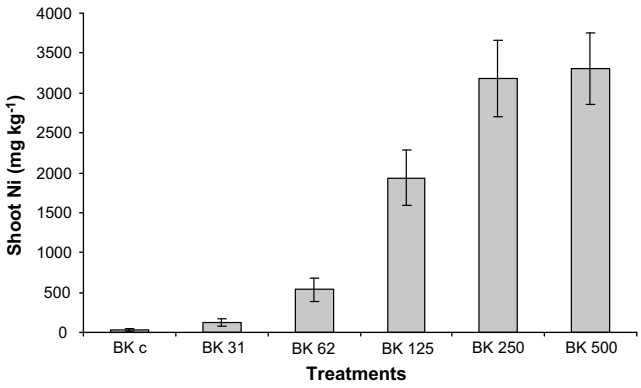

Fig. 3. Ni concentrations in the shoot biomass of *Berkheya coddii* under various Ni soil concentrations in the pot experiment. The error bars show standard deviation of the mean. Treatments without overlapping error bars are significantly different to each other.

There was evidence of morphological change in the root system and structure of *B. coddii* grown in the Ni-spiked soils compared to the control soil (Fig. 4). There was lower number of primary roots for each plant in the Ni-spiked soil (7–10 compared to 12–16 in the control soil) and the primary roots grew thicker with considerably less branching.

3.2. Root development over time in NR experiments

Fig. 5 shows neutron images of the roots of *B. coddii* (a, b and c) and *C. arietinum* (d, e and f) over the course of the experiment. The images were taken on the first week (a and d), the third week (b and e) and the fifth week (c and f) after the experiment started. Most of the root growth happened in the second and third weeks. In the third week, roots of both *C. arietinum* and *B. coddii* reached the container walls on the left and right side of the container (Fig. 5b and e). Roots of *C. arietinum*, after reaching the container wall, bounced back inside the container which shows that chickpeas need more space and a bigger container for natural root development.

3.3. Shoot and total root biomass in growth containers

Fig. 6a shows the shoot and root biomass of *B. coddii* in all the treatments. There was no significant difference in shoot biomass of the treatments except the treatment BK c/125 which was unexpectedly higher than the others. *B. coddii* had significantly more root biomass in the control soil (BK c), than in the BK 62 and BK 125 treatments. There was no significant difference between BK c/62 and BK c/125. In all the treatments except BK c and BK c/62, the root biomass was significantly lower than the shoot biomass. The highest shoot:root biomass ratio occurred in BK c/125 (1.95) and the lowest in the control (0.49).

Unlike the *B. coddii*, the shoot biomass of *C. arietinum* was unaffected by the Ni treatments (Fig. 6b). However, the root biomass was significantly reduced in the Ni treatments. While there was no significant difference between the shoot and root biomass of CP c, all other treatments had significantly higher shoot biomass than root biomass and therefore a higher shoot:root biomass ratio.

3.4. Root growth pattern in growth containers

Fig. 7a1–a5 shows the root development of *B. coddii* after five weeks in the control and Ni treatments. While in the control soil, (a1), roots have developed permeating most sections of the container, their development was perturbed in the Ni containing treatments in both homogenously or heterogeneously spiked soils (a2–a5). In agreement with the pot experiment, roots were thicker, and less laterally branched in all Ni-treated soils (BK c/62, BK c/125, BK 62, and BK 125) compared to BK c. The root growth pattern was similar in all Ni-spiked soils. This indicates there was no root response to Ni heterogeneity in soil.

For *C. arietinum*, root development was perturbed in all Ni treatments (Fig. 7b2–b5). In the treatment CP 125, roots only grew in the top 4 cm of the container. In the heterogeneously spiked soils, root growth was inhibited in the Ni-spiked segments of the containers. There were differences in the root growth of the top and the bottom of the Ni-spiked segments of the container. While the first marginal roots penetrated the top 5 cm of the Ni-spiked segment of the container, no roots grew in the bottom area of the Ni-spiked segment of the containers.

Fig. 4. Root system of *Berkheya coddii* grown in control soil (a) and in soil spiked with 125 mg kg^{-1} Ni (b). The primary roots appeared thicker with much less branching in the spiked soil.

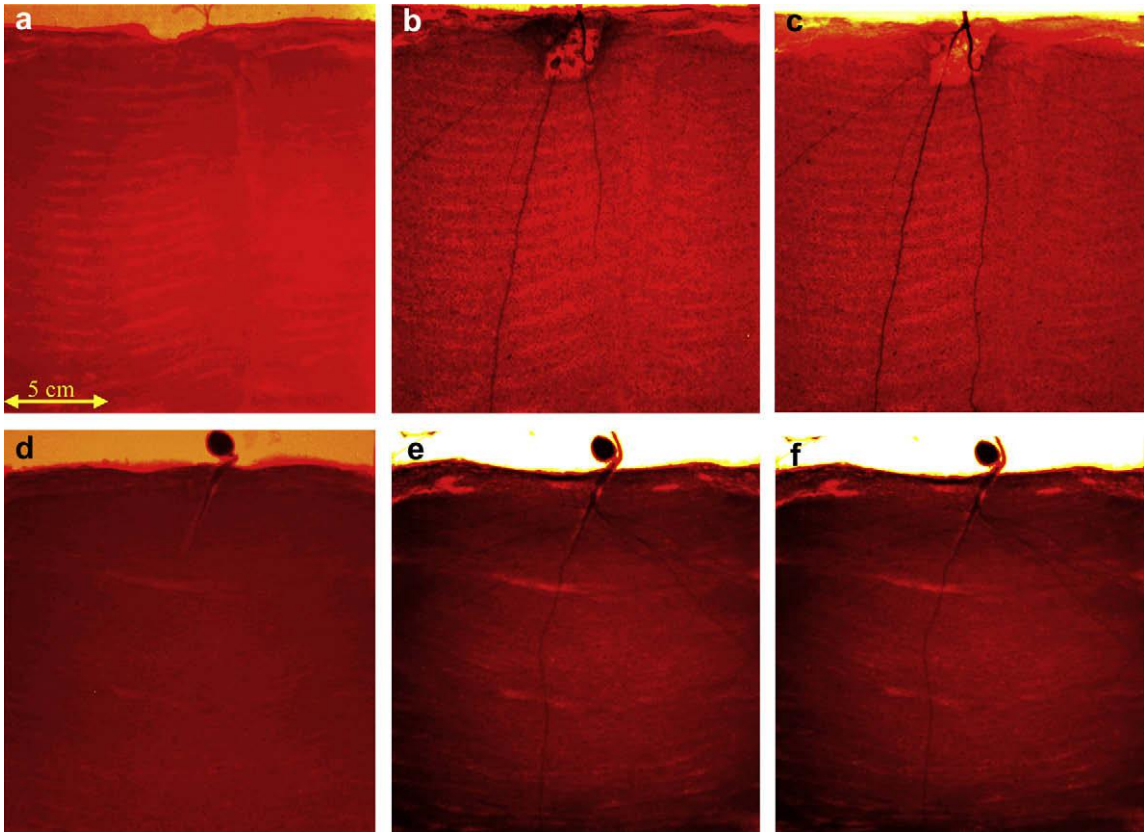

Fig. 5. Neutron images of roots of *Berkheya coddii* (a, b, and c) and *Cicer arietinum* (c, d and f) one week (a and c), three weeks (b and d) and 5 weeks (c and f) after the start of the experiment.

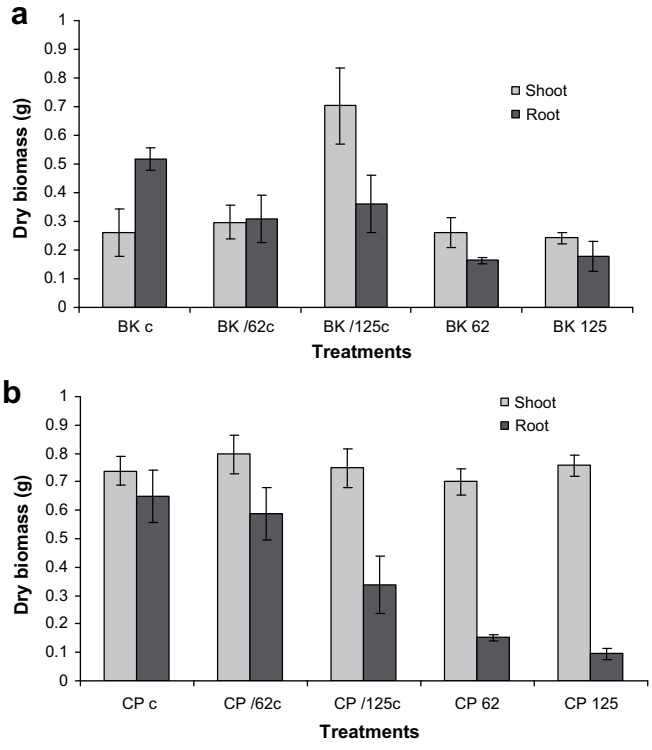

Fig. 6. Measured total root and shoot biomass of *Berkheya coddii* (a) and *Cicer arietinum* (b) in growth containers after 5 weeks of growth. The error bars show standard deviation of the mean. Treatments without overlapping error bars are significantly different than each other.

3.5. Root allocations in each segment of the growth containers

Table 2 shows the mean percentage of root allocation of *B. coddii* in each segment of the growth containers for each treatment. *B. coddii* roots in control soil (BK c) were uniformly distributed among the segments of the growth containers except for segment B. This segment contained the thick and bulky apical part of the root (stem base) in all the treatments, therefore the highest percentage of root biomass always occurred in this segment. For the heterogeneously spiked treatments (BK c/62 and BK c/125), there was no preferential growth towards or away from the Ni-spiked segments (C and F). However, the roots in both treatments produced more biomass in the lower half of the container (segments A, B, and C) than the upper half (segments D, E, and F). For the homogeneously spiked treatments, there were no significant differences between the segments except for segment B, which contained the stem base.

Similar to *B. coddii*, roots of *C. arietinum* showed a uniform distribution in all the segments of the control except in the segment B (Table 3). However unlike *B. coddii*, they showed a significant decrease in root biomass in the Ni-spiked segments (C and F) in both heterogeneously spiked treatments (CP c/62 and CP c/125). While only segment F in the treatment CP c/62 showed a significant reduction in root biomass, both Ni-spiked segments C and F had significantly lower root biomass in treatment CP c/125. Furthermore, there was a significant difference in root biomass of segments C and F in treatment CP c/125. While there were no roots in segment F, roots grew in segment C, although significantly less than the unspiked segments. Root growth was hindered in both homogeneously spiked treatments (CP 62 and CP 125) and root biomass decreased considerably in the lower half of the containers.

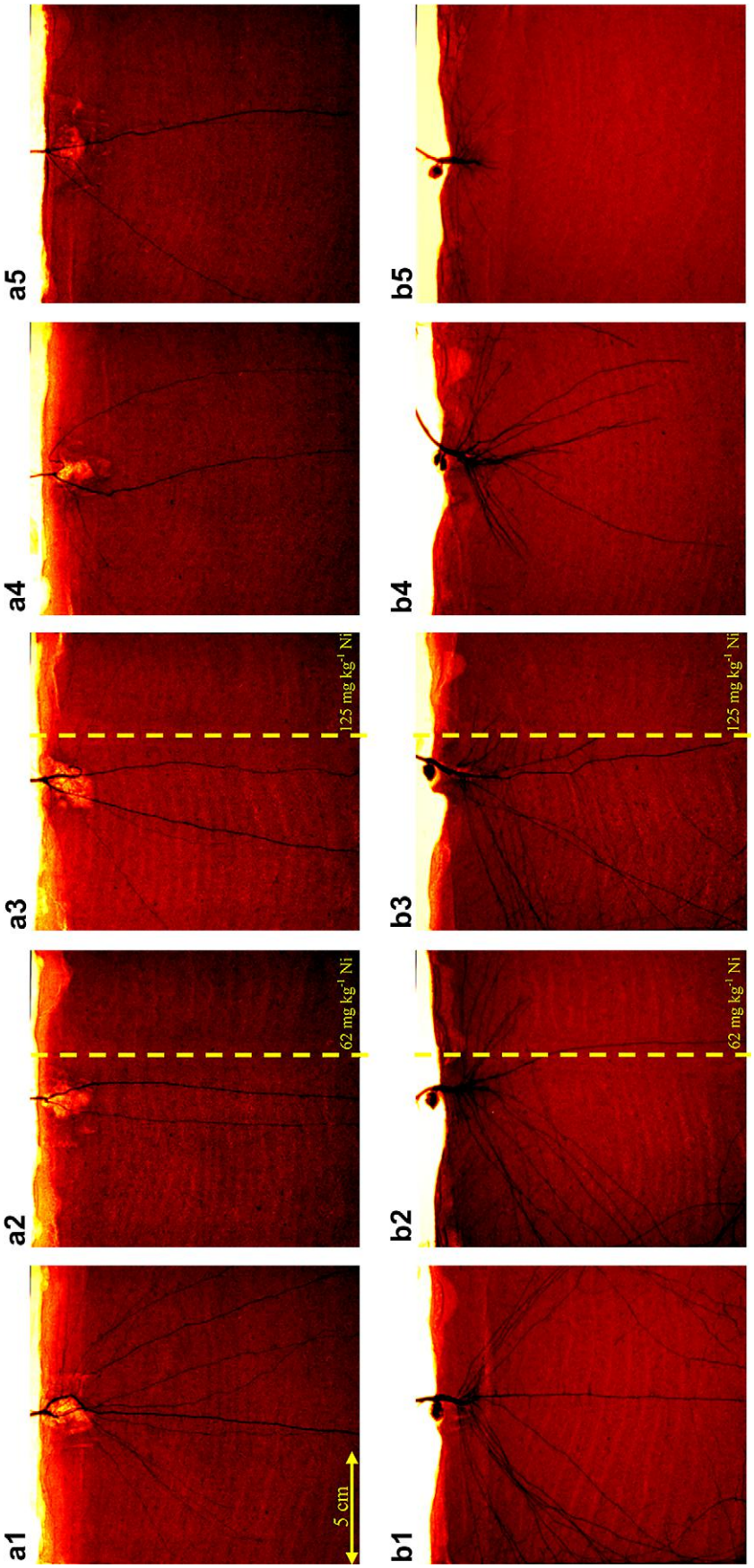

Fig. 7. Neutron radiographs of 5 weeks old *Berkheya coddii* (a) and *Cicer arietinum* (b) grown in control soil (a1 and b1), heterogeneously spiked with 62 mg kg^{-1} Ni (a2 and b2), heterogeneously spiked with 125 mg kg^{-1} Ni (a3 and b3), homogeneously spiked with 62 mg kg^{-1} Ni (a4 and b4), and homogeneously spiked with 125 mg kg^{-1} Ni (a5 and b5).

Table 2
Average percentage of *Berkheya coddii* roots in each segment of the growth container.

Segments	BK c	BK c/62	BK c/125	BK 62	BK 125
A	12.5 ± 2.2a[b]	3.3 ± 2.0a	5.3 ± 0.7a	8.1 ± 0.7a	11.4 ± 4.2a
B	27.1 ± 4.4b	51.6 ± 6.2b	41.5 ± 6.2b	51.9 ± 4.8b	52.7 ± 5.4b
C[a]	16.7 ± 4.9a	7.0 ± 4.2a	6.5 ± 0.8a	14.0 ± 1.9a	5.1 ± 4.1a
D	14.6 ± 2.9a	0.8 ± 0.1a	14.1 ± 0.2c	5.8 ± 2.1a	13.0 ± 4.8a
E	15.0 ± 4.7a	29.4 ± 2.9c	13.9 ± 4.6c	9.1 ± 2.8a	12.1 ± 4.5a
F[a]	14.1 ± 2.7a	7.8 ± 4.5a	18.6 ± 2.9c	11.0 ± 2.1a	5.6 ± 4.5a

Values are mean ± standard deviations, $n = 3$.
[a] Only segments C and F were spiked in treatments BK c/62 and BK c/125.
[b] Values sharing identical letters are not significantly different ($P > 0.05$).

3.6. Ni concentrations in above-ground biomass

Fig. 8 shows Ni concentration in dry above-ground biomass of *B. coddii* and *C. arietinum* grown in different treatments. Ni concentration in the dry above-ground biomass of *B. coddii* ranged from 13.9 ± 4.7 mg kg^{-1} in control soil to 1524.8 ± 137.1 mg kg^{-1} in BK 125. All the treatments significantly differed in their Ni concentrations. For *C. arietinum* however, there was no significant difference between CP 62 and CP 125 and between CP c and CP 62/c treatments and the Ni concentrations ranged from 10.6 ± 4.9 mg kg^{-1} in the control soil to 70.3 ± 19.1 mg kg^{-1} in CP 125.

4. Discussion

Root architecture of *B. coddii* changed in presence of Ni in soil compared to the control soil (Fig. 4). Thicker roots with considerably less branching were found in the spiked soil. Similar results for other hyperaccumulator plants have been reported in literature. Schwartz et al. (1999) showed that *T. caerulescens* developed shorter roots in clusters in the presence of Zn and rather finer and longer roots in unspiked soil. *B. coddii* produced lower root biomass in spiked soil compared to the control soil. Less branching, fewer fine roots, and lower root biomass means that the root surface area of *B. coddii* was reduced in the spiked soil. We speculate that this might be a morphological change to tolerate high concentration of Ni in the environment. More research is needed to understand fully the nature and the magnitude of these changes.

Our results did not show any preferential proliferation of *B. coddii* towards the Ni-spiked segments of the growth container in any of the heterogeneous treatments. Therefore, unlike what has been observed for some hyperaccumulator plants such as *T. caerulescens* (Schwartz et al., 1999; Whiting et al., 2000), root-foraging is not a metal-acquisition strategy in *B. coddii* at these concentrations. We did not observe any significant difference between the root biomass of *B. coddii* in the spiked segments of the heterogeneously spiked soil with 125 and 62 mg kg^{-1}. Foraging may occur at lower soil Ni concentrations. However, this is unlikely because the Ni concentrations that we used in our experiments are some tenfold lower than those reported by other workers (Robinson et al., 1997; Brooks et al., 2001). Therefore, a foraging strategy would have significantly increased the Ni concentration of the plant.

Table 3
Average percentage of *Cicer arietinum* roots in each segment of the growth container.

Segments	CP c	CP c/62	CP c/125	CP 62	CP 125
A	15.7 ± 3.0a[b]	14.8 ± 3.0a	24.9 ± 6.8a	21.3 ± 4.2a	19.9 ± 4.9a
B	37.9 ± 5.3b	32.7 ± 2.8b	36.1 ± 11.8a	46.9 ± 5.4b	53.4 ± 6.2b
C[a]	12.0 ± 4.9a	10.9 ± 4.7a	3.0 ± 0.9b	18.8 ± 1.1a	26.6 ± 2.8a
D	12.7 ± 2.7a	19.9 ± 4.8c	26.8 ± 8.9a	6.0 ± 1.9c	0.0 ± 0.0c
E	5.7 ± 4.6a	12.9 ± 0.9a	9.0 ± 2.6c	2.0 ± 4.5c	0.0 ± 0.0c
F[a]	15.8 ± 3.9a	8.6 ± 1.2d	0.0 ± 0.0d	4.9 ± 0.5c	0.0 ± 0.0c

Values are mean ± standard deviations, $n = 3$.
[a] Only segments C and F were spiked in treatments CP c/62 and CP c/125.
[b] Values sharing identical letters are not significantly different ($P > 0.05$).

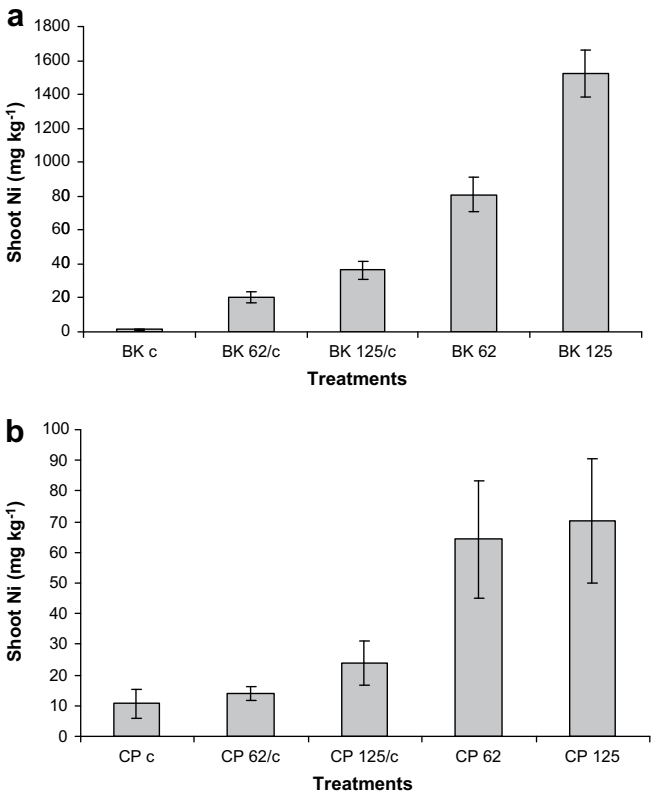

Fig. 8. Ni concentrations in the above-ground biomass of *Berkheya coddii* (a) and *Cicer arietinum* (b) after 5 weeks of growth in soil homogeneously and heterogeneously spiked with nickel. The error bars show standard deviation of the mean. Treatments without overlapping error bars are significantly different than each other.

B. coddii showed a high shoot:root ratio in the Ni-spiked treatments and disproportionally low shoot:root biomass ratio in the control soil (Fig. 6a). We speculate that this might be explained by the metabolic requirement of *B. coddii* for Ni. Studies on the possible physiological role of Ni in *B. coddii* could answer this question.

Root growth and biomass of *B. coddii* was significantly higher in the heterogeneously spiked soils compared to the homogeneous ones with the same concentrations of Ni in soil (Fig. 6a). Similarly, Haines et al. (2002) found greater growth of *T. caerulescens* in heterogeneous rather than homogeneous Zn treatment (even greater than the control). He associated this increase with stimulations due to higher nutritional requirement for Zn and differences in nutrient acquisitions by metal additions. In some plants greater growth can be achieved in habitats with heterogeneously distributed nutrients than with homogeneous ones (Wijesinghe et al., 2001; Hutchings and John, 2004). But whether soil heterogeneity causes plants to use the supply of resources more efficiently is not yet clear.

The non-accumulator species *C. arietinum*, however, showed a linear decrease in root biomass from control to heterogeneously and homogeneously spiked soils (Fig. 6b), which indicates that increasing Ni concentration in soil hindered root development. Interestingly, the shoot biomass did not show any significant change among the treatments despite severe reduction in root biomass. This might be explained by the large nutritional storage in the seeds.

Root biomass of *C. arietinum* was higher in the segment C (upper part of the container) of the container than the segment F (lower part of the container) in all the heterogeneously spiked treatments (Table 2). This indicates that there were more roots growing into the spiked segment in the early stage of root growth than the later stages. Whether this could be due to signalling or discrimination of the Ni-spiked segments in the later stage of root growth is yet to be studied. Unlike *B. coddii*, the root development of *C. arietinum* was more severely hindered in the heterogeneously spiked soil with 125 mg kg^{-1} Ni compared to the heterogeneously spiked soil with 62 mg kg^{-1}.

Ni concentrations in the above-ground biomass of *C. arietinum* did not exceed 93 mg kg^{-1} (Fig. 8b). There was no significant difference in the above-ground biomass of *C. arietinum* in various treatments. However, *C. arietinum* grown in the heterogeneously spiked soils showed significantly lower Ni concentrations in the above-ground biomass than the homogeneously spiked treatments. This can be explained by the root biomass reduction of *C. arietinum* in the Ni-spiked segments of the growth containers.

5. Conclusions

In summary, this study has demonstrated that unlike some other hyperaccumulator plants such as *T. caerulescens*, the roots of *B. coddii* do not proliferate preferentially towards Ni-rich patches in soil. However, its root morphology changes in the Ni-spiked soil. On the other hand, *C. arietinum* seems to avoid the Ni-spiked patches in soil. We recommend neutron radiography combined with image analysis technique to be used as a fast and quantitative screening method for root response of various plants to physical and chemical heterogeneities in soil.

Acknowledgements

This study was funded by the Swiss National Science Foundation. Dr. Héctor Conesa had a grant from Fundación Séneca of Comunidad Autónoma de Murcia (Spain).

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