Physiological responses of the nickel hyperaccumulator Bornmuellera emarginata under varying nickel dose levels and pH in hydroponics

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RESEARCH ARTICLE

Physiological responses of the nickel hyperaccumulator *Bornmuellera emarginata* under varying nickel dose levels and pH in hydroponics

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Abstract

Background and aims The nickel hyperaccumulator *Bornmuellera emarginata* (Brassicaceae) is a species adapted to thrive on naturally nickel-enriched ultramafic soils in the Balkans and a promising candidate for use in nickel agromining. The main aim of this study was to provide insight into the physiological mechanisms of nickel hyperaccumulation in *B. emarginata.*

Methods *Bornmuellera emarginata* was cultivated under various nickel exposure concentrations (control, 1, 10, and 100 µM nickel in solution), and different pH levels of the hydroponic solution for four weeks. During this period, the plants underwent assessment for various physiological parameters, including photosynthetic pigments, leaf relative water content, tolerance index, and metal accumulation in plant tissues.

Results The results show that the translocation factors and bioconcentration factors were > 1 even at 1 µM nickel in solution. This confirms the ability of *B. emarginata* to hyperaccumulate nickel (up to 6600 mg kg^{-1}) over a wide range of nickel concentrations in hydroponics. Nickel at 100 µM (a concentration that is an order of magnitude higher than the highest soil solution nickel concentration found in ultramafic soils) induced only mild physiological stress symptoms (e.g. a minor proline response). Alterations in the solution pH did not cause any significant effect on nickel accumulation in the plants.

Conclusions *Bornmuellera emarginata* is a highly adapted nickel-tolerant and nickel hyperaccumulating species that shows very little stress responses even to extreme nickel exposure concentrations in hydroponics. This species shows interesting trade-off responses between nickel and other metals, including non-competitive uptake of zinc. The potential for this species to accumulate zinc should therefore be further explored.

Keywords Nickel · Bornmuellera emarginata · Physiology · Nickel agromining

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Introduction

Metals and/or metalloids are released by natural phenomena or anthropogenic activities following increased industrial development and have become environmental issues that pose severe threats to

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ecosystems and human health (Sytar et al. 2021). Ultramafic soils are derived from magmatic rocks and typically contain > 500 mg kg^{-1} nickel (Ni) (Proctor 2003). Due to their origin and characteristics (high metal concentrations, low nutrients, and Ca/Mg ratio), soils derived from ultramafic bedrock impose what has been called the "serpentine syndrome" (Jenny 1980). These atypical soils exert a strong selective pressure on plants native to ultramafic habitats (Konečná et al. 2020). As a consequence, ultramafic soils frequently host a high level of endemism and specialized plant species that have developed mechanisms allowing them to grow in these environments (van der Ent et al. 2015b). To meet their metabolic requirement for development, plants use metals such as manganese (Mn), Ni and zinc (Zn) at trace levels (Srivastava et al. 2017). However, some metals highly enriched in ultramafic soils have no biological function and may impact growth, nutritional balance, and photosynthesis at high concentrations (Mohammed et al. 2011). Plants evolved physiological mechanisms which enable them to survive when facing high metal concentrations in the soil (Ghori et al. 2019). Most of the plants growing on metalliferous soils are "excluders" and reduce root metal uptake and translocation to aerial parts, while "indicators" accumulate metals in their aboveground tissues and the plant metal concentration reflect prevailing concentrations in the soil (Baker 1981; Baker and Walker 1990; Kraemer 2010). Hyperaccumulators are a group of plants that accumulate specific metals or metalloids at extremely high concentrations in their shoots and maintain low metal concentration in their roots (Kraemer 2010; van der Ent et al. 2013). To be classified as a hyperaccumulator, a plant accumulates more than 10,000 mg kg^{-1} Mn, 3000 mg kg^{-1} Zn, 1000 mg kg^{-1} As, Co, Cu, Ni, or 100 mg kg^{-1} Cd (Reeves et al. 2018). Currently, more than 700 hyperaccumulator species, of which 500 hyperaccumulate Ni, have been recorded globally (Reeves et al. 2018).

The evolution of hyperaccumulator plants resulted in their enhanced capacity to efficiently take up and detoxify high prevailing metal concentrations, that would otherwise be toxic (van der Ent et al. 2013, 2015a, b, 2019). This key trait is being exploited in novel technologies aimed at remediating contaminated soil in phytoextraction (Chaney 1983) and in phytomining (van der Ent et al. 2015a, b; Nkrumah et al. 2016). In this approach, the hyperaccumulation trait is put at work in plants to sequester Ni (or other target metals) in above-ground tissues that can be harvested to produce fine Ni chemicals or pure metals for use in industry. This whole agronomic chain which aims at producing metals in this way is known as 'agromining' or 'metal farming' (Li et al. 2003a; van der Ent et al. 2015a).

Hyperaccumulation involves molecular and physiological adaptations that include increased mobilization and uptake of metals in the roots, decreased sequestration of metals in root vacuoles, efficient transport of metals from roots to shoots through xylem, and effective sequestration and compartmentalization of metals to the leaves (Verbruggen et al. 2009; Kraemer 2010). Nickel uptake in roots relies on Ni^{2+} concentration in the medium, soil or solution acidity, competition with other divalent metals, and organic matter (Chen et al. 2009). Nickel uptake is mediated by high-affinity transporters in hyperaccumulator plants including IREG1-2, IRT1, NRAMPs (Merlot et al. 2014). Nickel uptake can be inhibited by the presence of Zn^{2+} in hydroponic solution in *Noccaea pindica* or *N. caerulescens*, but Ni has only a minor effect on Zn^{2+} uptake (Taylor and Macnair 2006; Deng et al. 2014). Likewise, Ni absorption in *Odontarrhena* (formerly *Alyssum*) *bertolonii* roots is lowered by Ca^{2+} (Gabbrielli and Pandolfini 1984) and translocation from roots to shoots is strongly decreased by excess Zn^{2+} in the solution (Deng et al. 2014); while the presence of Ca^{2+} enhances Ni^{2+} accumulation in *Berkheya coddii* (Boyd and Martens 1998).

Once Ni has entered the root it is complexed with citrate, malate and other carboxylic acids (Montargès-Pelletier et al. 2008; Kraemer 2010). Histidine is constitutively high in the roots of *Odontarrhena lesbiaca* compared to the non-hyperaccumulator *Brassica juncea* (Kerkeb and Kra 2003) and enhances Ni mobility by suppressing Ni sequestration into root vacuoles facilitating its radial transport in the root symplast (Kozhevnikova et al. 2014). Nickel is loaded from root to xylem vessels through specific transporters, but these have not yet been discovered (Kraemer 2010; Van der Pas and Ingle 2019). In the xylem sap, Ni is present as the free hydrated cation (Centofanti et al. 2013) or chelated with histidine, malate, and citrate in the *O. lesbiaca* (Kramer et al. 1997), or as the Ni-nicotianamine complex in the xylem sap of *N. caerulescens* (Mari et al. 2006). Once in the xylem, Ni movement

follows the xylem flow from transpiration and root growth induced by the water potential of the ion supply in the xylem (Eapen and D'Souza 2005). The final step in the physiological process of Ni transfer and accumulation from the root to the shoot, is the storage in the leaf epidermis, although palisade mesophyll cells represent a secondary compartment when Ni concentrations further increase in the leaves (Küpper et al. 2001; Broadhurst et al. 2004; Jaffré et al. 2018). At the subcellular level, Ni is mainly stored in leaf vacuoles, as evidenced by the Ni^{2+}/nH^{+} antiport activity driven by the vacuolar ATPase, whose function is to transport Ni into leaf vacuoles (Ingle et al. 2008). Here, Ni is complexed by carboxylic acids, such as citric acid, malic, and malonic acid in many different Ni hyperaccumulator species (Brooks et al. 1981; Montargès-Pelletier et al. 2008; Callahan et al. 2012).

Bornmuellera emarginata (Boiss.) Resetnik (synonyms: *Leptoplax emarginata* (Boiss.) O. E. Schulz, *Peltaria emarginata* (Boiss.) Hausskn.) (Resetnik et al. 2014) is endemic to ultramafic soils in Greece with a discontinuous distribution from the Pindus Mountains, Mt. Smolikas, and the island of Evvia (Chardot et al. 2005; van der Ent et al. 2019). *Bornmuellera emarginata* is a promising candidate for Ni agromining due to its strong foliar accumulation capacity which can reach up to 34,400 mg kg^{-1} Ni (Reeves et al. 1980). Carboxylic acids (citrate, malate) are the main chelators implicated in Ni complexation in *B. emarginata* (Montargès-Pelletier et al. 2008).

Currently, there is limited knowledge on the physiological mechanisms of Ni hyperaccumulation in *B. emarginata*. Therefore, this study aims examining a *B. emarginata* population originating from Evvia Island (Greece) and to evaluate the responses to Ni dose levels in hydroponics and the influence of solution pH on plant Ni accumulation.

Materials and methods

Plant materials and growth conditions

Bornmuellera emarginata seeds originated from different locations in Mantoudi on the island of Evvia (Greece) were sampled and stored in a greenhouse at 30 °C for one month to complete the ripening process and then at 4 °C until sowing. The seeds were subsequently germinated in organic potting soil for two weeks and six seedlings considered as a biological replicate were transferred to 15 L containers with Hoagland nutrient solution (Hoagland and Arnon 1950) spiked with different Ni dose levels (0, 1, 10, 100 µM $NiSO_4$) in Experiment 1. In Experiment 2, the Ni solution concentration was kept constant at 100 µM and the pH was varied at pH 5.5, 6.5, or 7.5. Both experiments were conducted in a climate chamber with a light intensity of 400 µmol m^{-2} s^{-1}, a room temperature of 22 °C day/15 °C night, a photoperiod of 16 h and a humidity of 80%. The hydroponic solutions were changed twice a week for the duration of the experiments.

Growth parameters measurement and metal content

At the end of both experiments, whole plants were harvested and carefully washed with deionised water, then separated into roots and shoots. The biomass was measured and then ground, and 0.5 g was digested with 5 mL HNO_3 and 2.5 mL H_2O_2. After cold digestion for 16 h, the samples were placed in a DigiPREP system for 180 min and filtered prior to ICP-AES analysis. Fresh roots were utilized to measure root components, including root length, root area, and root diameter, using the RhizoVision software (Seethepalli et al. 2021). The tolerance index was calculated in each experimental condition to assess the relative tolerance to the Ni exposure concentration using the equation of Turner and Marshall (1972) as follows:

$$\text{Ti}\% = 100 \times \text{Dry roots or leaves in Ni treatment} / \text{Dry roots or leaves in the control}$$

The rate of accumulation of Ni from the solution to the plants was assessed in all treatments by calculating the translocation factor and the bioaccumulation factor. The translocation factor is defined as the average of the ratio of leaf to root Ni concentrations, while the bioaccumulation factor is defined as the average of the ratio of soil/solution to leaf Ni concentrations (Macnair 2003; Roccotiello et al. 2015).

Chlorophyll and carotenoid content

Chlorophyll and carotenoids were analysed in all samples from each condition in Experiment 1 (0, 1, 10, 100 µM Ni) from the leaf for photosynthetic pigments. Five to ten milligrams of lyophilised leaves were crushed in 1 mL of chilled 80% ethanol using a pestle and mortar. The sample was centrifuged at 10,000 × g for 10 min at 4 °C and the supernatant was collected. The chlorophyll content was determined following the method of López-Hidalgo et al. (2021). The carotenoid content was determined following the same procedures as described above using wavelengths of 470, 649, and 664 nm.

Proline and relative water content determination

Proline content was measured using the methods described by Bates et al. (1973). Briefly, a fresh sample of 0.5 g was homogenized with 10 mL 3% sulfosalicylic acid. After centrifugation at 12,000 × g for 5 min, 2 mL of the supernatant was added to the reaction mixture of 2 mL of glacial acetic acid and 2 mL of acid ninhydrin then incubated for 1 h at 100 °C. The reaction was terminated by placing the tube in an ice bath. After 4 mL of toluene was added, the solution was vigorously mixed. The light absorbance of the toluene phase was measured at 520 nm using a UV-Vis spectrophotometer, and the proline concentration was determined using a standard curve prepared using proline.

The water status of the plant under the Ni exposure was assessed by measuring the relative water content in leaf tissues according to Yamasaki and Dillenburg 1999. This method comprises of direct measurement of the fresh weight, then, after 24 h of imbibition of the samples under continuous shaking, the turgid weight is measured, finally samples are dried for 24 h at 60 °C and the dry weight is measured. The fresh, dry and turgid weights were recorded for each plant.

Statistical analysis

All treatments for each condition were replicated six times, and the results are reported as mean value ± standard error. Tests of significance were tested using Fisher's exact test with R software.

Results

Biomass and metal accumulation in response to varying nickel dose levels

No visible symptoms of toxicity (such as foliar chlorosis) were observed in any of the Ni treatments. The average total dry weight of the plants was not significantly different in response to the Ni dose levels ($P < 0.05$) (Fig. 1a), showing that *B. emarginata* is tolerant to at least 100 µM Ni in hydroponic solution. However, the lowest biomass was recorded in the 10 µM Ni treatement and the highest biomass in the 1 µM Ni treatment, with an average shoot weight of 2.06 ± 1.26 and 3.51 ± 1.59 g dry weight respectively (Fig. 1a). In the presence of Ni, no significant effect on root total biomass and its components (root length, area, and volume) (Supplementary data) were observed in *B. emarginata* (Fig. 1b). However, at the highest Ni treatment level (100 µM Ni), the root diameter (Fig. 1c) significantly decreased compared to the control ($p < 0.05$). Regarding metal accumulation, Ni concentrations in the roots and shoots increased with increasing Ni concentration in the solution (Fig. 2). The mean shoot Ni concentration was 5 ± 2, 90 ± 40, 1010 ± 390 and 5010 ± 1300 mg kg^{-1} at Ni dose rates of 0, 1, 10, 100 µM, respectively. Therefore, we confirm that Ni hyperaccumulation (1000 mg kg^{-1}) occurred in response to 10 µM Ni in the solution (Fig. 2), indicating the ability of *B. emarginata* to accumulate Ni over a wide Ni concentration range. Leaf Ni concentrations were strongly and positively correlated (r = 0.99) with Ni concentrations in the solution. The translocation and bioconcentration factors of Ni changed along with the increase of Ni concentrations (Table 1). All were over 1 in all treatments and were significantly different for each treatment. However, the translocation and bioconcentration factors were much higher at 10 µM with an average of 3.38 and 101 respectively (Table 1).

The shoot metal concentrations (Fe, Zn, Mn, and Cu) and correlations with Ni are shown in Fig. 3 and summarized in Table 2. The metal accumulation in shoots significantly varied with increasing Ni dose levels in solution, as seen in Fig. 3. Specifically, Fe accumulation decreased from 275 mg kg^{-1} to 47.7 mg kg^{-1} between 0 and 100 µM Ni, respectively. Additionally, the concentrations of Mn and Cu significantly decreased with increasing Ni concentrations,

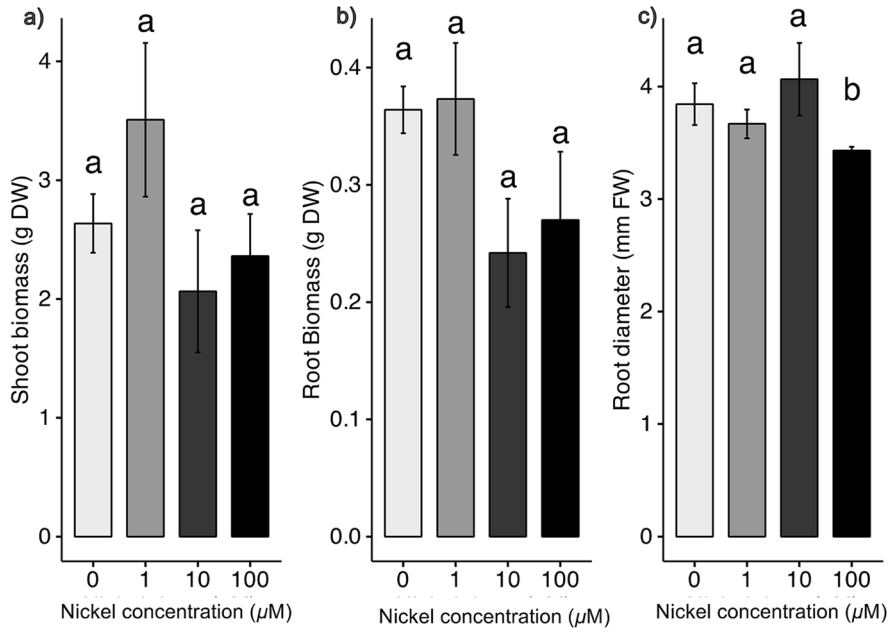

Fig. 1 Total biomass of *Bornmuellera emarginata* exposed to different Ni dose levels in hydroponics in Experiment 1. (**a**) Shoot biomass of plants treated with Ni. (**b**) Root biomass. (**c**) Root diameter of plants. Bar represents the standard error of the mean $n=6$. Means labelled with different letters indicate significant differences at $p<0.05$ using Fisher's exact test. DW: dry weight, FW: fresh weight

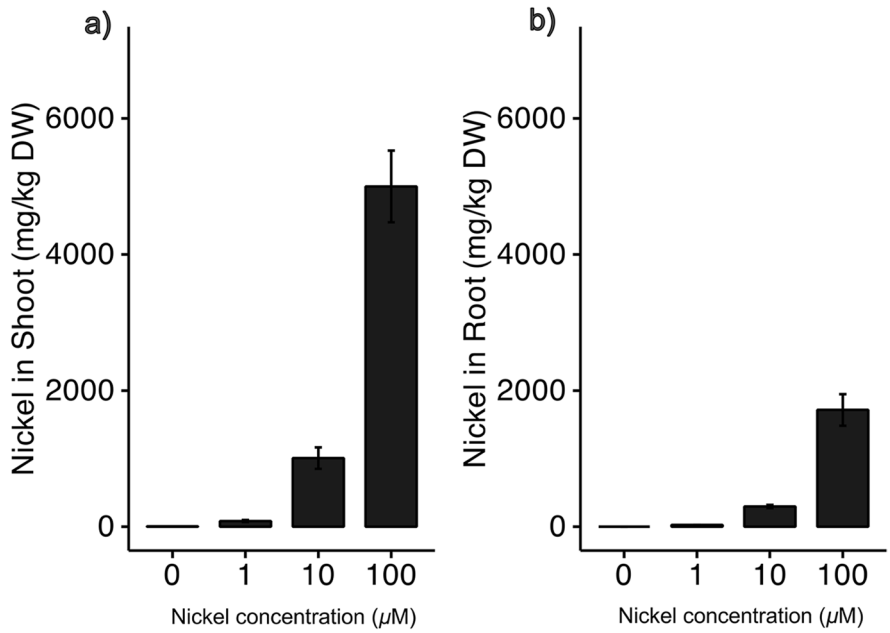

Fig. 2 Nickel accumulation in shoots and roots of *Bornmuellera emarginata* exposed to different Ni dose levels in Experiment 1. Bars represent the standard error of the mean $n=6$

from 180 to 50.8 mg kg^{-1} for Mn, and from 10.6 to 3.4 mg kg^{-1} for Cu (control and 100 µM Ni dose levels, respectively). However, the presence of Ni did not result in a significant difference in Zn accumulation. Table 2 shows the correlation analysis, revealing a negative correlation between metals (Fe, Zn, Mn, and Cu) and Ni, with *Pearson's* correlation coefficients of -0.26, -0.44, -0.56, and -0.42, respectively. Notably, Zn exhibits no statistically significant correlation with Ni ($p<0.05$).

The value of the tolerance index showed maximum values in the first treatments (control, 1 µM Ni) and no significant reduction was observed thereafter up to 100 µM Ni. Considering all the treatments, the decrease

Table 1 Translocation (TF), Bioconcentration factor (BF) and Tolerance index (Ti) of *Bornmuellera emarginata* in response to Ni dose levels in hydroponics. Data are means of six replicates. Means with different letters are significantly different at $p < 0.05$ (Fisher's exact test)

Ni concentration (μM)	TF	BF	Ti (100%)
0	-	-	-
1	3.14 a	84.0 a	101 a
10	3.38 a	101 a	66.5 a
100	2.91 a	50.0 b	74.2 a

TF Translocation factor, *BF* Bioconcentration factor, *Ti* Tolerance index

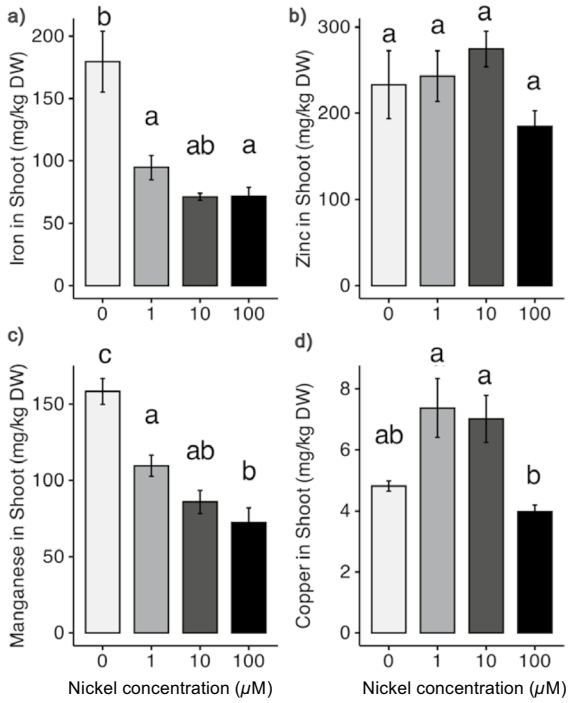

Fig. 3 Iron, zinc, manganese, and copper concentrations in plant shoots of *Bornmuellera emarginata* in Experiment 1 are shown in panels (**a**) Fe, (**b**) Zn, (**c**) Mn, and (**d**) Cu. Bars represent the standard error of the mean $n = 6$. Means labelled with different letters indicate significant differences at $p < 0.05$ using Fisher's exact test

of the tolerance index did not reach a value lower than 50% (Table 1). These values confirm the extremely high tolerance of *B. emarginata* to Ni. Typically, metal stress has a significant effect on photosynthetic activity, the most fundamental and complex physiological

Table 2 Correlation coefficients for Ni, Zn, Fe, Mn, and Cu concentrations in plant shoots of *B. emarginata* under Ni dose levels in hydroponics

Correlation Coefficient	Ni	Zn	Fe	Mn	Cu
Ni	1				
Zn	-0.26	1			
Fe	-0.44*	-0.04	1		
Mn	-0.56*	0.32	0.81*	1	
Cu	-0.42*	0.52*	-0.11	0.13	1

Asterisks (*) denote significant differences at $p < 0.05$ (Pearson)

process in all green plants. However, total chlorophyll (Chl a + Chl b) and carotenoids (Supplementary Fig. S1) in the shoot did not change significantly in the Ni treatments. However, at 10 μM Ni there was a slight reduction in chlorophyll and carotenoids compared to the other Ni treatments. Exposure of *B. emarginata* to Ni resulted in a modification of the water exchange according to the increasing Ni dose level in the solution. The relative water content, an important parameter indicating the water status in the plant, was lower with increasing Ni concentrations in solution. The highest relative water content of 93% was observed in the control condition (0 μM Ni) and the lowest relative water content was recorded in the highest Ni dose level (100 μM Ni) (Fig. 4) with an average value of 78.9%. No significant difference was observed between the treatments, except in the control and in the 100 μM Ni dose level ($p < 0.05$). Proline accumulation was assessed in response to Ni exposure across various conditions, and a noteworthy reduction in proline accumulation from 0.53 to 0.13 μmol g^{-1} fresh weight was found between the control (0 μM) and the 10 μM Ni dose level (Fig. 4). However, no statistically significant differences were observed between the control and the other Ni treatments (e.g. 1 and 100 μM Ni).

Biomass and metal accumulation in response to varying pH in solution

The total shoot and root biomass remained unaffected by the pH variation in solution in the experiment. While no significant differences were observed between the treatments, a slight reduction in root biomass was noted (Supplementary Fig. S2). The average biomass recorded was 2.98, 2.66, and 3.3 g DW

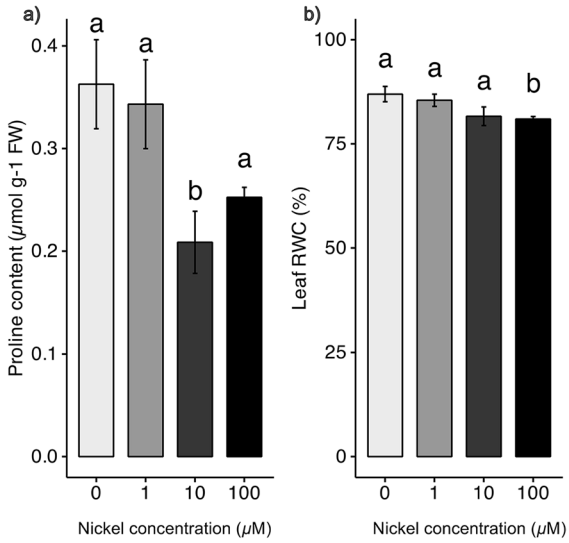

Fig. 4 Physiological traits of *Bornmuellera emarginata.* (**a**) Proline content of plants dose with Ni in solution, and (**b**) Relative water content of plants dose with Ni in Experiment 1. Bars represent the standard error of the mean $n=6$. The letter above indicates values that differ significantly from control at $P<0.05$ using Fisher's exact test

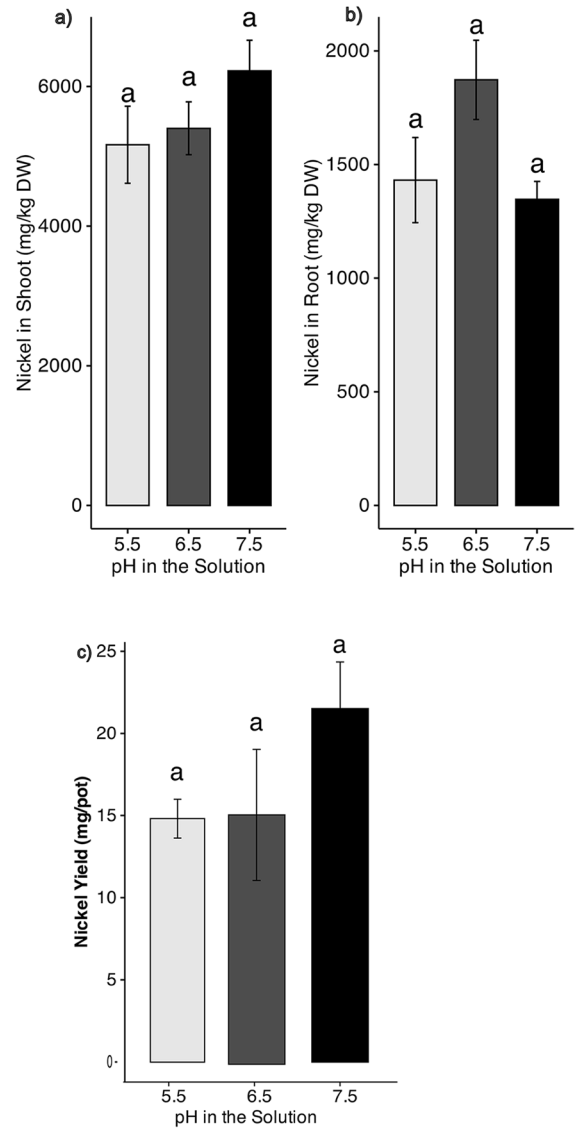

Fig. 5 Nickel concentrations in plant shoots, and Ni yield of *Bornmuellera emarginata* in Experiment 2 are shown in panels (**a**) Ni Shoot, (**b**) Ni Root, and (**c**) Nickel Yield. Bars represent the standard error of the mean $n=6$. Means labelled with different letters indicate significant differences at $p<0.05$ using Fisher's exact test

for pH 5.5, 6.5, and 7.5, respectively (Supplementary Fig. S2). The Ni yield, calculated as the Ni concentration in the shoot multiplied by the shoot biomass, was not impacted by the variation in solution pH (Fig. 5).

The Ni concentrations, analysed using ICP-AES, showed no statistically significant increase with the increasing solution pH, with average values of 5170, 5400, and 6230 mg kg^{-1} Ni for pH 5.5, 6.5, and 7.5, respectively (Fig. 5). Interestingly, the highest Ni shoot concentration was observed at pH 7.5, although there was no statistical difference compared to the other two conditions. The translocation factor, the ratio of root Ni concentration to shoot, Ni concentration was significantly affected between pH 6.5 and 7.5 (Fig. 6). However, no significant impact on the bioaccumulation factor was observed following pH variation (Fig. 6). All of the translocation factor values recorded are above 1, but increased significantly from 2.95 to 4.62 between pH 6.5 and 7.5.

The concentrations of metals (Fe, Zn, Mn, and Cu) in the shoot in response to solution pH are presented in the Fig. 7 and show that an increase in pH significantly decreased the accumulation of Fe and Cu in *B. emarginata*, but there was no significant difference or Zn and Mn. Specifically, the accumulation of Fe decreased from 77.7 mg kg^{-1} to 48.0 mg kg^{-1} as the pH increased from 5.5 to 7.5. Furthermore, shoot concentrations of Cu were influenced by the elevated pH, decreasing from 5.94 to 2.5 mg kg^{-1} at pH 5.5 and 7.7 with 100 µM Ni, respectively (Fig. 7). However, the presence of Ni did not lead to a significant difference in Zn and Mn accumulation, with values ranging

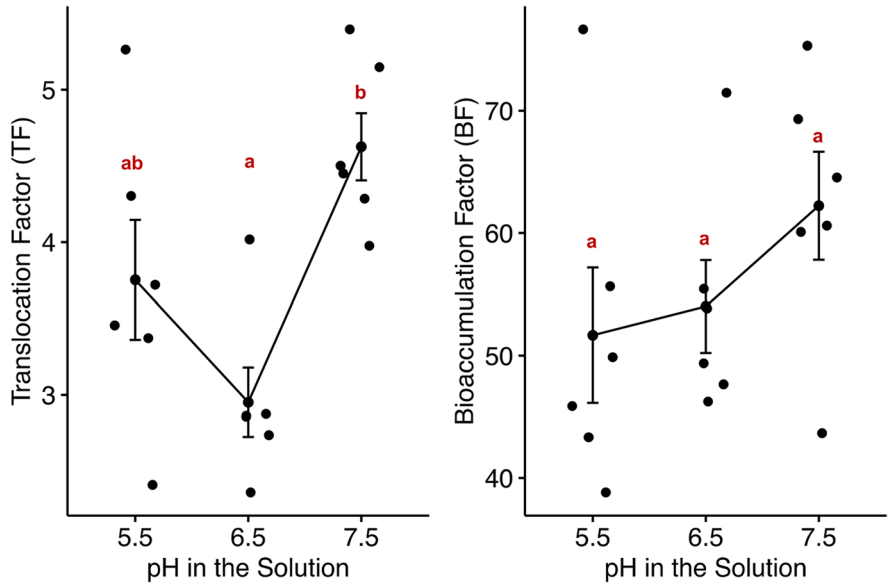

Fig. 6 Nickel accumulation factor of *Bornmuellera emarginata.* (**a**) Translocation and (**b**) Bioaccumulation Factor following pH variation in Experiment 2. Bars represent the standard error of the mean $n=6$. Letters indicate values that differ significantly from control at $p<0.05$

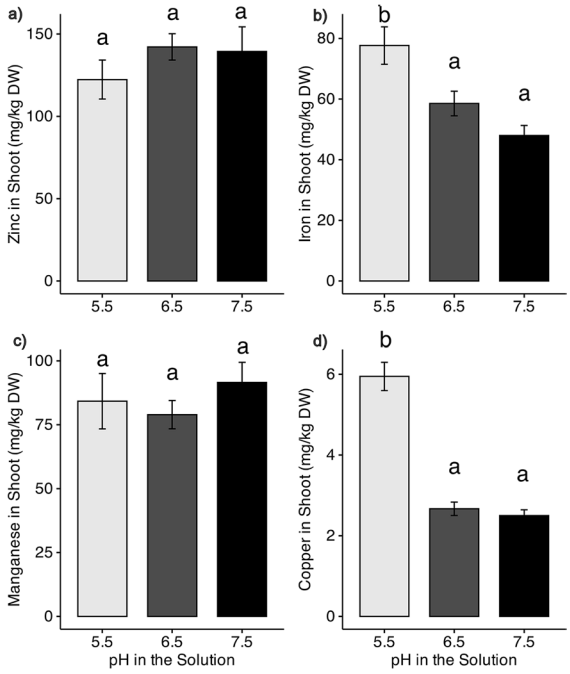

Fig. 7 Iron, zinc, manganese, and copper concentrations in plant shoots of *Bornmuellera* in Experiment 2 are shown in panels (**a**) Zn, (**b**) Fe, (**c**) Mn, and (**d**) Cu. Bars represents the standard error of the mean $n=6$ Means labelled with different letters indicate significant differences at $p<0.05$ using Fisher's exact test

from 84.2 mg kg^{-1} at pH 5.5 to 91.56 mg kg^{-1} at pH 7.5 for Mn, and an average Zn concentration of 122, 142, and 139 mg kg^{-1} for pH 5.5, 6.5, and 7.5, respectively.

Discussion

Effect of varying Ni dose levels in hydroponics

Bornmuellera emarginata showed no signs of toxicity when exposed to elevated Ni concentration up to 100 µM Ni in hydroponic solution, which makes it an extremely Ni tolerance species, as expected of a genuine hyperaccumulator. The plant Ni concentrations increased with the increase of Ni in the solution (Bazihizina et al. 2024). The threshold (1000 mg kg^{-1}) (Brooks et al. 1977) for Ni hyperaccumulators was reached when plants were exposed to 10 µM Ni in solution, with a mean value of 1010 ± 390 mg kg^{-1} DW in leaves. The Ni concentration in the root (Fig. 2) was always lower than the Ni in the shoot, which is in agreement with the fact that hyperaccumulator species store more metal in the shoot than in the roots, in contrast to excluders (Deng et al. 2018).

The mean shoot Ni concentrations (5010 ± 1300 mg kg^{-1}) at 100 µM Ni in solution (Fig. 2) were much higher than that obtained by Robinson et al. (2003) with *Berkheya coddii* grown in hydroponics (with 4375 and 3210 mg kg^{-1} for old and young leaves, respectively). The translocation and bioconcentration factors (Table 1) were greater than 1 in all treatments, confirming the hyperaccumulation capacity of *B. emarginata*. The decrease in translocation factor (3.38–2.91) between 10 and 100 µM Ni

may indicate that Ni translocation is reduced when the concentration threshold in the upper part is exceeded. This trend has been shown in the Lellingen population of *N. caerulescens*. Metal translocation from root to shoot was reduced, suggesting a down-regulation of the translocation system (Assunção et al. 2003). This response contrasts with the Ni hyperaccumulators *O. lesbiaca* and *O. bertolonii*, with no decrease in the Ni root:shoot ratio with increasing Ni concentration, even at high levels (Galardi et al. 2007; Adamidis et al. 2014).

In our study, exposure to Ni exhibited a detrimental effect on the accumulation of Fe and Mn, whilst it had a lesser impact on Cu, and had no observable effect on Zn. Particularly, Ni led to a reduction in Fe accumulation, significantly differing between the control and treatment conditions. This contrasts with previous findings indicating an increase in Fe accumulation under Ni exposure (Deng et al. 2019). In hydroponics, increasing Ni concentrations were subjected to the Ni-Zn hyperaccumulator *Noccaea caerulescens*, resulting in elevated Fe accumulation, as reported by Deng et al. (2019). Additionally, under Ni exposure, *Arabidopsis thaliana*, a non-accumulator species, accumulated high levels of Fe, as observed by Nishida et al. (2012). This phenomenon can be attributed to the induction of Fe deficiency by the presence of Ni, potentially triggering the expression of the *IRT1* gene associated with the Strategy I (reducing) of Fe homeostasis (Nishida et al. 2011, 2012; Deng et al. 2019). Furthermore, we have found that the Ni exposure negatively impacted Mn accumulation and in lesser extent Cu, where the concentration increased at 1 and 10 µM Ni exposure. The substantial impact of Ni on Mn accumulation suggests competition between the two divalent elements Ni^{2+} and Mn^{2+}. This has been shown previously in different species, including hyperaccumulator and non-accumulator (Mizuno et al. 2005; Milner et al. 2013; Deng et al. 2019; Sabir et al. 2022).

Interestingly, our results show that Ni exposure had no discernible effect on Zn accumulation, as its concentration remained relatively stable. This aligns with findings in *N. tymphaea* (formerly *Thlaspi pindicum*), where no significant effect on Zn accumulation was reported during growth in the presence of 100/100 µM Zn/Ni (Taylor and Macnair 2006). These results differ from those of Deng et al. (2019), who reported a reduction in Zn accumulation in *N. caerulescens* following Ni exposure and an even bigger reduction in Ni accumulation and translocation (root-to-shoot) was observed with high Zn concentrations (Deng et al. 2014). This discrepancy may be attributed to the fact that Ni is transported with low-affinity transporters, while Zn is acquired through high-affinity transporters, making its accumulation less susceptible to variations in Ni concentration in the solution (Assunção et al. 2001).

Total shoot and root biomass did not differ significantly with Ni concentration (Fig. 1). However, a slight, although non-significant, increase in biomass occurred at low concentrations (1 µM) which may be the hormesis effect (Calabrese and Blain 2009). This stimulation phenomenon has been previously reported in other studies of hyperaccumulators plants (Krämer et al. 1996; Küpper et al. 2001; Bettarini et al. 2021). Our results are in agreement with Robinson et al. (2003), Adamidis et al. (2014) and Roccotiello et al., (2016), who found no significant reduction in biomass of *B. coddii*, *O. lesbiaca* and *Alyssoides utrilicata*, respectively. Moreover, Deng et al. (2019) observed no significant difference in biomass yield of *N. caerulescens* cultivated under Ni exposure up to 100 µM.

Total chlorophyll and carotenoid content did not differ significantly according to Ni dose level, although a slight reduction was observed at 10 µM Ni in solution, this did lead to visible leaf chlorosis in the plants. A similar result was found in *O. moravensis* and *O. chalcidica*, where a stable amount of Chl a and Car was maintained over a range of Ni dose levels (Scartazza et al. 2022). A significant difference was observed for water content following Ni dose level in solution. The decrease in relative water content of 6.88% at 100 µM Ni could indicate that the plant was under mild osmotic stress (Fig. 4). Our results are in agreement with Scartazza et al. (2022), who reported a decrease in leaf water content of 37.8% and 11.3% in both *Odontarrhena muralis* and *O. moravensis*, respectively, at 1 mM Ni compared to the control. On the other hand, this response contrasts with the results of Roccotiello et al. (2016), where a stable water content was maintained in *A. utrilicata* under Ni treatment. The differences observed between our results and those of Roccotiello et al. (2016) may be due to the fact that in the latter study the experiments were carried out in soil and not in hydroponics.

Our study revealed intriguing variations in proline (Fig. 4) accumulation patterns under Ni exposure,

showcasing a distinct trend between the treatment conditions. Notably, a significant difference emerged between the control and the treatment at 10 µM Ni, while no such difference was recorded in the remaining conditions. This outcome is somewhat unexpected, given the conventional understanding that plants tend to accumulate higher proline content in response to stress conditions. Typically, under stress, plants amass these compatible solutes as part of a protective mechanism, contributing, among other factors, to reactive oxygen species detoxification, cellular osmotic adjustment, and the preservation of membrane integrity (Hayat et al. 2012). The response observed in our results prompts further exploration to decipher the underlying mechanisms governing proline dynamics in *Bornmuellera emarginata* under different concentrations of Ni exposure.

Impact of pH variation in hydroponics on Ni accumulation

It has been reported that increasing soil pH was associated with increasing Ni accumulation in the Ni hyperaccumulator *O. chalcidica* (previously *Alyssum murale*) (Li et al. 2003a, b), but in *Berkheya coddii* and *O. bertolonii* a reduction of Ni accumulation followed an increased pH (Robinson et al. 1999). Our results on *B. emarginata* showed no significant impact on Ni accumulation in response to varying pH levels in hydroponic solution. However, we found a marginal rise in Ni shoot concentration at pH 7.5. Our findings align with the results of Li et al. (2003a, b), who showed an increase in Ni concentration with rising soil pH in the hyperaccumulators *O. chalcidica* and *O. corsica*, even though their study was conducted in soil. Additionally, this study is consistent with the results reported in the tropical Ni hyperaccumulators *Phyllanthus rufuschaneyi* in which the reduction of soil pH reduced the shoot Ni concentration (Nkrumah et al. 2019). This contrasts with the results reported by (Robinson et al. 1999), where a decrease in Ni concentration was linked to an increase in soil pH. The authors showed that the Ni hyperaccumulators *Berkheya coddii* and *O. bertolonii* were influenced in their Ni shoot concentration by variations in pH.

Varying solution pH did not impact Zn or Mn accumulation in *B. emarginata,* but it did have a negative effect on Fe and Cu accumulation. Interestingly, we have observed that the increase of solution pH significantly reduced the accumulation of Fe and Cu. These results are in contrast with the finding of Kukier et al. (2004) who reported a stable trend of Cu concentration throughout a pH range in *Alyssum* plants. However, they observed a decrease of Zn concentrations in plants while the pH was raised (Kukier et al. 2004).

Conclusions

This study showed the high Ni tolerance of *B. emarginata*, with no significant impact on Ni accumulation observed under varying pH levels in a hydroponic system, while Ni shoot accumulation positively correlated with Ni concentration in the solution. The highest dose level (100 µM Ni) led to mild osmotic stress, resulting in a reduction in leaf relative water content. The findings from pH variation in hydroponics differ from those in soil, where pH changes typically impacts Ni accumulation in the plant. This suggests that soil Ni availability is influenced by various factors, and further investigations into the effects of different hydroponic conditions on *B. emarginata* are warranted to better understand the primary factors influencing Ni uptake.

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Author contributions SNL and GE collected the samples in the field. SNL, GE, AVDE conceived and designated the experiments. SNL cultivated the plants and conducted the experiments. SNL acquired the data and conducted the elemental analysis. All authors contributed to write, review, and edit the manuscript.

Data availability The data that support this study will be shared upon reasonable request to the corresponding author.

Declarations

Conflicts of interest The authors declare no conflicts of interest relevant to the content of this manuscript.

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